EVALUATION OF SELECTIVELY FERTILIZED COCONUT HYBRIDS (*Cocos nucifera* L.) FOR WATER USE EFFICIENCY THROUGH STABLE ISOTOPE DISCRIMINATION.

By

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"EVALUATION OF SELECTIVELY FERTILIZED COCONUT HYBRIDS (*Cocos nucifera* L.) FOR WATER USE EFFICIENCY THROUGH STABLE ISOTOPE DISCRIMINATION"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled **"EVALUATION OF SELECTIVELY FERTILIZED COCONUT HYBRIDS (*Cocos nucifera* L.) FOR WATER USE EFFICIENCY THROUGH STABLE ISOTOPE DISCRIMINATION''** is a record of research work done independently by Ms. Aisha Renju N.A under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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EXTERNAL EXAMINER

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Aisha Renju N.A

DEDICATED

TO

MY FAMILY

&

TEACHERS

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LIST OF ABBREVIATIONS

ABA	Abscicic acid
APX	Ascorbate peroxidase
ATP	Adenosine tri phosphate
CDB	Coconut Development Board
F.C	Field Capacity
GB	Gangabondam
KG	Keraganga
KS	Kerasree
KVK	Krishi Vigyan Kendra
LEA	Late Embryogenic Abundant
MYD	Malayan Yellow Dwarf
NADPH	Nicotinamide adenine dinucleotide phosphate
S.F	Selectively fertilized

Introduction

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) has been cultivated in Kerala since ages and has an important role in socio-economic and cultural activities of people. The palm is amenable to both plantation and homestead management and it can be either a major crop or a minor one in a homestead garden of mixed crops. With coverage of 8.2 lakh ha, coconut occupies 38 per cent of the net cropped area and provides livelihood to over 3.5 million families in Kerala. The importance of coconut, "the Tree of Heaven" or "Kalpavriksha" can be appreciated when we consider its innumerable uses to mankind. Among major plantation crops in India, coconut ranks first in area, production and productivity with a production share of 91.47 % (NHB, 2015). Hence coconut as a crop is raising tremendous opportunities and scope for value addition industries and thereby a way to uplift vulnerable and backward classes of Indian economy.

Lack of scientific approach in crop management technology adoption, uneconomic holding size, poor quality of planting materials used, debilitating root wilt disease in southern districts, climate change and dry spell in northern districts, high input cost and non availability of labours for agricultural works, low price of the coconut etc. are factors of low yield and production of coconut in the state. Various measures are being undertaken by central and state government to minimize the effect of these factors through KISSAN Kerala, KVKs, Research stations etc. But efforts and research works are still at the stage of infancy to sustain coconut production against biotic and abiotic stresses. Drought is the second most important factor reducing the productivity of coconut next to dreaded root (wilt) disease.

Coconut is frequently exposed to soil and atmospheric drought because it is a perennial palm with long productive life span (Rajagopal and Kasturi Bai, 2002; Gomes *et al.,* 2002). The development of drought tolerant coconut varieties is important and urgent area of research. Yield is the most integrative and commonly used

parameter to evaluate genotypes for their tolerance to drought or any other environmental stresses (Wachira *et al.,* 2002). However, yield-based evaluation is a complex and time consuming exercise with a perennial tree crop like coconut which has a long generation period of about 15 years for yields to stabilize, and the fruits have a long development period taking about one year from the opening of an inflorescence for the nuts to mature. Setting of button nuts, enlargement of nuts and development of nut water and kernel takes place sequentially at different times of this year-long maturation period (Ranasinghe *et al.,* 2003). The impact of a drought on yield, especially on the different nut components, will therefore vary with the development stage exposed to the drought. Hence, the development of an alternative methodology for rapid selection of drought tolerant palms is most desirable.

The effects of water deficit on the physiology, growth and productivity of coconut have been widely reported (Gomes and Prado, 2010) where some important traits such as stomatal control of water status in stressed and non-stressed plants, as well as the search and validation of morphological, physiological and biochemical indicators of drought tolerance, have been highlighted as potential criteria for breeding and selection of tolerant genotypes (Gomes and Prado, 2010). *In vitro* pollen selection followed by selective fertilization is another feasible and cost effective approach in this direction. This technique is characterized by artificially imposing the desired selective pressure during pollination and fertilization so that the pollen grains which are tolerant to selection pressure only will germinate and fertilize the ovule.

Drought tolerant varieties underlies the mechanism to combat water stress by maintaining tissue turgor via osmotic adjustment, which allows plants to maintain growth under water stress and reduced transpiration rate; proving themselves to be water use efficient under water limited situations. Water use efficiency is defined as the amount of biomass accumulated per unit amount of water transpired or yield of plant product per unit of crop water use. Considerable genetic variation in WUE exists among plant genotypes. Though gravimetrical method of measuring WUE facilitates

assessment of intrinsic genotypic variation for WUE (Kumar *et al*., 1998), nuclear and isotopic techniques is gaining popularity owed to its accuracy and reliability.

Isotopic composition of plant biomass is a function of the isotopic fractionation that occurs at different stages during photosynthetic carbon fixation process. The fractionation broadly occurs at the stomatal diffusive stage and at the site of carboxylation by Rubisco. The isotopic ratio of ^{13}C to ^{12} C in plant tissue is less than the isotopic ratio of ^{13}C to ^{12}C in the atmosphere, indicating that plants discriminate against ^{13}C during photosynthesis. Theoretical and empirical studies have demonstrated that carbon isotope discrimination is highly correlated with plant WUE. Analysis of carbon isotope discrimination has conceptual and practical advantages over measuring WUE by instantaneous measurement of gas exchange or whole plant harvests.

In the event of declining coconut production due to drought in Kerala, the present study was undertaken with the objective to evaluate the selectively fertilized coconut hybrids for water use efficiency, and to study the mechanism of water stress tolerance in coconut, and to estimate genetic variability in coconut for water use efficiency through stable isotope discrimination.

Review of Literature

2. REVIEW OF LITERATURE

Cocos nucifera (*2n=32*) is a perennial tropical species of the Arecaceae family, one of the most important in the Monocotyledonous class. Coconut palms require hot climates with an annual mean air temperature between 22-34ºC, absence of temperatures below 15ºC, solar radiation incidence of 300-900 Wm^{-2} and relative air humidity between 60-90%. Coconut trees are found in areas where rainfall varies from 1300 to 2500 mm, with 150 mm being considered the ideal monthly average for adequate growth and productivity of fruits (Rajagopal *et al*., 1990). Coconut palms can be grown on a wide range of soils, providing they are free draining (Murray, 1977; Purseglove, 1972).

The slender, leaning, ringed trunk of the coconut tree rises to a height of up to 25 m (80 feet) and is surmounted by a graceful crown of giant, featherlike leaves. The palm has an adventitious root system as typical of a monocot. The roots are localized generally at the lowermost region of the stem known as 'bole'. Coconut inflorescence is termed as spadix, which is 1.2m to 1.8m long, stout, erect, androgynous and simply branched; branches (spikes) bear one or more female flowers. Mature fruits, botanically known as 'drupe' isovoid or ellipsoid in shape, 300–450 mm (12–18 inches) in length, and 150–200 mm in diameter, have a thick, fibrous husk surrounding the familiar single-seeded nut of commerce. A hard shell encloses the insignificant embryo with its abundant endosperm, composed of both meat and liquid.

Coconut is frequently exposed to soil and atmospheric drought because it is a perennial palm with long productive life span (Rajagopal and Kasturi 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 Ramadasan, 1999). The basic physiological principle in drought studies of coconut is to look for the varieties which can conserve

water and maintain leaf turgidity during adverse conditions. This can be assessed by investigating physiological and biochemical aspects of coconut under drought.

Coconut genotypes respond differently to drought stress in relation to the rate of internal dehydration as well as to the rate of gas-exchange recovery upon resuming irrigation. Research on water relations in coconut have shown interesting results such as the response of palm to seasonal (Passos *et al*., 2003, 2005), or experimental (Gomes *et al*., 2007) water deficit or to osmotic stress (Silva Junior *et al*., 2002). These reports indicated aspects such as stomatal control of water status in stressed and non-stressed plants, as well as the search and validation of morphological, physiological and biochemical indicators of drought tolerance, as potential criteria for breeding and selection of tolerant genotypes.

2.1 GROWTH AND PHYSIOLOGICAL PARAMETERS

Plants are affected by environment during all phases of growth and development. The three most ecologically important environmental factors affecting plant growth are temperature, water (precipitation), and light. Water deficit is a major problem worldwide, limiting the growth and productivity of many crop species, especially in rain fed agricultural areas (Passioura, 2007). Evaluating various growth parameters like plant height, leaf area, SLA, biomass accumulation, RGR, NAR, etc. under any stress situation like water deficit will help to know the growth performance of the crop genotype under that particular stress. Crop performance may vary from crop to crop and between varieties. Hence in the context of screening for drought tolerance, measuring the growth parameters is crucial and mandatory.

2.1.1 Plant Height

Cell growth is one of the most drought-sensitive physiological processes due to the reduction in turgor pressure (Taiz and Zeiger, 2006). Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998). Impaired

mitosis, cell elongation and expansion result in reduced plant height, leaf area and crop growth under drought (Nonami, 1998; Kaya *et al*., 2006, Hussain *et al*., 2008).

Reduced plant height in two species of thyme in response to water deficit conditions was observed (Alavi-Samani *et al*., 2013). Water stress as a very important limiting factor for plant growth and development affects both elongation and expansion growth (Shao *et al*., 2008).

2.1.2. Leaf Area

Leaf area was greatly influenced under water stress. Leaf area increased with the plant age in all the water levels but it decreased with the severity of stress. Leaf size is determined by cell production and expansion, which are controlled in a coordinated manner during leaf organogenesis (Tsukaya, 2006). Leaf area, of coconut palm "cv. Brazilian Green Dwarf" was significantly retarded, by 26%, in plants exposed to drought stress (Gomes *et al*., 2010). El-Juhany and Aref (2005) reported a 77% reduction in total leaf area of *Conocarpus erectus* seedlings subjected to low water supply.

2.1.3 Specific Leaf Area (SLA)

Reduction in SLA under severe water stress in cowpea may be ascribed as an adaptive mechanism to reduce water loss from evaporative surfaces (Hayatu and Mukhtar, 2010). Reduced leaf area results in reduced transpiration surface (Namirembe *et al*., 2008) and may be a drought avoidance strategy for the plants. SLA is a marker for the regulation of plant leaf following abiotic stress factors including drought (Monclus *et al.*, 2006).

The leaves had higher tissue density under drought than under wetter conditions, which tends to decrease the SLA (Castro-Dıez *et al.,* 2000; Navas and Garnier, 2002). Similar SLA response to water stress had been reported for two legume species (Villagra and Cavagnaro 2006) and in *Eucalyptus globulus* (Coopman *et al*., 2008).

2.1.4 Biomass accumulation

Short-term responses of coconut to water stress such as low biomass accumulation and partitioning, reduced stomatal conductance to water vapor (g_s) and leaf water potential (w) which often impair photosynthesis (A) and transpiration (E) have been extensively documented (Gomes and Prado, 2010). Decreases in carbon assimilation in both tall and dwarf genotypes in response to atmospheric and soil water deficit have been reported (Prado *et al*., 2001; Gomes *et al*., 2008).

Inhibition of plant growth when subjected to water deficit conditions has been demonstrated in perennial grass (Xu *et al*., 2009), olive (Guerfel *et al*., 2009), *Sesuvium portulacastruni* (Slama *et al*, 2006) and Dianthus (Alvarez *et al*, 2009). Besides photosynthesis, dry matter production and its partitioning are largely influenced by the water availability (Rajagopal *et al.,* 1989).

2.1.5 Root and Shoot growth

Environmental stresses on the plant generally decreases both root growth and shoot growth. Amos and Walters (2006) summarized the effects of several soil environmental variables on corn root: shoot ratios. Kondo *et al* (2000) measured a decrease in root: shoot ratio as corn experienced greater water stress. Gregory (2006) concluded that dry soils induce plants to develop a more extensive root system and include deeper rooting, greater total weight, and greater total root length when compared with well-watered plants. Shoot growth was proportionately more affected than root growth, leading to an increased root: shoot ratio as stress increased (Benjamin *et al*., 2014). The shoot, leaf, root and whole plant dry weights decreased with increased water deficit in rice (Sikuku *et al*. 2010). The root: shoot ratio of unstressed plants decreases as the plant develops. Decline in root and shoot dry weight under water deficit may be attributed to root damage and death thereby reducing the sink activity of the roots leading to the built up of carbohydrates (Munns and Termaat, 1986).

2.1.6 Relative Growth Rate (RGR)

Water deficit caused a significant reduction in mean relative growth rate of the trees. Decreasing relative growth rate under water stress conditions has been reported by other authors (Ibrahim, 1995; El-Juhany and Aref, 1999). Galmés *et al.* (2005) concluded that the decrease in RGR caused by water deficit was mainly explained by decreases in SLA.

Drought significantly decreased plant height, RGR and leaf size, while increased root/total biomass ratio, and all these responses in plant structure and growth pattern are often considered as an important acclimation strategy to severe water deficit over long time scales (Li *et al* 2000).

RGR is sensitive to drought and may drastically reduce if plants are exposed to drought stress (Khalil *et al*, 2011). Drought might reduce gas exchange, the growth of expanding tissues by reducing cellular expansion and productivity (Sanchez-Blanco *et al*., 2002).

2.1.7 Net Assimilation Rate (NAR)

Photosynthesis in coconut palms was reduced significantly when exposed to water deficit stress (Gomes *et al*., 2008), in order to limit the CO_2 assimilation rate through the stomatal apertures (Cornic, 2000). Limited CO_2 assimilation through stomatal pores of water-deficit stressed plants leads to low photosynthesis, (Chaves, 1991; van Heerden *et al*., 2007; Dias and Bruggemann, 2010). A great reduction of growth rate was observed in dwarf coconut under severe drought stress (Gomes, 2006).

Short-term responses of coconut to water stress such as low stomatal conductance and water potential which often impair net photosynthetic rate and transpiration rate have been extensively documented (Passos *et al*., 1999; Rajagopal and Kasturi Bai, 2002). Carbon assimilation rate is impaired in both tall (Prado *et al*., 2001) and dwarf genotypes (Passos *et al*., 2003, 2005; Gomes *et al.,* 2007) in response to atmospheric and soil water deficit.

Water stress reduce plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al*., 2008; Farooq et al., 2009). Stomatal conductance decreases with water deficit, internal CO_2 concentrations in the leaf are predicted to be reduced, thus causing a slower rate of photosynthesis (Cramer *et al*., 2007).

2.1.8 Phyllochron

The phyllochron has been estimated as a constant value in several species (Dellai *et al*., 2005). Severe drought stress increased phyllochron compared to optimum irrigation condition in Sunflower (Gholinezhad *et al.*, 2012).Water stress tended to lengthen the phyllochron of perennial ryegrass (Volaire *et al.*, 1998). In wheat, the phyllochron interval is also affected by water stress and temperature (Baker, 1986).

2.1.9 Cumulative Water Transpired

Coconut has been considered as extravagant in water consumption. There is now strong evidence for efficient stomatal control of plant water status at least under mild to moderate water deficit (Prado *et al*., 2001; Passos *et al*., 2003; 2005; Sousa, 2006; Gomes *et al*., 2007). According to Nogueira *et al*. (1998) the water requirement of coconut depends on factors such as age, height, leaf area, environmental conditions and soil type.

Compared to the tall varieties, some evidence suggests that dwarf varieties use water more prodigally due to its elevated transpiration rate (IRHO-CIRAD,1992), greater number of stomata per unit leaf surface (stomatal frequency) and lower wax content on the leaf surface (Rajagopal *et al.*, 1990), as well as a poorer stomatal control of water loss (Passos and Silva, 1990).

2.1.10 Relative Water Content (RWC)

Maintenance of high leaf water status is the basis of drought tolerance mechanisms in coconut (Rajagopal and Kasturi Bai, 2002). The foliar photosynthetic

rate in higher plants is known to decrease as the relative water content and leaf water potential decreases (Lawlor and Cornic, 2002). The decrease in the water potential 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). When water stress is reduced from -1.0 to -2.0 MPa, cells become smaller and leaves develop less, resulting in a reduced area for photosynthesis and at these water potentials, ion transport is slowed and may also lead to a decrease in yield (Medrano *et al*., 2002).

The relative water content (RWC) was studied in drought tolerant and drought sensitive genotypes of cotton during induction of water stress and posterior recovery (Parida *et al*., 2008). It is believed that leaf water potential and RWC are reliable parameters for quantifying the plant drought stress response (Siddique *et al*., 2000; Bayoumi *et al.,* 2008).

As observed by Jeykumar *et al.* (2007), the photosynthetic rate was strongly influenced by RWC of the leaf in papaya cultivars. Drought resistance varieties showed consistently higher leaf water potential in their tissues than susceptible types under soil moisture deficit (Natarajan and Kamaravelu, 1993). Plants under severe drought conditions had a small but significant decrease in RWC of leaves and a higher decrease in gas exchange parameters in legumes (Ludlow and Muchow, 1990). Retention of more water in leaf is known to be important to drought tolerance (Hafid *et al*, 1998).

2.1.11 Membrane Integrity

Cell membrane stability as measured by the electrolyte leakage technique has been used as a tolerance index for drought (Sayar *et al*., 2008; Yang *et al*., 2009). The ion leakage is an indicator of cell membrane stability and integrity, which is commonly considered as one of the best physiological components of drought tolerance in plants (Kocheva *et al.*, 2004; Xu *et al*., 2008). 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). When a cell suffers dehydration, the rigidity of the cell wall can provide mechanical protection;

however, this organelle is permeable, thus allowing desiccation if a stronger stress is applied (Verslues *et al*., 2006).

Konwar (2009) found a reduction in MSI in upland rice under moisture stress condition. The reduction in MSI is related to production of ROS which causes damage to membrane lipid and protein. Sairam *et al*. (2008) stated that the genotypes that showed higher MSI under water stress possessed higher activities of glutathione reductase and peroxidase enzyme.

A consequence of the altered membrane integrity is the increase of the cell permeability which is accompanied by electrolyte leakage from the cell (Blum and Ebercon, 1981). The physical state of cell membranes is known to be very sensitive monitor of the most diverse environmental changes. This feature was suggested to render cell membranes an ideal location for the primary temperature stress sensor. Membrane stability has also been associated with water and high temperature in various crops plants (Sairam *et al.*, 1997). Tolerance to drought evaluated as increase in cell membrane stability under water deficit conditions was well differentiated between cultivars (Premachandra *et al.*, 1993).

2.1.12 Epicuticular Wax (ECW)

The plant cuticle and waxes have many important functions. They reduce the loss of water, reflect or attenuate radiation, form the basis of phyllosphere, protect plant tissues against penetration by fungi, bacteria and insects, as well as from mechanical damage (by wind, rain, soil particles etc.), reduce water retention on the plant surface, and provide a self-cleaning surface.

The plant cuticle plays an important role in protecting plants against water loss. The outermost layer is epicuticular wax, which may be amorphous in form or possess a semi crystalline or crystalline structure (Jenks and Ashworth, 1999). The cuticle plays a fundamental protective role against water loss, particularly when stomata are closed (Jenks and Ashworth, 1999). Shivashankar *et al.* (1993) reported that the level of ECW was higher in stress condition. When drought progressed, stomata get closed and water

loss occurred through the leaf cuticle without CO_2 fixation. Higher deposition of ECW decreased cuticular permeability of water loss and increases the crop albedo (Blum and Ebercon, 1981). The cuticle, made up of major component called cutin forming a layer of hydrophobic material on plant aerial organs, provides an effective barrier against desiccation and also protects the plants from abiotic and biotic stresses (Beattie and Marcell, 2002). Particularly the ECW influenced the wettability of the leaf and thus affected the plant water use efficiency, the opportunity for gas exchange and the leaching of the solutes (Jenks and Ashworth, 1999). Leaf ECW played a critical role in plant environmental stress resistance and their modification had greater potential in crop improvement (Jenks *et al.*, 1996). Waxes are involved in the plant's defense against abiotic stress. The strongly hydrophobic waxes play an important role in water retention by limiting non stomatal water loss (Kerstiens, 1996; Riederer and Schreiber 2001; Jenks, 2002).

Protective role of epicuticular wax has also been reported in coconut (*Cocos nucifera*) by Kurup *et al.*(1993) who observed a clear indication of a negative relationship between the epicuticular wax content of coconut leaves and transpiration rate. It was reported for two single coconut genotypes that lupeol methyl ether, isoskimmiwallin, and the skimmiwallin are the major components of coconut epicuticular wax (Escalante *et al.,* 2002).

2.1.13 Stomatal Characteristics

2.1.13.1 Stomatal Frequency

Plant stomata, the vital gate between plant and atmosphere may play a central role in plant/vegetation responses to environmental conditions, which have been and are being investigated from molecular and whole plant perspectives, as well as at ecosystem and global levels (Nilson and Assmann, 2007). Response of stomatal density to drought was reported by Galmes *et al*. (2007). Many studies have shown that water deficit leads to an increase in stomatal density (Zhang *et al.*, 2006), and a

decrease in stomatal size (Spence *et al.*, 1986), indicating this may enhance the adaptation of plant to drought (Martinez *et al.*, 2007).

Leaf morphological traits, including stomatal density and distribution, and epidermal features may affect gas exchange quite remarkably and their relationships with key environmental factors such as light, water status, and CO_2 levels (Nilson and Assmann, 2007). Stomatal density was also negatively correlated with stomatal length under different water conditions in some Jujube leaves (Liu *et al.*, 2006) and *Platanus acerifolia* leaves (Zhang *et al.*, 2004).

Nevertheless, different effects of abiotic factors on stomatal size may depend on plant species/varieties (Maherali *et al.*, 2002; Liu *et al.*, 2006). Stomatal densities of leaves from several varieties of Jujube also have similar patterns under a drought severity gradient: initially increasing, then declining (Liu *et al.*, 2006). However, wheat stomatal density always increases with continually increasing drought severity (Zhang *et al.*, 2006).

2.1.13.2 Stomatal index

Several reports have shown that the stomatal density and its index increase with water stress (Yang and Wang, 2001; Zhang *et al.*, 2006). Leaf stomatal density and the stomatal index (the percentage of stomatal number to total cell number on a given leaf area) may be affected by cell expansion, depending on leaf development, ageing, and position (Ceulemans *et al.*, 1995; Lecoeur *et al.*, 1995).

2.1.13.3 Stomatal conductance (gs)

Stomata, highly specialized cells involved in gas exchange, can account for a high water loss through leaf transpiration; the adaptation to drought consists in stomata closure during stress (Blum, 1996). This adaptation implies the accumulation of gases such as CO_2, which diminish photosynthesis (Bohnert and Sheveleva, 1998). Both stomatal and non-stomatal factors have been demonstrated to contribute to the reduction in assimilation during a period of water deficit and during the recovery phase after resuming irrigation (Gomes *et al*., 2008). Stomatal closure is often considered as

an early physiological response to water deficit, which results in decreased photosynthesis, through limited CO_2 availability in the mesophyll (Cornic, 2000). Light, temperature and vapor pressure deficit (VPD) have influence on water relations of coconut, with stomatal regulation playing a pivotal role in the control of water balance (Gomes *et al*., 2002).

Stomata sensitivity to VPD in dwarf coconut was demonstrated by (Gomes *et al*., 2002). The stomatal and non-stomatal limitation was generally accepted to be the main determinant of photosynthesis under drought stress (Farooq *et al*., 2009). Passos and Silva (1990) suggested that coconut stomata would not exert an effective control of water loss during the hottest and driest hours of the day. However, seasonal and diurnal changes in leaf gas exchange have demonstrated a different response of stomatal conductance despite a similar response of Ψ in both tall and dwarf varieties (Prado *et al*., 2001; Passos *et al*., 2003).

Stomatal closure has a greater effect on photosynthesis than on transpiration because of the additional resistance associated with diffusion of CO_2 relative to H_2O in the leaf (Cowan 1982, Nobel 1991). All the palms irrespective of the genotype were equally sensitive to soil water depletion and responded to water deficit by decreasing the g_s (Rajagopal *et al,* 1990). As shown by Jayasekara *et al*. (1993) and Ranasinghe *et al.* (2003) stomatal regulation was the key factor controlling the water balance of coconut.

2.1.14 Transpiration Rate.

Under severe water stress condition, water loss was minimized by a steep decline in transpiration (Srivastava *et al*., 1996). Stomatal closure has a greater effect on photosynthesis than on transpiration because of the additional resistance associated with diffusion of CO_2 relative to H_2O in the leaf (Cowan, 1982 and Nobel, 1991). An early response to water deficit is a reduction in leaf area and plant growth, which allows plants to reduce their transpiration, thus increasing water use efficiencies (WUE) (Xu and Zhou, 2005; Monclus *et al.*, 2006; Aguirrezabal *et al.*, 2006). The instantaneous

control of the transpiration stream by the stomata is an important defense mechanism used by many species in arid environments in order to avoid excessive water loss by transpiration (Gucci *et al*., 1996; Nogueira *et al*., 1998; Silva *et al*., 2004) and eventual death by desiccation (Silva *et al*., 2000).

2.1.15 Chlorophyll Stability Index (CSI)

The chlorophyll stability index is an indicative of the maintenance of photosynthetic pigments under drought situation. (Ananthi *et al.*, 2013). The CSI is a single parameter used to measure frost (or) drought resistance of a plant. Sairam *et al.* (1996) reported that both drought stress and temperature stress decreased membrane stability, chlorophyll content and chlorophyll stability index in all wheat genotypes. The high chlorophyll stability indices help the plants to withstand stress through better availability of chlorophyll. This leads to increased photosynthetic rate and more dry matter production (Madhan Mohan *et al.,* 2000). Mohan *et al.* (2000) reported that high CSI value means that the stress did not have much effect on chlorophyll content of plants. CSI has been used as an indicator of stress tolerance in rice as reported by Gomez and Rangasamy (2002) and (Yogameenakshi, 2002). Drought tolerant varieties had higher Chlorophyll Stability (Natarajan and Kumaravelu, 1993). Meenakumari *et al.* (2004) reported that chlorophyll content as well as Chlorophyll stability index decreased during drought environments. Konwar (2009) stated that the Chlorophyll stability index decreased with increasing water stress in rice.

Chlorophyll stability is a function of temperature and it is found to correlate with drought tolerance. Chlorophyll stability index is a measure of integrity of membrane or heat stability of the pigments under stress conditions (Kaloyereas, 1958).

2.1.16 Water Use Efficiency (WUE)

Water use efficiency is usually defined as the crop yield per unit of water consumed as crop evapotranspiration (Doorenbos and Pruitt, 1977) or the ratio of grain weight or biomass yield to crop evapotranspiration (Zhang *et al*.,2004), expressed in units of kilograms per cubic meter of water. Water use efficiency is an important tool

to improve the sustainable agriculture in irrigated areas (Azevedo *et al*., 2003). WUE has been shown to vary among varieties and also among ecotypes of the same variety (Prado *et al.,* 2001; Gomes *et al*., 2002). Hatfield *et al*. (2001) observed that WUE is influenced by crop morphology, soil conditions, agricultural practices and atmospheric variables.

Transpiration efficiency (TE) which is WUE at the leaf level is determined by the delicate interplay between transient photo-system activity, sub stomatal cavity CO_2 concentration and stomatal activity (Farquhar *et al*., 1989). Drought resistance was found to be associated with low WUE when analyzed by isotope discrimination under limited water supply (Araus *et al*., 2003; Solomon and Labuschagne, 2004). A drought resistant *Coffea canephora* clone had relatively lower WUE than a drought susceptible one, where resistance was associated with deeper roots and presumably greater water use (Pinheiro *et al*., 2005). Crop WUE has long been known to increase with increasing drought stress and reduced water supply (Myers *et al*., 1984). This has been more recently confirmed with isotope discrimination analysis (Li *et al.,* 2000; Peuke *et al*., 2006).

Coconut has been considered as extravagant in water consumption. Coconut palm exhibits efficient stomatal control of plant water status at least under mild to moderate water deficit (Prado *et al.,* 2001; Passos *et al*., 2005; Sousa, 2006; Gomes *et al*., 2007). According to Nogueira *et al.* (1998) the water requirement of coconut depends on factors such age, height, leaf area, environmental conditions and soil type. Compared to the tall varieties, dwarf varieties use water more prodigally due to its elevated transpiration rate (IRHO-CIRAD, 1992).

2.1.17 Stable Isotope Discrimination

Farquhar and Richards (1984) defined the term carbon isotope discrimination (ΔC) as the molar ratio of $^{13}C/^{12}C$ (Ra) in atmospheric CO_2 the carbon source for plants divided by the same ratio in the plant (product (Rp***)*.** Plants of C_3 species have C-isotope ratios that contain a contribution from discrimination at stomata and principally from

Rubisco. The closer the ratio to unity, the greater the extent of discrimination during carboxylation (Condon *et al*., 2004). Measuring Δ^{13} C in dry matter of C_3 species provides an assimilation-weighted average value of C_i / C_a, over the life of the plant material being analyzed (Condon *et al*., 2006). The association of transpiration efficiency and ^{13}C comes about because of the independent associations of both transpiration efficiency and Δ^{13} C with the ratio Ci / Ca (Condon *et al*., 2006). Stable carbon isotope ratios have been determined by isotope ratio mass spectrometry (IRMS), (Vaughn *et al.,* 2004).

In contrast to gas exchange techniques that provide measurements of photosynthesis rates at a single point in time, leaf carbon isotopic composition integrates the ratio of intercellular (pi) to air CO_2 concentration (pa) for longer periods. The basis of the biochemical discrimination against ^{13}C in C_3 plants lies with the primary carboxylating enzyme, ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco) which discriminates against 13C because of the intrinsically lower reactivity of 13C compared with ^{12}C (Farquhar *et al*., 1982; Brugnoli and Farquhar, 2000). Thus the isotopic composition reflects the effect of the plant water status on photosynthesis throughout the growing season (Farquhar and Richards, 1984). Plants discriminate against the heavier ^{13}C when they fix carbon through photosynthesis (O'Leary, 1988), and so the ratio of ^{13}C to ^{12}C in plant tissues is lower than that in the atmosphere. Because the extent of discrimination is directly related to the intercellular CO_2 concentration (Ci) (Farquhar and Richards 1984, Farquhar *et al*. 1989), the relationship between $\delta^{13}C$ and WUE is connected by Ci.

Abiotic stresses such as soil-water deficit (Farquhar and Richards, 1984; Ehdaie *et al.,* 1991; Condon *et al*., 1992: Merah *et al*., 1999), result in lower values of $\Delta^{13}C$ because they result in some degree of stomatal closure, causing Ci / Ca to be lower. In C_3plants, stable carbon isotope discrimination (Δ) has been used to assess genotypic variation in WUE and physiological responses to environmental factors (Hubick *et al*.1986, Martin and Thorstenson 1988, Johnson *et al.,* 1990). The natural $^{13}C/^{12}C$ ratio

of C_3 plant tissue is related quantitatively to the ratio of intercellular CO_2 to atmospheric CO_2 (Ci/Ca) contents, a parameter which reflects a balance between assimilation of CO2by photo-synthetic activity and the supply of CO2through stomatal diffusion (Farquhar *et al*., 1989).

Low delta is reasonably correlated with high TE (Hall *et al*., 1994). Delta was therefore taken to represent crop WUE (Condon *et al*., 2002). Crop WUE as estimated under rain fed conditions by carbon isotope discrimination analysis or any other method is an elusive ratio (Reynolds and Tuberosa, 2008). Genotype x Environment (G x E) interactions for $\Delta^{13}C$ are potentially large because environment influences can have a very large effect on $\Delta^{13}C$ values measured in plant dry matter, especially under conditions of declining soil water and rising evaporative demand and the $\Delta^{13}C$ value of all genotypes may not respond in the same manner or at the same time to these environmental perturbations (Condon *et al.,* 2006). Genetic studies in wheat have shown a preponderance of additive gene effects for Δ^{13} C (Rebetzke *et al*., 2006) and transpiration efficiency (Solomon and Labuschagne, 2004).

Carbon isotope discrimination has been used as a selection criterion in wheat (*Triticum aestivum*) breeding, and as a result water-use efficient wheat varieties have been released commercially (Condon *et al*., 2002; Rebetzke *et al*., 2002) for dryland agriculture. Negative correlation between WUE and carbon isotope discrimination was reported in sunflower (Lauteri *et al*., 1993), *Triticum turgidum* L. (Condon *et al*., 1990), *Oryza sativa* L. (Dingkuhn *et al.,* 1991) and *Eucalyptus microtheca* (Li C, 1999).

Atmospheric CO2 undergoes oxygen isotopic exchange with leaf water and soil water, and changes in the $\delta^{18}O$ of CO_2 can be used to study spatial and temporal variation in the net exchange of CO2 in terrestrial ecosystems, and its underlying causes, dominated by photosynthesis and respiration (Farquhar and Lloyd, 1993; Cuntz *et al*., 2003). O_2 is released by the plant during photosynthesis. The isotopic composition of evolved oxygen is identical to that of the substrate water in the chloroplast thylakoids (Cuy *et al.*, 1993). Oxygen isotope composition ($\delta^{18}O$) of leaf

material could be used to pick up differences in leaf to air water vapour composition (Farquhar *et al.*, 1994).

Measurement of $\Delta^{13}C$ and $\Delta^{18}O$ in organic matter could be combined to separate photosynthetic capacity effects on TE from g_w (Conductance to diffusion of water) effects (Farquhar *et al*., 1998). Changes in the oxygen isotope ratios of atmospheric O2 are revealed on long time scales in the Dole effect (Dole *et al*., 1954), where atmospheric oxygen is currently enriched in 18O by 23.5‰ compared with mean ocean water. Measurements of the isotopic composition of water vapor have been used to estimate transpiration fluxes in rice (*Oryza sativa*) crops (Brunel *et al*., 1992). The isotopic composition of water vapor plays a direct role as it is a key component in the calculation of leaf water isotopic enrichment (Lee *et al*., 2006).

2.2 BIOCHEMICAL PARAMETERS

2.2.1 Pigment Composition

Chlorophyll content of the plant influences photosynthetic rate. Water stress caused a fall in leaf chlorophyll. Increasing intensity of water stress decreased leaf chlorophyll content and tended to increase the chlorophyll a /b ratio (Zhu and Huang, 1994). Makhmudov (1983) reported that moisture stress inhibited biosynthesis of the precursor of chlorophyll in wheat leaves which ultimately reduced the chlorophyll content. Thimman (1980) stated that water stress induced leaf senescence leading to decreased photosynthetic activity with the accompanying degradation of proteins and chlorophyll. Asharaf and Mahmood (1990) stated that total chlorophyll content of the leaf declined under water stress conditions. It may be due to decreased synthesis and increased degradation of chlorophyll in leaves under water stress (Dekov *et al.,* 2000). The chlorophyll content of leaf tissue varies with cultivars, age of the crop, growth stages, light and temperature (Kumar and Singh, 1996). A photosynthetic potential is directly proportional to the quantity of chlorophyll present in the leaf tissue (Carter, 1991). Ramesh babu *et al. (*1982) stated that there is an increase in total chlorophyll content under moisture stress condition.

Drought can also lead to pigment degradation (Hendry *et al*., 1987), thus causing irreversible water-deficit damage to the photosynthetic apparatus (Clarke *et al*., 1996). Photosynthetic pigment degradation in water deficit stressed palms is a general physiological response in plants subjected to water withholding or reduced soil water content

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). 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 declines in electron transfer, ATP and NADPH production and eventually CO_2 fixation process (Zhang *et al*., 2006).

2.2.2 Total Soluble Protein

Drought conditions bring about quantitative and qualitative changes in plant proteins. In general, proteins in the plant leaves decreases during water deficiency due to the suppressed synthesis, more pronouncedly in C_3 than in C_4 plants. Water stress alters gene expression and consequently, the synthesis of new proteins and mRNAs. The main proteins those synthesized in response to water stress are LEA, desiccation stress protein, proteins those respond to ABA, dehydrins, cold regulation proteins, proteases, enzymes required for the biosynthesis of various osmoprotectants, the detoxification enzymes. Heat-shock proteins (HSPs) and late embryogenesis abundant (LEA)-type proteins are two major types of stress-induced proteins during different stresses including water stress (Taiz and Zeiger, 2010). Heat Shock Proteins (HSP), highly accumulated during stress and also known as molecular chaperones, are widely

distributed in nature; they are involved in protein folding and assembly, as well as in the removal and disposal of non-functional proteins (Wang *et al*., 2004). LEA proteins are expressed at basal levels and can be induced to high levels during osmotic and drought stress (Ingram and Bartels, 1996; Barrera-Figueroa *et al*., 2007).

A decrease in the protein concentration would be a typical symptom of oxidative stress and has frequently been observed in drought stressed plants (Seel *et al.,* 1992; Moran *et al.,* 1994). Protein degradation might be the result of increased activity of protease or other catabolic enzymes, which were activated under drought stress, or due to fragmentation of proteins due to toxic effects of reactive oxygen species resulting in reduced protein content (Davies 1987). Reduction in quantity of soluble protein can be related to the reduced rate of protein biosynthesis and increased breakdown of protein under limited environment as observed by Gong *et al.* (2005) in wheat, Rodriguez *et al*. (2002) in sunflower and Mafakheri *et al*. (2011) in chickpea under drought stress condition.

2.2.3 Osmoprotectants

Osmotic adjustment is a cellular adaptive mechanism vital for stress-tolerant plants, allowing for plants to continue growing in the case of drought (Silva *et al*., 2010). It is usually defined as a decrease in the cell sap osmotic potential, resulting from a net increase in intracellular solutes rather than from a loss of cell water (Kusaka *et al*., 2005). As a consequence, the cell's osmotic potential is diminished; this, in turn, attracts water into the cell by tending to maintain turgor pressure (Pérez-Pérez *et al*., 2009). According to Martinez *et al*. (2005), compatible solubles, such as sugars, glycerol, proline or glycinebetaine, can also contribute to this process.

Soluble sugars and proline are two kinds of the most important compatible solutes in plants (Chaves *et al.,* 2003; Ben Ahmed *et al.*, 2009; Hessini *et al*., 2009). Besides their roles in osmotic adjustment, they may protect membranes from damages and stabilize the structures and activities of proteins and enzymes (Iyer and Caplan,

1998; Samuel *et al*., 2000; Villadsen *et al*., 2005; Lee *et al*., 2008; Ben Ahmed *et al.,* 2009; Hessini *et al.*, 2009). Associations between osmotic adjustment and cellular membrane stability under drought stress were suggested more recently (Chandra Babu *et al.* 2004).

Despite osmotic adjustment has been reported as an important component of drought tolerance mechanisms in various coconut genotypes (Kasturi Bai and Rajagopal, 2000), the importance of such strategy for coconut drought tolerance is still unclear (Gomes and Prado, 2010).

2.2.3.1 Free Amino Acids

Amino acids have traditionally been considered as precursors and constituents of proteins. Amino acids can play wide roles in plants including acting as regulatory and signalling molecules. As observed by Rai and Sharma (1991), amino acids promoting stomatal opening also promoted K influx into the guard cells while amino acids inhibiting stomatal opening inhibited K influx into the guard cells.

Accumulation of free amino acids is also significant under water stress and may be due to induced hydrolysis of proteins as reported in crops like *Arachis hypogeae* and *Vicia faba* (Purushotham *et al.,* 1998; EI Tayab, 2000). The increase in free amino acids could contribute to the tolerance of the plant to water deficit through an increase in osmotic potential or as a reserve of nitrogen principally for the synthesis of specific enzymes (Navari-Izzo *et al.,* 1990). The increase in aspartate, glutamate, proline, alanine and valine compound due to increase in free amino acids content in stressed leaves could help maintain energy fluxes of the chloroplast (Ashraf, 2004).

Kraus *et al.,* (1995) reported that increased accumulation of FAA during stress conditions could be an indicator of the adaptive nature of the coconut palms to cope up with the adverse conditions during summer months. The accumulation of free amino acids in response to drought stress is well documented (Kasturi Bai and Rajagopal, 2000; Asha and Rao, 2002; Tan *et al*., 2006; Sircelj *et al*., 2007; Maricle *et al*., 2008).

Similar results were observed in Marsh grasses (Maricle *et al.*, 2008) and in Sorghum (Yadav *et al*., 2005) under drought stress condition.

Amino acid accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species *Radix astragali* (Tan *et al*., 2006). These accumulations may occur in response to the change in osmotic adjustment in cellular contents (Greenway and Munns, 1980; Shao *et al*., 2007).

2.2.3.2 Sugar

A complex essential role of soluble sugars in plant metabolism is well known as products of hydrolytic processes, substrates in biosynthesis processes, energy production but also in a sugar sensing and signaling systems. Recently it has been claimed that, under drought stress condition, even sugar flux may be a signal for metabolic regulation (Kishor *et al*., 2005). Sugars and amino acids are the major solutes responsible for osmotic adjustment (De Costa, 2001). Trehalose, a disaccharide, is accumulated under drought stress and functions during embryo and flower development, as well as in the regulation of carbon metabolism and photosynthesis (Phillips *et al*., 2002; Iturriaga *et al.,* 2009).

In a study with cowpea (*Vigna unguiculata*) subjected to water stress and recovery treatment, Souza *et al*. (2004) showed that carbohydrate metabolic changes revealed an accumulation of soluble sugars in water-stressed leaves, which also persisted for one day after re-watering. The accumulation of sugars in response to drought stress is also quite well documented (Watanabe *et al.*, 2000; Izanloo *et al*., 2008). Soluble sugars also contributed to improving drought tolerance of peas (Sánchez *et al*., 1998), sugar beets (Choluj *et al*., 2008) and black poplars (Regier *et al*., 2009). In two mango cultivars, a cultivar, which exhibited more active accumulations of soluble sugars and proline, also revealed higher resistance to drought than the other one (Elsheery and Cao, 2008).

2.2.3.3 Starch

Starch may play an important role in accumulation of soluble sugars in cells. The carbohydrate status of the leaf, which is altered in quantity and quality by water deficits, may act as a metabolic signal in the response to stress (Chaves *et al.*, 2003). Starch synthesis is, in general, strongly depressed, even under moderate water deficits (Chaves, 1991). Starch depletion in grapevine leaves was noted by Patakas and Noitsakis (2001) in response to drought stress.

2.2.4 Phenol

Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks. (Yildrim *et al*., 2001; Mathew and Abraham, 2006). Phenolics can neutralize light falling on the leaves through its transformation into blue fluorescence, which is no longer damaging, and can even be used for photosynthetic quantum conversion (Bilger *et al.,* 2001). The phenolic compounds are very reactive structures due to the presence of the benzene ring (Bilger *et al*., 2001). It is known that drought stress predisposes plants to injury of the photosynthetic apparatus through its interaction with UV or/and visible radiation (Garcia-Plazaola and Becerril, 2000).

Water deficit led to an increase in the intensity of the blue fluorescence originating from plant phenolics, mainly from ferulic acid (Cerovic *et al*., 2002; Meyer *et al*., 2003; Morales *et al*., 2005; Hura *et al*., 2006). Drought stress significantly limits plant growth, crop productivity and changes its behaviour regarding the biosynthesis of bioactive compounds such as phenolic compounds (Al-cázar *et al*., 2006). Water deficit has been suggested to cause both increases and decreases in the concentration of various phenolic components (Horner, 1990). Drought stress may increase the formation of reactive oxygen species (ROS) which can damage plants by oxidizing photosynthetic pigments, membrane lipids, proteins, and nucleic acids (Reddy *et al*., 2004).

2.3 STRESS TOLERANCE PARAMETERS

2.3.1 Proline

Proline is a compatible solute that can have a major role in osmotic adjustments and may also have a number of protective roles (Verslues *et al.*, 2005). Proline is a non-protein amino acid that forms in most tissues subjected to water stress and together with sugar, it is readily metabolized upon recovery from drought (Singh *et al*., 2000). In addition to acting as an osmoprotectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger (Sharma and Dietz, 2006), as a solute that protects macro-molecules against denaturation and as a means of reducing acidity in the cell (Kishor *et al*., 2005). Proline accumulation in leaves of drought-stressed plants and its role as an osmolyte or osmoprotectant has been the theme of a long-standing debate (Seki *et al*., 2007; Szabados and Savoure, 2009). Proline might regulate the osmotic balance of the cell thus relieving the negative effect of stress (Reddy *et al.,* 2004).

Proline accumulation is considered the first response of plants exposed to salt stress and water-deficit stress to reduce leaf osmotic potential; the kinetics of accumulation of this solute depend on the intensity and duration of the stress (Ashraf & Foolad, 2007). Plants, producing a higher amount of proline under water stress conditions may be considered as varieties with relatively greater drought tolerance (Jharna *et al.*, 2003).

In *Cocos nucifera*, Jayasekara *et al.* (1993) found high levels of proline in leaves of tolerant genotypes during the dry season. However, the high contents of proline detected in coconut leaflets submitted to dry air (mainly in dwarf varieties) were unlikely to be directly associated with drought tolerance (Kasturi Bai and Rajagopal, 2004). Regarding osmoregulation in the coconut palm, proline's contribution to the overall osmotic adjustment in ecotypes of Brazilian Green Dwarf was recently shown to be low (Gomes *et al*., 2006; Gomes & Prado, 2007) In tolerant coconut genotypes, the enzyme systems protecting the membrane lipids (Shivashankar

et al., 1991), as well as the protective action of proline and high membrane stability as indicated by electrolyte leakage measurements (Gomes, 2006) explain, at least partially, the low sensitivity of the chloroplast membranes to drought stress. Proline enrichment in oil palm has been demonstrated as one of the most evident biochemical indices under water deficit stress (Chaum *et al*., 2010; Cao *et al.*, 2011; Chaum *et al*., 2011). The high contents of proline detected in coconut leaflets submitted to dry air (mainly in dwarf varieties) were unlikely to be directly associated with drought tolerance (Kasturi Bai and Rajagopal, 2004).

Sundaresan and Sudhakaran (2006) also observed a higher accumulation of proline in drought tolerant Cassava as compared to drought susceptible Cassava. Vendruscolo *et al*. (2007) stated that proline might confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than as an osmotic adjustment In olive trees, proline levels in both young and old leaves of plants exposed to water deficit stress were higher than in plants grown under well-irrigated conditions (Ahmed et al,, 2009). Proline content in mature leaves of drought-stressed sugar beet was 1.5 times higher than in well-irrigated plants (Choluj *et al.,* 2008).

2.3.2 Antioxidants

Drought stress usually leads to oxidative stress due to stomatal closure (Lei *et al.,* 2006; Ozkur *et al*., 2009), which causes the over-reduction of photosynthetic electron chain (Bacelar *et al*., 2007; Ben Ahmed *et al*., 2009) and high formation of reactive oxygen species (ROS) in chloroplasts and mitochondria (Fu and Huang, 2001). ROS including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-) and singlet oxygen ($1\ O_2$) could disrupt normal metabolisms of plants through oxidative damages to lipids, proteins, nucleic acids, and photosynthetic pigments and enzymes (Fu and Huang, 2001; Ozkur *et al.,* 2009). Under various abiotic stresses the extent of ROS production exceeds the antioxidant defense capability of the cell, resulting in cellular damages (Almeselmani *et al*., 2006).

Since ROS are mainly produced in chloroplasts, the photosynthetic activity is compromised during stress; drought tolerance is unequivocally related to efficient antioxidant cellular processes (Kranner *et al.,* 2002; Montero-Tavera *et al,* 2008). Factors participating in antioxidative activity are non-enzymatic and enzymatic (Bartels and Sunkar, 2005); among the former are vitamins C and E, glutathione, flavonoids, alkaloids, carotenoids, polyamines, etc. Enzymatic activities include catalase, super-oxide dismutase, peroxidase and metallothionein (Seki *et al.,* 2007).

The balance between ROS production and activities of antioxidative enzymes determines whether oxidative signalling and/or damage will occur (Moller *et al*. 2007). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Sudhakar *et al*., 2001). Various reports underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to environmental stresses in several plant species, such as rice (Guo *et al.* 2006), foxtail millet (Sreenivasulu *et al*. 2000), tomato (Mittova *et al*. 2000), sugar beet (Bor *et al.* 2003), wheat (Khanna-Chopra & Selote 2007) and barley (Acar *et al.* 2001).

2.3.2.1 Non-Enzymatic antioxidants

2.3.2.1. a Carotenoids

Carotenoids are a large class of isoprenoid molecules that are synthesized de novo by all photosynthetic and many non -photosynthetic organisms (Jaleel *et al.*, 2009). In chloroplast, the carotenoids function act as accessory pigments in light harvesting, but perhaps a more important role is their ability to detoxify various forms of activated oxygen and produced as a result of excitation of the photosynthetic complexes by light (Surender *et al.*, 2013). The carotenoids act as competitive inhibitor for the formation of singlet oxygen and this is aided considerably by their proximity to chlorophyll in the light harvesting complex. This method of protection is especially critical as light intensity increases above saturating level (Demming-Adams and Adams, 1993).

Chen and Creeb (1991) found increased level of carotenoid content under drought conditions Total chlorophyll and total carotenoids concentrations in water deficit stressed olive plants decreased sharply, showing >50% reduction when exposed to extreme water deficit stress for 60 days by withholding watering, especially in the "Chemleli" cultivar, leading to reduced Fv/Fm (Chlorophyll flouroscence) and Pn (Photosynthesis) (Boughalleb and Hajlaoui, 2011). Different effects on C, have been reported for coconut (Gomes *et al.*, 2008) and olive (Ahmed *et al*., 2009; Guerfel *et al.,* 2009). The Carotenoid levels in water deficit stressed olive plants decreased significantly (Ahmed *et al;* 2009), while they were maintained in coconut (Gomes *et* al, 2008). The Carotenoid is believed to play an important role in protection against photo-oxidative damage, as represented by low NPQ (Muller *et al.,* 2001; Omasa and Takayama, 2003).

2.3.2.1. b Ascorbic acid

Ascorbic acid is essential in cell physiology as a quencher of ROS and donor of electrons for APX mediated H_2O_2 detoxification. (Navabpour *et al*., 2003). An increase in ascorbic acid content in leaf and stem tissues of cowpea under drought stress has suggested it to be a necessary response for efficient destruction of O_2 under stress conditions (Manivannan *et al*., 2007).

2.3.2.2 Enzymatic antioxidants

2.3.2.2. a Peroxidase (POX)

Peroxidase (POX), an iron heme protein, accelerates the reduction of H_2O_2 with a concurrent oxidation of a substrate, mostly located in cell wall; it is also involved in oxidation of phenol compounds as the key enzyme for polymerization towards the synthesis of lignin (Gaspar *et al*., 1991; Ozdemir *et al*., 2004). POX is a major enzyme scavenging H_2O_2 in chloroplasts produced through dismutation of O_2^-catalyzed by superoxide dismutase. Several physiological processes are dramatically affected by peroxidase over-production, and severe wilting was found in transgenic plants (Arora *et al*., 2002).

Increase in POX activity in leaves of drought tolerant and sensitive maize cultivars has also been reported earlier under water deficit stress (Djakovic *et al*., 2002). An increase in POX activity in drought tolerant as well as sensitive maize genotypes at seedling stage under osmotic stress has been reported (Kolarovic *et al*., 2009). Increase in CAT and POX activity is supposed to be an adaptive trait possibly helping to overcome the damage to the tissue metabolism by reducing toxic levels of H_2O_2 produced during cell metabolism and protection against oxidative stress (Sudhakar *et al*., 2001).

2.3.2.2. b Catalase

Catalase is a tetrameric heme protein, occurring in almost all aerobic organisms, and one of the few enzymes showing dual activity: it has hyperoxidase activity (catalytic activity) when it catalyzes the breakdown of hydrogen peroxide into water and oxygen. It also shows peroxidase activity (Luhova *et al*., 2003). In environmental stresses conditions such as drought, high activities of CAT enzymes are important for plants to tolerate stresses. Catalase is essential for the removal of H_2O_2 produced in the peroxisomes by photorespiration (Noctor *et al*., 2000). Catalase, which degrades H_2O_2 into water and oxygen, is one of the major antioxidant enzymes (Scandalios *et al*., 1997).

Role of CAT in averting the cellular damage under unfavorable conditions like water stress has been suggested (Reddy *et al.,* 2004). An increase in CAT activity under drought stress has been reported in wheat (Luna *et al*., 2004)

2.3.2.2. c Superoxide Dismutase (SOD)

SODs constitute the first line of defence against reactive oxygen species (ROS). SODs belong to a large and ubiquitous family of metallo enzymes in aerobic organisms. SOD can protect PS II from superoxide generated by oxidative and water stress (Martinez *et al.*, 2001).

Higher expression MnSOD, FeSOD can protect the photosynthetic apparatus via protecting the relevant proteins and lipids, leads to tolerance under drought stress.

In the tolerant genotype, increased number of MnSOD, Cytosolic SOD, transcripts were associated with protection of the plant an against oxidative stress (Shiriga *et al*., 2014). Wang *et al*. (2005) has reported that in presence of Mn SOD drought tolerance was in increased in transgenic rice. By the increase in drought stress intensity, superoxide dismutase activity increased extensively in lentil cultivars (Moghadam *et al.*, 2013)

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 the coconut palms with change in leaf water potential (Kasturi Bai *et al*,2005). Adaptive mechanism in coconut palms under field stress conditions have been reported by Shivashankar *et al*.(1991), Chempakam *et al.* (1993) and Kasturi Bai *et al*. (1996).

Responses at the molecular level

In recent years, much molecular information has been generated on the response of plants to environmental stresses. Plants respond to environmental stresses such a drought by the induction of both regulatory and functional sets of genes (Ramanjulu and Bartels, 2002; Bartels and Sunkar, 2005).Drought tolerance is considered a quantitative trait, involving the participation of a complex set of genes. The studies that have established this have been performed on model plants as well as in drought tolerant species (Seki *et al.,* 2007; Ozturk *et al.,* 2002; Way *et al.,* 2005; Montalvo-Hernandez *et al.,* 2008). When drought stress is perceived by the plant, changes in the expression pattern have been monitored, ranging from genes whose products are involved in early response such as signal transduction, transcription and translation factors; to late response genes, such as water transport, osmotic balance, oxidative stress and damage repair (Ramanjulu and Bartels, 2002, Shinozaki and Yamaguchi-Shinozaki, 2007; Knight and Knight, 2001; Zhu, 2002). Adaptive responses are observed as a consequence of such changes, including early flowering, growth inhibition, among others (Bray, 2002).

Materials and Methods

3. MATERIALS AND METHODS

The experiment was undertaken with objective to evaluate selectively fertilized coconut (*Cocos nucifera.* L) hybrids for water use efficiency, and to study mechanism of water stress tolerance in coconut, and to estimate genetic variability in coconut for water use efficiency through Carbon Isotope Discrimination. To achieve this objective, pot culture experiment was conducted at Department of Plant Physiology, College of Agriculture, Vellayani (2014-15). Biochemical analyses were done at monthly intervals and the growth parameters were studied after completion of the experiment.

3.1 EXPERIMENT DETAILS

3.1.1 Location (Plate 1)

The field experiment was conducted in rainout shelter located in College of Agriculture Vellayani, situated at 8 ^{0} 30'N latitude and 76 ^{0}9'E longitude at an altitude of 29 m above mean sea level.

3.1.2 Planting material

One year old seedlings of seven coconut varieties were procured from RARS, Pilicode and CRS, Balaramapuram.

3.1.3 Layout of the Experiment and design

The experiment was laid out in CRD with two treatments and three replications. Seven coconut varieties and hybrids were chosen for the study which included Keraganga, Kerasree, Malayan Yellow Dwarf, Gangabondam, West Coast Tall, Kerasree (Selectively fertilised) and Keraganga (Selectively fertilised).

3.1.4 Experimental set up and Methodology (Plate 2)

3.1.4.1 Planting and Initial sampling

Coconut seedlings were planted in polybags (56 cm x 30cm) filled with potting mixture (dried cow dung, sand and soil in the ratio of 1:1:1) of known weight. Equal sets of seedlings were kept under each treatment. Seedlings were properly maintained for two months with adequate irrigation, fertilizers, pest and disease management as

Plate 1. Experimental location

Coconut seedlings in polybags

Electronic weighing balance

Plate 2. Experimental set up and methodology

per package of practices by KAU. Initial sampling was done to know the average initial biomass for each coconut genotype. Samples were oven dried at 80 ^{0} C to constant weight and dry weights were noted.

3.1.4.2 Treatments

Study included two set of treatments.

T_1 – 100 % F.C

T_2 - 50 % F.C

Under each treatment, each variety was kept under three replications, making a total of 42 seedling units in the experiment.

3.1.4.3 Gravimetry

Estimating water use efficiency by gravimetrical method was one of the major components of the technical programme. Equipment used for the study included an electronic weighing balance of 50 kg (Plate 2) and measuring cylinder.

Methodology

The day before the start of the experiment, all the units (Seedling + Filled polybags) were fully irrigated and kept for drainage. Next day, each unit was weighed to know its weight at 100 % field capacity. Difference in weight gave the quantity of water in each unit at 100 % F.C. Accordingly, water to be applied for half the set of seedlings at 50 % F.C were also computed.

Every day in the morning, each unit was weighed to know the water lost through transpiration and the value was noted. The units were then irrigated using measured quantity of water to maintain the respective field capacities. Exposed soil surfaces were covered with plastic sheet pieces to minimise soil evaporation. Along with the experimental seedling sets, a set of reference seedlings each from every variety were maintained to know the average biomass accumulation in each variety. Biomass accumulated was assessed monthly and the computed value was added to the daily weight of each unit and irrigation was done accordingly.

3.1.4.4. Final sampling (Plate 3)

Final sampling was scheduled, after samples were taken for estimation of stable isotope discrimination and biochemical analyses. After three months marked as end of the experiment, all the coconut seedlings were uprooted, and roots, shoots, leaves and nut of each seedling was individually dried in the oven at 80 ^{0}C to know the final dry weight.

3.1.4.5 Assessing genotypic variation for water use efficiency

Leaf samples were collected from adult palms of thirty coconut varieties from RARS, Pilicode; the samples were dried in the oven at 80 ^{0}C, finely powdered, properly labelled and were sent for stable isotope discrimination.

3.2 OBSERVATIONS

3.2.1. GROWTH PARAMETERS AND PHYSIOLOGICAL PARAMETERS

3.2.1.1 Plant Height

Length of the longest leaf was measured in metres

3.2.1.2 Leaf Area

Leaf area was measured by plotting the leaves in a graph paper and counting the number of milli squares covered by the leaf

3.2.1.3 Specific Leaf Area

Leaflets were seperated from the index leaf and area was measured graphically. Leaflets were dried at 80^{0}C for 2 days and the dry weight was taken. SLA was calculated using the formula.

$$SLA\ (cm^2\ g^{-1}) = \text{Leaf area / dry weight}$$

3.2.1.4 Total Dry Matter Accumulation

Initial sampling was done and plant samples were dried in a hot air oven at 80^{0}C to constant weight to find average biomass of individual varieties and recorded as W_1.At the end of experiment, final sampling were done for all seedlings, oven dried and weight recorded as W_2. Biomass comprises of shoot, root and leaves. Biomass accumulation can be calculated as

Total dry matter accumulation = $W_2 - W_1$ and expressed in kilograms

3.2.1.5 Relative Growth Rate

Relative growth rate (RGR) was determined using the formulae of Williams (1946) and expressed in mg $g^{-1}day^{-1}$.

$$RGR = \log_e W_2 - \log_e W1 / t2 - t1$$

where, W_1 and W_2 = plant dry weight (g) at time t_1 and t_2 respectively

$t_2 - t_1$ = time interval in days

3.2.1.6 Net Assimilation Rate

The method proposed by Williams (1946) was used for calculating the net assimilation rate (NAR) on leaf dry weight basis and the values were expressed as mg $cm^{-2}day^{-1}$.

$$NAR = W_2-W_1 / t_2-t_1 \times \log_e L_2 - \log_e L_1 / L_2 - L_1$$

where, W_1 and W_2 = leaf dry weight (mg) at t_1 and t_2 respectively

L_1 and L_2 = leaf area (cm^2) at t_1 and t_2 respectively

$t_2 - t_1$ = time interval in days

3.2.1.7 Phyllochron

The phyllochron is the intervening period between the sequential emergence of leaves. Phyllochon was expressed in days

3.2.1.8 Cumulative Water Transpired

Water transpired was measured using gravimetric method. Weight of the individual seedling units were measured daily, and the difference in weight of the individual unit from its weight under respective field capacity gave the water transpired by that unit. Meanwhile, the biomass accumulated over a regular period (one month) by the reference seedling of each variety was added to the daily weightage of individual seedling unit and water transpired was calculated accordingly. Sum of all the values of

water transpired over three months gave the cumulative water transpired and was expressed in litres.

3.2.1.9 Relative water content

Relative water content was calculated by measuring the fresh weight, dry weight and turgid weight of known number of leaf discs from the treatment plants. After measuring the fresh weight of the sample, it was submerged in distilled water for 3 hours and then the turgid weight was taken. The dry weight of the sample was measured after keeping the samples in oven at 80 o C for 3 consecutive days. The RWC of the treatments was calculated using the following formula and was expressed in percentage.

RWC = (Fresh weight - dry weight) / (turgid weight - dry weight) X 100

3.2.1.10 Membrane Integrity

Leaf discs (about 10 no) of test samples were taken in a 50ml beaker with 10ml distilled water. Initial EC was measured for detecting the small degree of leakage by the discs caused by the punching treatment using conductivity electrode (EC_a).After 30 minutes incubation the leakage of solutes in this bathing medium was measured (EC_b).Then the beakers were boiled at 100oC for 10 minutes and the EC was again recorded (ECc).The membrane integrity of leaf tissues was calculated using the following formula and was expressed in percentage leakage.

$$\% \text{ leakage} = ((EC_b - EC_a) / ECc) \times 100$$

3.2.1.11 Wax Content

Samples were collected from third fully opened leaves of plants and were cut into $10cm^2$ bits.10ml of chloroform was taken in beakers after noting down their initial weight .Leaf bits were dipped into chloroform for 30seconds.After removing the leaf bits the beakers were left for evaporation of chloroform .The final weight of beakers was noted after complete evaporation of chloroform. The difference between the final and initial weight of beakers would be noted as the wax content and would be expressed per unit leaf area. ECW was expressed as mg $cm.^{-2}$

3.2.1.12 Stomatal Characteristics

3.2.1.12. a Stomatal frequency and Stomatal Index

Stomatal count refers to the number of stomata per unit area of leaf. A thick mixture of thermocol and xylene was prepared and this was smeared on both the surfaces of leaves and allowed to dry. It was peeled gently after drying and the peel was observed under microscope and counted using a 40 x objective and 10 x eyepieces. The field of the microscope was measured using a stage micrometre and stomatal frequency per unit area was calculated.

Stomatal frequency = No. of stomata / Area of the microscopic field

Stomatal index is the percentage which indicates the number of stomata present to the total number of epidermal cells, each stomata being counted as one cell. Stomatal index was calculated by using following equation.

$$\text{Stomatal Index, } I = \frac{\text{No: of stomata per unit area}}{\text{(No: of stomata per unit area + No: of epidermal cells per unit area)}} \times 100$$

3.2.1.12. b Transpiration Rate and Stomatal Conductance

Transpiration rate and Stomatal conductance was measured using the SAI-1 Porometer of company Delta T Devices and were expressed in milli moles $m^{-2}s^{-1}$.

3.2.1.13 Chlorophyll Stability Index

One gram of fresh green leaf is taken and divided into two lots of 0.5 gram each. One lot (Control) is stored in room temperature (26 ^{0}C) and the other lot is put in empty test tube standing in boiling water bath for 1 hr. Total chlorophyll content of two lots were measured by DMSO method. Finally Chlorophyll Stability Index was calculated using the formula

Chlorophyll Stability Index (%) = (Total Chl. Content in Heated lot / Total Chl. Content in Control lot) X 100

3.2.1.14 Water Use Efficiency

Water use efficiency is defined as kilograms of biomass accumulated per unit of water transpired and WUE is expressed as gkg^{-1}

Water Use Efficiency = Biomass accumulated (gram) /Water transpired (kilogram)

3.2.1.15 Stable Isotope Discrimination

The carbon isotope discrimination ratio (CID) and oxygen isotope ratio was determined for calculating the isotope discrimination. The third fully opened leaves of experimental plants were collected, oven dried at 80^0C and were ground to a very fine powder. The samples were sent to the National Facility for stable isotope studies at the Department of Crop Physiology, GKVK, UAS, Bangalore where they were analysed using the isotope ratio mass spectrophotometer (Plate 4) coupled with the elemental analyser for the continuous flow measurement of carbon and oxygen isotope ratios in plant samples.

3.2.2 BIOCHEMICAL PARAMETERS

3.2.2.1 Pigment composition

Estimation of Chlorophyll and Carotenoids (DMSO method)

A weighed quantity of sample (0.5g) was taken and cut into small bits. These bits were put in test tubes and incubated overnight at room temperature, after pouring 10 ml DMSO: 80% acetone mixture (1:1 v/v). The coloured solution was decanted into a measuring cylinder and made up to 25 ml with the DMSO-acetone mixture. The absorbance was measured at 663, 645,480 and 510nm using a spectrophotometer. The chlorophyll content was measured by substituting the absorbance values in the given formulae and was expressed as mg g^{-1}

Chl a = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times V/1000 \times 1/$ Fresh weight

Chl b = $(22.9 \times A_{645} - 4.68 \times A_{663}) \times V/1000 \times 1/$ Fresh weight

Total Chl (a + b) = $(8.02 \times A_{663} + 20.2 \times A_{645}) \times V/1000 \times 1/$ Fresh weight

Carotenoid = $[(7.6 \times A_{480}) - (1.49 \times A_{510}) \times V] / (w \times 1000)$

Plate 3. Final Sampling

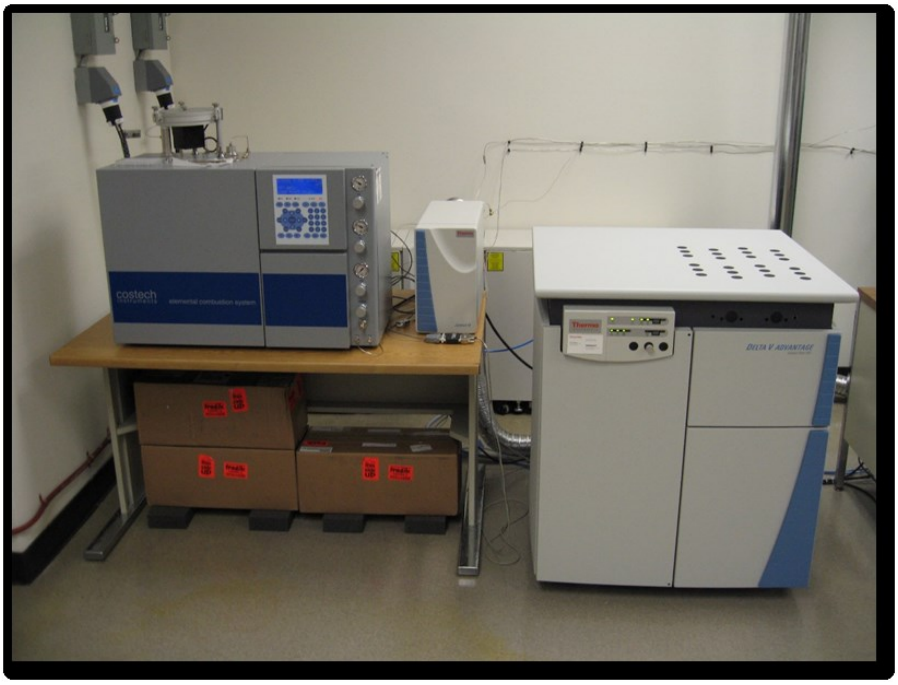

Plate 4. IRMS facility

3.2.2.2 Estimation of Total soluble protein (Bradford method)

The total soluble protein of leaf samples were estimated using simple protein dye binding assay of Bradford (1976) using bovine serum albumin as the standard. One hundred milligram of CBB 250 was dissolved in 50 ml of 95% ethanol. To this 100 ml of 85% (w/v) orthophosphoric acid was added. The resulting solution was diluted to a final volume of 200 ml with distilled water. 0.1g of leaf samples were taken from third fully opened leaves and was ground to a thin paste and soluble protein was extracted with 10 ml of phosphate buffer (pH 7.8). The extract was centrifuged at 5000 rpm for 10 minutes. To the 20µl of the supernatant a known volume (5 ml) of diluted dye binding solution was added. The solution was mixed well and allowed to develop a blue colour for at least 5 min but no longer than 30 min and the absorbance was measured at 596 nm. The protein content was calculated using the BSA standard in the range of (10-100µg). The protein content was expressed as mg g^{-1} FW.

3.2.2.3 Estimation of total free amino acid

The total free amino acids were estimated following the Ninhydrin method (Moore and Stein, 1948). The plant sample was weighed (500 mg) and ground in a mortar and pestle with a small quantity of acid washed sand. To this homogenate 5-10 ml of 80% ethanol was added. The solution was filtered and centrifuged. The filtrate or the supernatant was saved for further use. This extraction was repeated twice with the residue and the supernatants were pooled. The volume was reduced by evaporation and the extract was used for the quantitative estimation of total free amino acids. Ninhydrin solution (1 ml) was added to 1 ml of extract and the volume was made up to 2 ml using distilled water. The test tube was heated in a boiling water bath for 20 minutes. The contents were mixed after adding 5 ml of the diluents. The intensity of the purple colour was read at 570 nm, in a colorimeter, against a reagent blank, after incubation of 15 minutes. The reagent blank was prepared as above by taking 0.1 ml of 80% ethanol, instead of extract. The standard Leucine (50mg) was dissolved in50 ml of distilled water in a volumetric flask. The stock standard of 10 ml was diluted to 100 ml in another volumetric flask to make the working standard solution. A series of volume from 0.1 to 1 ml of this standard solution was

prepared to give a concentration range of 10µg-100µg. The procedure was followed as that of sample and the absorbance of purple colour was read at 570 nm. A standard curve was drawn using absorbance versus concentration. The concentration of total free amino acid in the sample was determined from the standard graph and was expressed as % equivalent of leucine.

3.2.2.4 Estimation of Reducing sugars (DNS Acid Method)

The estimation of reducing sugars in plants was done following Dinitro Salicylic acid (DNS) method (Somogyi, 1952). The sample was weighed (100 mg) and the sugars were extracted with hot 80% ethanol, twice. The supernatant was collected and evaporated by keeping it on a boiling water bath at 80^0C. The sugars were dissolved by adding 10 ml water. Aliquots of 0.5 to 3 ml were pipetted out into test tubes and the volume was equalized to 3ml with distilled water in all the test tubes. To this 3 ml of DNS reagent was added. The test tubes were heated in a boiling water bath for 5 minutes. Rochelle salt solution (40%, w/v) (1 ml) was added to the test tubes when the contents were hot. Then the test tubes were cooled and the intensity of dark red colour was read at 510 nm. A series of the standard, Glucose, (0 to 500 µg) was run and a standard curve was plotted. The amount of reducing sugars in the sample was calculated from the standard graph.

3.2.2.5 Estimation of Starch

The estimation of starch in plants was done following the Anthrone method (Mc Cready *et al*., 1950). A known quantity of plant sample (0.1 g) was homogenized in hot 80% ethanol to remove sugars. The homogenate was centrifuged and residue was retained. The residue was washed repeatedly with hot 80% ethanol till the washing to give any colour with anthrone reagent. Then the residue was dried well over a water bath. The dried residue was mixed with 5ml water and 6.5 ml 52% perchloric acid and was extracted at 0^oC for 20 min. This solution was centrifuged and the supernatant was saved. The extraction was repeated using fresh perchloric acid. The supernatants after centrifugation was pooled and made up to 100 ml. An aliquot of 0.1 ml of the supernatant was taken and again made up to 1 ml using distilled water. The standard was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution and

made up the volume to 1 ml in each tube using distilled water. Anthrone reagent (4 ml) was added to both the sample and standard test tubes. These test tubes were heated for eight minutes in a boiling water bath and cooled rapidly. The intensity of colour change from green to dark green was measured at 630 nm. The glucose content in the sample was calculated using the standard curve. This value was multiplied by a factor of 0.9 to arrive at the starch content.

3.2.2.6 Estimation of Phenols

Quantification of phenols was done by Folin-Ciocalteau method (Mayr *et al.,* 1995). Phenol was estimated from 0.5g of leaf samples and reflexed in 10 ml 80% methanol for 20 min. The tissue was ground thoroughly in a mortar with pestle and filtered through a double layered cheese cloth. The filtrate was subjected to centrifugation at 1000 rpm for 10 min. The supernatant was collected and made to a known volume using 80% methanol. 0.1 ml aliquot was drawn to a test tube and made up to 3 ml using 80% methanol. To this, 0.5 ml of Folin-Ciocalteau reagent and 2 ml 20% Na2CO3 were added. It was kept in a boiling water bath for 5 min till a white precipitate was formed and was then again centrifuged at 5000 rpm for 5 min. The absorbance of the clear supernatant was read at 650 nm against the blank. Standard curve was prepared using different concentrations of catechol and expressed in catechol equivalents as microgram per gram leaf tissue on fresh weight basis.

3.2.3 STRESS TOLERANCE PARAMETERS

3.2.3.1 Estimation of Proline

Proline estimation was done following the Acid Ninhydrin method (Bates *et al*., 1973). Fresh leaves (500mg) were homogenized in 10 ml of aqueous sulphosalicylic acid (3%). The homogenate was centrifuged at 6000 rpm for 15 min. A two ml aliquot of the supernatant was mixed with an equal volume (2 ml) of acetic acid and ninhydrin and heated in boiling water bath for 1 h. The reaction was terminated on ice bath and extracted with 4 ml of toluene. The extract was vortexed for 20 s and the chromatophore-containing toluene was aspirated from the aqueous phase and

absorbance determined photometrically at 520 nm using toluene for a blank. A standard curve was drawn using absorbance versus concentration. The concentration of proline in the sample was determined from the standard graph and was expressed as

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

3.2.3.2 Non-Enzymatic Antioxidants

Estimation of ascorbic acid

The ascorbic acid content in plants was estimated volumetrically by the method explained by Harris and Ray (1935). Working standard solution (5 ml) was pipetted out into a 100 ml conical flask. 4%oxalic acid was added to it and titrated against the dye (V_1ml). End point was noted on appearance of pink colour which persisted for a few minutes. The sample (0.5-5g) was weighed and ground in a mortar with pestle using 15ml 4% oxalic acid. The homogenate was filtered through a double layered cheese cloth. The filtrate was made up to a known volume and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and made up to 25ml using oxalic acid. 5.0ml aliquot was pipetted into a conical flask to which 10ml of 4% oxalic acid was added. This was titrated against Dichlorophenol indophenol (DCPIP) solution, until the appearance of pink colour (V_2ml). The amount of ascorbic acid is calculated as follows: Ascorbic acid (mg/100g) = (0.5 mg /V1ml) x (V2/ 5 ml) x (100/ weight of sample).

3.2.3.3 Enzymatic antioxidants

3.2.3.3.a Estimation of Peroxidase (POX)

The peroxidase activity in plants was estimated following the method described by Reddy *et al*. (1995). A 20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the sample, clarified by centrifugation and the supernatant was used for the assay. To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5ml of H2O2was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

3.2.3.3. b Estimation of Catalase (CAT)

The CAT activity in plants was quantified following the method described by Luck (1974). A 20% homogenate of the sample was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay. The H_2O_2-phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

3.2.3.3. c Estimation of Superoxide dismutase (SOD)

SOD activity of plants was quantified following the method described by Kakkar *et al*. (1984). The samples (0.5g) were ground with 3.0ml of potassium phosphate buffer, centrifuged at 2000g for 10 minutes and the supernatants were used for the assay .The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of0.2ml of NADH. The mixture was incubated at 30°C for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

Results

4. RESULTS

Kerala, as the name pronounces, is the land of 'Kera' or Coconuts. But at present, Kerala ranks third in production and fourth in productivity (CDB, 2013).This decline in production is greatly attributed to various environmental stresses. Drought is the major abiotic stress form that reduces coconut production especially in the northern Kerala, next to root (wilt) disease. Current situation is in need of drought tolerant coconut genotypes with higher water use efficiency.

Today's physiological research greatly relies on isotopic studies to screen out water use efficient crop genotypes based on natural discrimination that occur for carbon and oxygen isotopes. Hence, efforts are made in the present study to evaluate water use efficient coconut genotypes based on isotope discrimination and various physiological and biometrical studies. The results obtained are presented in this chapter

4.1 GROWTH AND PHYSIOLOGICAL PARAMETERS

Various growth parameters analyzed includes plant height, leaf area, specific leaf area, biomass accumulated, cumulative water transpired, relative growth rate, net assimilation rate and phyllochron. Significant variations were observed in all these parameters between different coconut varieties

4.1.1 Plant Height

Plant height varied significantly among varieties under both the treatments. Keraganga had shown highest treatment mean value for plant height under 100 % F.C (2.60 m) and 50% F.C (1.80 m). Under both the treatments, MYD recorded lowest plant height with mean values of (1.55 m) and (0.73 m) under T_1 and T_2 respectively. Under T_1, selectively fertilized hybrid of Keraganga varied significantly for plant height from other hybrids, dwarfs and its S.F hybrid companion. Also under T_1, dwarfs recorded significant variation for their height but there was no significant difference between the normal hybrids i.e. Kerasree and Keraganga. Under T_2, plant height varied significantly among selectively fertilized hybrids but dwarfs and WCT were on par. Effect of water stress on plant height is depicted in Table 1.

4.1.2 Leaf Area

All varieties had shown significant variation in leaf area under both the treatments. Selectively fertilized hybrid of Keraganga had the highest treatment value mean under T_1 (45.46 m^2) and T_2 (61.73 m^2). Kerasree (9.85 m^2) and MYD (14.46 m^2) recorded lowest mean value in T_1 and T_2 respectively (Table 1). Under 50 % F.C, Gangabondam had no significant variation from MYD, and also Keraganga was on par with S.F hybrid of Kerasree.

4.1.3 Specific Leaf Area

Effect of water stress on specific leaf area is presented in Table 1. Though all varieties had significant variation in case of SLA, selectively fertilized hybrid of Keraganga had the highest treatment mean value in both T_1 (1710.84 $cm^2\ g^{-1}$) and T_2 (1423.25 $cm^2\ g^{-1}$). Also for 100 % F.C and 50 % F.C, Kerasree S.F had shown no significant variation from WCT and, Kerasree had shown no significant variation from MYD. The lowest mean value for SLA under 100 % F.C was for Keraganga (655.63 $cm^2\ g^{-1}$) and that under 50 % F.C was for Kerasree (874.27 $cm^2\ g^{-1}$).

4.1.4. Total Dry Matter Accumulation

With respect to water use efficiency, total dry matter accumulated is of great importance to screen out drought tolerant types. In this study, all varieties had shown significant variation in total dry matter accumulation under both the treatments as presented in Table 2. Under 100 % F.C, Keraganga S.F accumulated maximum dry matter (366.67g) being on par with Kerasree S.F. All other varieties under T_1 had no significant variation from each other and was on par with MYD which was the lowest dry matter accumulator (34.33 g). Under 50 % F.C, Kerasree S.F had the highest mean value for dry matter accumulation (118.00 g) for which it was significant from other varieties. Keraganga accumulated least under T_2 (15.67 g) and was on par with the dwarfs, WCT and Kerasree.

Table.1 Effect of water stress on Plant height, m; Specific leaf area, cm^2g^{-1} and Total leaf area, m^2

Varieties& Hybrids	Plant height		Specific leaf area		Total leaf area	
	100 % F.C	50 % F.C	100 % F.C	50 % F.C	100 % F.C	50 % F.C
Keraganga	2.60	1.80	655.63	1037.00	16.29	39.33
Kerasree	2.19	1.52	758.75	874.27	9.85	28.74
MYD	1.55	0.73	798.96	923.10	12.65	14.46
Gangabondam	1.84	0.79	911.28	1242.75	19.20	15.63
WCT	2.20	1.02	1414.73	1327.20	21.43	23.36
Kerasree S.F	2.11	1.19	1372.29	1268.08	40.60	47.46
Keraganga S.F	2.26	1.66	1710.84	1423.25	45.47	61.73
CD (0.05)	0.183	0.266	61.435	70.391	2.341	1.918

Table 2. Effect of water stress on Total dry matter accumulation, g

Varieties &Hybrids	**100 % F.C**	**50 %F.C**
Keraganga	74.33	15.67
Kerasree	92.67	72.67
MYD	34.33	24.00
Gangabondam	65.33	37.00
WCT	55.00	44.67
Kerasree S.F	296.67	188.33
Keraganga S.F	366.67	118.00
CD (0.05)	119.605	64.666

Table 3.Effect of water stress on root weight, shoot weight and total biomass, g

Varieties &Hybrids	Root weight		Shoot weight		Total biomass	
	100 % F.C	**50 % F.C**	**100 % F.C**	**50 % F.C**	**100 % F.C**	**50 % F.C**
Keraganga	60.00	81.67	783.33	405.00	843.33	486.67
Kerasree	50.33	150.00	811.33	393.66	861.67	543.67
MYD	45.00	60.00	646.67	530.00	691.67	590.00
Gangabondam	103.33	76.67	680.00	698.33	783.33	775.00
WCT	78.33	76.67	711.67	605.00	790.00	681.67
Kerasree S.F	233.33	106.67	1583.33	1101.67	1816.67	1208.33
Keraganga .F	233.33	166.67	1433.33	1151.33	1666.67	1318.00
CD (0.05)	114.522	46.066	212.724	64.826	120.343	65.662

4.1.5 Root Weight, Shoot Weight and Total Biomass

Effect of water stress on root weight, shoot weight and total biomass is presented in Table 3.

Growth of roots which are water extracting organs and shoots which are water losing organs (transpiration) gets modified under water deficit in drought tolerant types. Keraganga S.F had highest mean for root weight under T_1 (233.33 g) and T_2 (166.67g). Lowest mean for root weight under T_1 (45.00 g) and T_2 (60.00 g) was recorded by MYD. S.F hybrids were on par under T_1, and under T_2 S.F hybrids were on par with Kerasree and also WCT had no significant variation from the dwarfs.

Shoot weight under T_1 was also highest for S.F hybrids with a mean of (1583.33g) for Kerasree S.F and was lowest for MYD with a mean of (646.67 g). Under T_2, highest and lowest mean were reported by Keraganga S.F (1151.33 g) and Kerasree (393.66 g) respectively. Under both the treatments, S.F hybrids were on par for shoot growth. But, MYD and WCT varied significantly from all other varieties under 50 % F.C.

In case of total biomass, highest mean under T_1 was shown by Kerasree S.F (1816.67 g) and there was significant variation from all other varieties; lowest mean was shown by MYD (691.67g). Gangabondam and WCT were on par with the lowest mean for total biomass under 100 % F.C Under T_2, Keraganga S.F was significantly higher than other varieties with a mean of (1318.00 g);the lowest mean (486.67g) was shown by Keraganga which was on par with Kerasree. Normal hybrids were also on par with each other and with Gangabondam under T_2. WCT and dwarfs varied significantly from all other varieties under T_2 for the total biomass.

4.1.6 Relative Growth Rate

RGR of coconut varieties and hybrids is presented in Table 4. For all the coconut varieties under evaluation, there was significant variation among varieties for relative growth rate both under T_1 and T_2. However highest mean values for RGR were shown by Kerasree S.F under T_1 (0.008 mg g^{-1} d^{-1}) and T_2 (0.008 mg g^{-1} d^{-1}). Lowest

mean values under T_1 was recorded by Keraganga S.F (0.001 mg g^{-1} d^{-1}) and that under T_2 was given by Keraganga (0.001 mg g^{-1} d^{-1}).

4.1.7 Net Assimilation Rate

NAR of coconut varieties and hybrids is presented in Table 4. Considering the parameter, behavior of coconut varieties under both the treatments showed no marked difference except for the highest means. Under 100 % F.C, highest mean value was shown by MYD (0.257 g $m^{-2}d^{-1}$) and the lowest mean value was shown by WCT (0.024 $gm^{-2}d^{-1}$). Except for MYD, WCT was on par with rest of the varieties under treatment T_1. Under 50 % F.C, highest mean under treatment T_2 was recorded by MYD (0.121 $gm^{-2}d^{-1}$) and the lowest mean was given by Keraganga (0.005 $gm^{-2}d^{-1}$). S.F hybrids were on par with WCT for NAR under 50 % F.C.

4.1.8 Phyllochron

Number of leaves matters for effective biomass production in all crop genotypes. All varieties had shown significant variation for the parameter under both the treatment conditions as shown in Table 5. Under T_1 (100 % F.C), Keraganga, its S.F hybrid, Kerasree and WCT were on par for the highest mean value (23.0 days). Kerasree S.F had the lowest mean value (13.57 days) under T_1 condition, and also MYD and Gangabondam were on par under 100% F.C. In case of 50 % F.C, highest (48.33 days) and lowest mean (19.5 days) value was shown by WCT and MYD respectively S.F hybrids and Gangabondam had no significant variation among themselves under 50 % F.C.

4.1.8 Cumulative Water Transpired

A drought tolerant genotype always conserves water and controls water loss through transpiration. Hence, WUE also relies on cumulative water transpired over the experimental period. Table 6 shows the effect of water stress on cumulative water transpired by different coconut varieties and hybrids. Selectively fertilized hybrid of Keraganga lost highest quantity of water (46.67 kg) through transpiration under 100 % F.C and varied significantly from all other genotypes. Lowest volume of water (30.42

kg) under T_1 was transpired by Gangabondam and had shown no significant variation from MYD and WCT. Under 50 % F.C, highest (39.38 kg) and lowest (21.66 kg) volume of water was transpired by Kerasree and WCT respectively. Irrespective of the quantity under T_2, S.F hybrids were on par for the water transpired over the experimental period. WCT under T_2 was on par with Gangabondam for the cumulative volume of transpired water.

4.1.9 Relative Water Content

Effect of water stress on relative water content is depicted in Table 7. Maintaining the leaf water status is very important to withstand ill effects of water deficit. During first month, highest leaf water status under T_1 was shown by Kerasree S.F with a treatment mean of (84.49 %) and was on par with Keraganga, its S.F hybrid and the dwarf varieties. Lowest mean was shown by WCT (75.76 %). In treatment T_2 also, highest and lowest mean were shown by Kerasree S.F (86.19 %) and WCT (72.7 %) respectively.

During second month of stress, dwarf varieties behaved comparatively better under both T_1 and T_2 treatments. Gangabondam had the highest treatment mean (87.75%) under T_1 and MYD had the highest mean value (90.13%) under T_2. If it was Keraganga that had shown the lowest mean value (75.31%) under T_1, its S.F hybrid had the lowest mean value (70.93%) under T_2 condition.

During third month, S.F hybrids had shown a signification variation in keeping a better leaf status under both treatment situations. Under T_1 and T_2, highest treatment mean value was shown by S.F hybrids of Keraganga (87.88 %) and Kerasree (80.19%) respectively. MYD recorded lowest mean value under T_1 (74.27%) and T_2 (68.70%). WCT, Gangabondam and Keraganga was on par with the highest treatment mean under T_2 condition.

Table 4. Effect of water stress on Relative growth rate, mg $g^{-1}day^{-1}$ and Net assimilation rate, g $m^{-2}day^{-1}$

Varieties &Hybrids	Relative growth rate		Net assimilation rate	
	100 % F.C	**50 %F.C**	**100 % F.C**	**50 %F.C**
Keraganga	0.004	0.001	0.039	0.005
Kerasree	0.005	0.006	0.040	0.013
MYD	0.002	0.005	0.257	0.121
Gangabondam	0.004	0.002	0.043	0.021
WCT	0.003	0.003	0.024	0.025
Kerasree S.F	0.008	0.008	0.048	0.029
Keraganga S.F	0.001	0.004	0.045	0.011
CD (0.05)	0.003	0.003	0.034	0.015

Table 5. Effect of water stress on Phyllochron, number of days

Varieties/Hybrids	**100 % F.C**	**50 %F.C**
Keraganga	23.00	40.00
Kerasree	23.00	45.00
MYD	18.00	19.50
Gangabondam	16.00	27.50
WCT	21.33	48.33
Kerasree S.F	13.57	27.50
Keraganga S.F	23.00	30.00
CD (0.05)	2.378	7.47

Table 6. Effect of water stress on cumulative water transpired, kg

Varieties & Hybrids	First month		Second month		Third month		Cumulative water transpired	
	100 % F.C	**50 % F.C**	**100 % F.C**	**50 % F.C**	**100 % F.C**	**50 % F.C**	**100 % F.C**	**50 % F.C**
Keraganga	16.85	12.77	5.08	5.09	17.31	14.27	39.24	32.13
Kerasree	20.52	16.59	6.92	7.98	13.98	14.82	41.42	39.38
MYD	13.48	8.82	6.27	4.52	12.93	8.57	32.68	21.92
Gangabondam	14.64	9.53	4.52	4.44	11.27	13.58	30.42	27.56
WCT	14.40	8.72	5.80	4.40	10.38	8.54	30.59	21.66
Kerasree S.F	20.27	15.07	6.65	5.90	11.53	8.75	38.45	29.72
Keraganga S.F	21.15	17.33	7.50	6.60	18.03	7.40	46.67	31.32
CD (0.05)	2.356	1.388	0.783	0.474	2.799	1.977	4.089	2.544

All the varieties except Kerasree and S.F hybrid of Keraganga under T_1 had shown a decrease in water potential which the increased in the third month. Kerasree had shown an increased water potential in the second month which then decreased in the third month. In T_2, all the varieties except WCT had shown a decreased water leaf water status in the third month after a steady increase in second month. Variety WCT had shown an increase in water content up to third month.

4.1.10 Membrane integrity

Drought susceptible genotypes cannot tolerate stress situations and is characterized by high membrane instability when exposed to any kind of stresses. Membrane integrity is expressed as percentage leakage of solutes. Effect of water stress on membrane integrity is shown in Table 8. During first month of treatment, WCT had shown highest mean value (17.34 %) and Keraganga had shown the lowest mean value (0.27 %) for solute leakage under 100 % F.C. Under 50 % F.C, highest (55.2%) and lowest (4.32 %) leakage of solutes were shown by Kerasree and Keraganga S.F respectively. Under T_1, there was significant difference between WCT and S.F hybrids but dwarfs were on par with the lowest mean. Under T_2 condition, Keraganga, Gangabondam, WCT and Kerasree S.F were on par.

During second month, under T_1, S.F hybrids exhibited the extreme mean values; Kerasree S.F with the highest mean (34.74%) and Keraganga S.F with the lowest mean (26.08 %). WCT, Gangabondam and Kerasree were on par with Kerasree S.F. Under T_2 condition, WCT had shown the lowest membrane integrity with highest leakage mean (38.89 %) and Keraganga had shown the highest membrane stability with lowest leakage mean value (21.89 %). There was no significant difference between Kerasree and Keraganga under T_2.

During third month, Kerasree exhibited highest mean value (33.66 %) for solute leakage in 100 % F.C with no significant variation from its S.F hybrid and WCT; and Keraganga had shown the highest mean for leakage (50.91%) in 50 % F.C with

significant variation from rest of the varieties. MYD had the maximum membrane stability with lowest percentage solute leakage under T_1 (21.05%) and T_2 (28.67%).

Under T_1 and T_2, Keraganga and its S.F hybrid had shown an increasing trend for the value. Both the dwarfs under T_1 had a decreasing trend in the third month and under T_2 they had shown increasing pattern in second and third month. Under water deficit, WCT had shown a decreasing value.

4.1.11 Epicuticular Wax

Significant wax deposition on cuticle is an adaptive mechanism to prevent excess transpirational water loss under water deficit condition. Table 9 shows wax deposition of coconut genotypes under water stress. During first month, MYD had shown maximum wax deposition with a highest mean (2.30 mg cm^{-2}) under T_1 and Kerasree shown highest mean value (3.60 mg cm^{-2}) under T_2. Keraganga S.F was significantly lower than other varieties for cuticular wax with the mean of 0.63 mg cm^{-2} and 1.03 mg cm^{-2} under T_1 and T_2 respectively.

During second month of treatment, similar results as that of first month was obtained. Under T_1, extreme values was shown by MYD (2.46 mg cm^{-2}) and Keraganga S.F (0.89 mg cm^{-2}); and under T_2 Kerasree had shown the highest mean (3.75 mg cm^{-2}) and Keraganga S.F had shown the lowest mean (1.16) mg cm^{-2}.

During third month, under T_1, MYD had shown the highest wax deposit (2.61 mg cm^{-2}) which was on par with Keraganga, and Keraganga S.F had shown the lowest wax deposit which was on par with Gangabondam. Under T_2 treatment, Kerasree was significantly higher than all other varieties for deposition of cuticular wax with a mean of (3.93 mg cm^{-2}) and unlike other two months, WCT had shown the least value with a mean of (1.27 mg cm^{-2}). S.F hybrids were on par with WCT under 50 % F.C.

Table 7. Effect of water stress on Relative water content, percentage

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	83.77	83.68	75.31	84.36	84.69	69.17
Kerasree	80.33	74.60	84.55	80.92	78.89	78.10
MYD	83.69	80.85	83.14	90.13	74.27	68.71
Gangabondam	82.63	79.64	87.75	81.49	83.61	78.30
WCT	75.76	72.70	78.09	72.08	76.10	79.87
Kerasree S.F	84.49	86.19	86.59	79.56	81.88	80.19
Keraganga S.F	82.32	80.87	84.78	70.93	87.88	75.93
C.D (0.05)	3.492	5.784	3.439	3.319	2.140	7.106

Table 8. Effect of water stress on Membrane integrity, percentage of solute leakage

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	0.27	13.36	26.19	21.89	29.97	50.91
Kerasree	6.48	55.20	32.93	22.71	33.67	28.99
MYD	3.25	4.97	30.64	27.03	21.05	28.67
Gangabondam	1.39	12.79	33.24	28.88	28.32	32.24
WCT	17.34	11.78	33.81	38.89	32.57	34.09
Kerasree S.F	5.65	14.08	34.74	30.31	32.76	33.45
Keraganga S.F	7.88	4.32	26.08	30.72	29.90	36.20
C.D (0.05)	2.991	2.378	2.693	2.123	3.369	3.364

Table 9. Effect of water stress on Epicuticular wax deposition, mg cm^{-2}

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	2.17	1.93	2.32	2.05	2.61	1.52
Kerasree	1.30	3.60	1.43	3.75	1.55	3.93
MYD	2.30	3.03	2.46	1.92	2.61	2.62
Gangabondam	0.92	2.40	1.12	2.57	1.30	2.73
WCT	1.19	1.57	1.45	1.75	1.70	1.27
Kerasree S.F	1.13	2.27	1.33	2.51	1.59	1.33
Keraganga S.F	0.63	1.03	0.89	1.17	1.20	1.40
C.D (0.05)	0.266	0.493	0.234	1.172	0.137	0.62

For all the varieties except MYD, WCT and Kerasree S.F under T1 and T2, wax deposition increased up to third month. Under T2, WCT and Kerasree S.F had increased value in the second month which later decreased in the third month, while for MYD in the third month the value increased after showing a decreased value in the second month.

4.1.12 Stomatal characteristics

Stomatal frequency and Stomatal index were two important aspects of stomata which will influence the transpiration behavior of the crop

4.1.12.1 Stomatal frequency

Stomatal frequency of different coconut genotypes under water stress is presented in Table 10. During first month, S.F hybrids had significant variation in between, and Keraganga S.F with a highest mean (73199.30 no cm $^{-2}$). Under the same treatment WCT was found with the lowest stomatal frequency with a mean of (31284.92 no cm $^{-2}$). Under T_2, mean values of S.F hybrids were at the extremes with significant variation, where Kerasree S.F had shown the highest mean (54388.30 no cm $^{-2}$) and Keraganga S.F had shown the lowest mean (31284.70 no cm $^{-2}$).

During second month of treatment, as that of first month Keraganga S.F recorded the highest mean (65994.65 no cm $^{-2}$) and WCT recorded the lowest mean (39646.18 no cm $^{-2}$) under T_1. Dwarfs and the hybrids (Kerasree and Keraganga) were on par. Under T_2, there was significant variation between the S.F hybrids themselves and with the rest of the varieties. Highest mean was given by Kerasree S.F (51843.58 no cm $^{-2}$) and lowest mean was given by Keraganga S.F (34190.07 no cm $^{-2}$).

During third month of treatment, under T_1, normal Keraganga hybrid recorded the lowest mean value (35009.31 no cm $^{-2}$) whereas its S.F hybrid recorded the highest mean value (58759.91 no cm^{-2}); S.F hybrids were on par for the treatment. Under T_2, normal hybrid of Kerasree had shown the lowest mean value (31620.11 no cm $^{-2}$) whereas its S.F hybrid had shown the highest mean value (50949.72 no cm^{-2}); Kerasree was on par with Keraganga and MYD under T_2 situation.

Under T_1, except for Gangabondam and WCT which had shown increased stomatal frequency up to third month, all other varieties had shown a decreasing trend. Under T_2, Gangabondam and Keraganga S.F had shown an increasing trend while all others had shown a decreasing trend.

4.1.12.2 Stomatal Index

Effect of water stress on stomatal index is presented in Table 11. During first month, Gangabondam recorded the highest treatment mean value under T_1 (25.94) and T_2 (24.02); and the variety varied significantly from rest of the varieties under both the treatment situations. Kerasree had shown the lowest mean value under T_1 (15.43) and Keraganga S.F had shown the lowest mean value under T_2 (8.5). Except the varieties at extremes, all other varieties were on par under T_1.

As that of first month, during second month also Gangabondam recorded the highest mean value for stomatal index under T_1 (28.86) and T_2 (28.42) with significant variation from all other varieties in both the treatments. Kerasree had shown the lowest mean value under T_1 (15.64) and Keraganga S.F had shown the lowest mean value under T_2 (9.67).

During third month of treatment, dwarfs had shown greater values for stomatal index; where Gangabondam had recorded highest mean value under T_1 (31.78) and T_2 (32.82). Under T_1, lowest mean was given by Keraganga (14.58) and its S.F hybrid had given the lowest mean value (10.81) under T_2.

Under T_1, Keraganga and its S.F hybrid exhibited a decreasing trend while all others had shown an increasing trend. Under T_2, only hybrids had shown a decreasing pattern while all others had shown an increasing pattern.

4.1.12.3 Stomatal conductance

The ease with which water vapour and gases move in and out of the leaf tissue especially during periods of water stress gives an idea about the behavior of the crop genotypes in drought situations. Effect of water stress on stomatal conductance is depicted in Table 12. During first month of treatment, S.F hybrid of Keraganga performed with the lowest mean of (34.13 milli moles m^{-2} s^{-1}) and (5.26 milli moles m^{-2} s^{-1}) under T_1 and T_2 respectively. MYD which shown the highest mean under T_1 (93.4 milli moles m^{-2} s^{-1}) had no significant variation from WCT and Gangabondam. But under T_2 condition, WCT recorded highest mean (47.2 milli moles m^{-2} s^{-1}) was significantly different from rest of the varieties.

During second month of treatment, Gangabondam was found with the highest mean under T_1 (83.03 milli moles m^{-1} s^{-1}) and T_2 (68.10 milli moles m^{-2} s^{-1}); and the lowest mean was given by Kerasree under T_1 (31.46 milli moles m^{-2} s^{-1}) and T_2 (28.56 milli moles m^{-2} s^{-1}). In both the treatments, Gangabondam and Kerasree varied significantly from rest of the varieties.

During third month, all varieties had shown significant variation between themselves under 100 % F.C; and highest and lowest means were, given by dwarfs MYD (44.63 milli moles m^{-2} s^{-1}) and Gangabondam (8.46 milli moles m^{-2} s^{-1}). But under 50 % F.C, dwarfs were closer to the lowest mean (4.26 milli moles m^{-2} s^{-1}) which was given by Kerasree and the highest mean (28.75 milli moles m^{-2} s^{-1}) was recorded by its S.F hybrid.

Under T_1, all the varieties except S.F hybrids had decreased stomatal conductance up to third month; S.F hybrids had increased value in the second month which then later decreased. Under T_2 all the varieties had shown a similar trend of increased value in second month which then decreased in third month.

Table 10.Effect of water stress on Stomatal frequency, no: of stomata /cm ^{2}

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	60782.12	45597.77	47374.30	40782.12	35009.31	33966.48
Kerasree	48610.04	42681.56	46425.09	37150.84	46284.18	31620.11
MYD	48491.62	36424.58	48156.42	36089.39	47821.23	35754.19
Gangabondam	49608.940	36648.04	49944.13	38324.02	50279.33	40000.00
WCT	31284.92	43333.40	39646.18	41675.98	41340.78	43351.96
Kerasree S.F	60997.73	54388.30	60204.19	51843.58	57154.82	50949.72
Keraganga S.F	73199.30	31284.70	65994.65	34190.07	58759.91	37094.92
C.D (0.05)	4214.408	2986.470	5340.957	1183.973	3413.475	4228.796

Table 11.Effect of water stress on Stomatal index

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	20.46	17.19	17.52	16.03	14.58	15.53
Kerasree	15.43	16.02	15.64	15.20	15.66	14.37
MYD	22.35	17.22	24.13	18.70	25.91	20.18
Gangabondam	25.94	24.03	28.86	28.42	31.78	32.82
WCT	16.76	16.33	21.28	17.52	25.79	19.38
Kerasree S.F	19.44	17.22	19.80	18.23	20.12	19.24
Keraganga S.F	21.09	8.50	20.19	9.67	19.98	10.81
C.D (0.05)	1.040	1.470	1.325	1.149	2.181	1.745

Table 12. Effect of water stress on Stomatal conductance, milli moles $m^{-2}s^{-1}$

Varieties/ Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	84.07	21.12	76.33	39.10	12.53	10.00
Kerasree	86.53	23.20	31.47	28.57	18.70	4.27
MYD	93.43	28.53	64.47	46.63	44.63	9.40
Gangabondam	89.57	17.83	83.03	68.10	8.47	8.73
WCT	91.50	47.20	65.13	58.83	12.87	17.87
Kerasree S.F	43.07	21.07	56.73	30.80	24.70	28.75
Keraganga S.F	34.13	5.27	69.56	47.13	15.77	17.73
C.D (0.05)	5.329	1.974	4.822	3.883	1.490	1.523

4.1.13 Transpiration rate

Effect of water stress on transpiration rate is shown in Table 13. Though rate of transpiration also relies on climatic condition, varietal variation is greatly considered to evaluate water use efficient types, especially under water deficit situation. During first month under T_1, highest mean value was given by Keraganga S.F (2.54 milli moles m^{-2} s^{-1}) and lowest mean value was given by WCT (1.64 milli moles m^{-2} s^{-1}). Keraganga S.F varied significantly from all other varieties except Keraganga and Gangabondam.

During second month of treatment, MYD had the highest value of mean under T_1 (1.47 milli moles m^{-2} s^{-1}) and under T_2 (0.97 milli moles m^{-2} s^{-1}). Likewise, Keraganga had the lowest mean value under T_1 (0.53 milli moles m^{-2} s^{-1}) and T_2 (0.23 milli moles m^{-2} s^{-1}). MYD and Keraganga had shown significant difference from all other varieties under 100 and 50 % F.C.

During third month of treatment, under T_1, dwarfs had recorded the extreme values; MYD with highest mean (1.79 milli moles m^{-2} s^{-1}) and Gangabondam (0.33 milli moles m^{-2} s^{-1}), with significant difference from each other and between other varieties. Under T_2, Kerasree S.F recorded highest mean value (1.50 milli moles m^{-2} s^{-1}) and its normal hybrid Kerasree recorded lowest mean value (0.15 milli moles m^{-2} s^{-1}), with significant variation from rest of the varieties.

Under T_1 and T_2, all varieties except S.F hybrids had a decreased transpiration rate up to third month. Under T_1, S.F hybrids had shown an increased value in the third month after getting decreased in second month. But under T_2, S.F hybrids had shown increased transpiration rate up to third month.

4.1.14 Chlorophyll Stability Index

Table 14 presents Chlorophyll stability index of coconut varieties and hybrids under water stress. During first month, MYD recorded highest mean value for CSI under T_1 (98.83 %) and T_2 (99.19 %) situations. MYD had no signification variation from Keraganga and Kerasree in 100% F.C; and under 50 % F.C MYD varied significantly from all other varieties except Keraganga S.F. Lowest mean value under

T_1 and T_2 were shown by Keraganga S.F (97.23%) and Gangabondam (97.56%) respectively.

During second month, WCT which recorded highest mean value (99.37%) under T_1 had no significant variation between MYD, Kerasree and Keraganga; and the lowest mean was given by Gangabondam (99.83%). Under T_2 treatment, treatments were found to be non-significant for the parameter.

During third month, MYD and Keraganga S.F had shown extreme values under both the treatments. Under T_1 MYD recorded a highest mean (99.07%) and Keraganga S.F with a lowest mean (97.50%). Under T_2, MYD had a highest mean (99.18) and Keraganga S.F with a lowest value (98.02 %) of mean.

For all the varieties under both the treatments, CSI had shown increasing trend in the second month which later decreased in third month.

4.2 WATER USE EFFICIENCY

Effect of water stress on water use efficiency of different coconut hybrids and varieties is depicted in Table 15. Selectively fertilized hybrids were found to be on par for water use efficiency under T_1 with a highest mean of (7.81 gkg^{-1}) for Keraganga S.F. All other varieties under T_1 had no significant variation from MYD (1.06 gkg^{-1}) which recorded the lowest mean value for the parameter. However under 50 % F.C, Kerasree S.F which was the most water use efficient variety with a mean value (6.30 gkg^{-1}) was significantly different from other coconut types. Keraganga S.F was on par with WCT was on par with dwarfs for WUE under T_2. Least water use efficient under 50 % F.C was Keraganga (0.48 gkg^{-1}) which was on par with the dwarfs.

4.3 CARBON ISOTOPE DISCRIMINATION

Discrimination values for the stable isotope of carbon (^{13}C) had shown significant variation among varieties under both soil moisture regimes as presented in Table 16. MYD recorded highest discrimination (21.91‰) under treatment T_1 which

varied in significantly from WCT. Keraganga S.F being on par with Gangabondam had shown the lowest value (20.18 $^{o}/_{oo}$) for carbon isotope discrimination under 100 % F.C. Highest discrimination value for stable carbon isotope under 50 % F.C was shown by Keraganga (21.49 $^{o}/_{oo}$) which had significant variation from other varieties. Dwarf variety Gangabondam was also on par with Kerasree and Keraganga S.F. Selectively fertilized hybrid of Kerasree has found to be least discriminated for ^{13} C under 50 % F.C with a mean value of (19.90 $^{o}/_{oo}$) and had shown no significant variation from WCT.

4.4 BIOCHEMICAL PARAMETERS

4.4.1 Pigment Composition

Table 17, 18 and 19 shows the effect of water stress on different pigment components of coconut genotypes during first, second and third month respectively

Chlorophyll a

During first month, under T_1, highest treatment mean (0.67 mg g^{-1}) was shown by Gangabondam which was on par with S.F hybrids and WCT. MYD was significantly lower than rest of the varieties (0.51 mg g^{-1}). In between S.F hybrids and among the normal hybrids, no significant variation was noticed. Under T2, Gangabondam was significantly higher than all other varieties (0.67 mg g^{-1}); and Keraganga S.F recorded the lowest mean value (0.22 mg g^{-1}).

During second month, under T_1 WCT was significantly higher (0.26 mg g^{-1}) than all other types and Gangabondam which had shown the lowest mean value (0.06 mgg-1) was on par with Keraganga S.F, Kerasree and MYD. Under T_2, Kerasree recorded highest mean (0.13 mg g^{-1}) and MYD recorded lowest mean (0.06 mg g^{-1}).

During third month under T_1, highest mean was recorded by Keraganga S.F (0.62 mg g^{-1}) with significant variation from other varieties and MYD recorded the lowest mean (0.36 mg g^{-1}) with Gangabondam.

Table 13.Effect of water stress on transpiration rate, milli moles m^{-2} s^{-1}

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	2.35	0.73	0.53	0.23	0.60	0.42
Kerasree	2.18	0.57	1.05	0.41	0.63	0.15
MYD	1.910	2.04	1.47	0.97	1.79	0.36
Gangabondam	2.40	2.08	0.82	0.26	0.33	0.29
WCT	1.64	1.31	0.76	0.63	0.39	0.61
Kerasree S.F	1.92	0.59	0.55	0.76	0.56	1.50
Keraganga S.F	2.54	0.14	0.66	0.49	0.75	0.85
C.D (0.05)	0.328	0.147	0.112	0.073	0.084	0.277

Table 14. Effect of water stress on Chlorophyll stability index, percentage

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	98.20	98.22	99.67	99.780	98.55	98.26
Kerasree	98.16	98.15	99.75	99.69	98.37	98.45
MYD	98.83	99.19	99.79	99.83	99.06	99.19
Gangabondam	98.01	97.56	99.83	99.72	98.89	98.15
WCT	97.28	98.55	99.38	99.74	98.67	98.77
Kerasree S.F	97.65	98.53	99.54	99.74	98.44	99.03
Keraganga S.F	97.23	99.09	99.59	99.77	97.50	98.02
C.D (0.05)	0.793	0.382	0.237	NS	0.482	0.365

Table15 .Water use efficiency of coconut genotypes under water stress, gkg^{-1}

Varieties &Hybrids	**100 % F.C**	**50 %F.C**
Keraganga	1.91	0.48
Kerasree	2.24	1.84
MYD	1.06	1.09
Gangabondam	2.19	1.35
WCT	1.79	2.04
Kerasree S.F	7.70	6.30
Keraganga S.F	7.81	3.79
C.D (0.05)	2.596	2.077

Table16. Carbon isotope discrimination of coconut genotypes under water stress, per mil (^{o} / $_{oo}$)

Varieties& Hybrids	**100 % F.C**	**50 % F.C**
Keraganga	20.50	21.49
Kerasree	21.28	20.41
MYD	21.91	20.82
Gangabondam	20.20	20.60
WCT	21.71	20.08
Kerasree S.F	21.32	19.90
Keraganga S.F	20.18	20.36
CD (0.05)	0.296	0.269

Under T_2, Kerasree had shown the highest mean value (0.13 mg g^{-1}) and MYD had shown the lowest mean value (0.06 mg g^{-1}), and Kerasree being significantly different from all others and MYD being on par with Keraganga and its S.F hybrid.

For all varieties under T_1 and T_2, chlorophyll a decreased in second month and increased in the third month

Chlorophyll b

During first month under T_1, WCT (0.89 mg g^{-1}) and Gangabondam (0.37 mg g^{-1}) had shown the highest mean value under T_1 and T_2 respectively. MYD had shown the lowest mean under both T_1(0.15 mg g^{-1}) and T_2 (0.09 mg g^{-1}) treatments All the varieties in both the treatments varied significantly from highest means whereas hybrids had shown no significant difference from the lowest mean under T_1.

During second month, under T_1, WCT was significantly higher (0.09 mg g^{-1}) than all other varieties but Gangabondam which recorded the lowest mean (0.016 mg g^{-1}) was on par with all the varieties except WCT. Under T_2, all the varieties were found be non-significant for Chlorophyll b content.

During third month of treatment, S.F hybrid of Keraganga recorded highest mean (0.36 mg g^{-1}) under T_1 with significant variation from other varieties and Gangabondam recorded the lowest mean value (0.15 mg g^{-1}) with no significant variation from MYD. Dwarfs varied significantly among themselves under T_2; Gangabondam with the highest mean (0.38 mg g^{-1}) and MYD with lowest mean (0.11 mg g^{-1}). S.F hybrids and normal hybrids had also shown significant variation among themselves for Chlorophyll b content.

For all varieties under T_1 and T_2, chlorophyll b decreased in second month and increased in the third month

Table 17. Effect of water stress on pigment composition during first month, mg g^{-1}

Varieties & Hybrids	100 % F.C				50 % F.C			
	Chl a	Chl b	Total chl	Carotenoids	Chl a	Chl b	Total chl	Carotenoids
Keraganga	0.59	0.19	0.78	0.02	0.58	0.19	0.77	0.020
Kerasree	0.62	0.24	0.73	0.02	0.58	0.21	0.79	0.02
MYD	0.51	0.15	0.49	0.02	0.31	0.09	0.39	0.02
Gangabondam	0.67	0.25	0.93	0.02	0.67	0.37	1.21	0.02
WCT	0.64	0.89	1.43	0.01	0.59	0.18	0.79	0.02
Kerasree S.F	0.67	0.26	0.92	0.02	0.59	0.18	0.76	0.02
Keraganga S.F	0.63	0.33	0.96	0.01	0.22	0.12	0.36	0.01
C.D (0.05)	0.077	0.123	0.200	NS	.050	.070	.090	NS

Table 18. Effect of water stress on pigment composition during second month, mg g^{-1}

Varieties & Hybrids	100 % .F.C				50 % F.C			
	Chl a	Chl b	Total chl	Carotenoids	Chl a	Chl b	Total chl	Carotenoids
Keraganga	0.14	0.03	6.45	0.07	0.07	0.02	0.11	0.04
Kerasree	0.08	0.03	0.11	0.07	0.13	0.03	0.18	0.06
MYD	0.06	0.02	0.10	0.05	0.06	0.02	0.08	0.03
Gangabondam	0.06	0.01	0.08	0.04	0.10	0.03	0.13	0.05
WCT	0.26	0.09	0.36	0.12	0.10	0.03	0.14	0.06
Kerasree S.F	0.11	0.04	0.14	0.07	0.09	0.04	0.12	0.04
Keraganga S.F	0.07	0.036	0.11	0.06	0.07	0.02	0.07	0.03
C.D (0.05)	0.028	0.025	NS	0.012	.020	NS	.050	0.017

Table 19. Effect of water stress on pigment composition during third month, mg g^{-1}

Varieties & Hybrids	100 % .F.C				50 % F.C			
	Chl a	Chl b	Total chl	Carotenoids	Chl a	Chl b	Total chl	Carotenoids
Keraganga	0.52	0.19	0.63	0.29	0.52	0.24	0.75	0.30
Kerasree	0.51	0.21	0.69	0.27	0.48	0.21	0.63	0.27
MYD	0.36	0.16	0.45	0.18	0.28	0.11	0.39	0.18
Gangabondam	0.37	0.15	0.51	0.21	0.53	0.38	0.96	0.29
WCT	0.52	0.25	0.70	0.29	0.47	0.18	0.65	0.29
Kerasree S.F	0.56	0.24	0.48	0.28	0.29	0.14	0.37	0.16
Keraganga S.F	0.62	0.36	0.96	0.40	0.53	0.28	0.82	0.25
CD (0.05)	0.061	0.034	0.163	0.044	.050	.030	.090	0.045

Total Chlorophyll

During first month, WCT and MYD recorded highest (1.46 mg g^{-1}) and lowest (0.49 mg g^{-1}) respectively under T_1; both the varieties varied significantly from rest of the varieties. S.F hybrids had shown no significant variation among themselves as with the case of their normal hybrids. Under T_2, Gangabondam was significantly higher (1.21 mg g^{-1}) than WCT, MYD, S.F and normal hybrids. Keraganga S.F which recorded the lowest mean value was on par with MYD.

During second month, variation among coconut varieties under T1 for total chlorophyll content was found to be non-significant. Under T_2, highest mean (0.183) was given by Kerasree and the lowest mean (0.07 mg g^{-1}) was given by Keraganga S.F; WCT was on par the highest mean and MYD was on par with the lowest mean.

During third month, Keraganga S.F was significantly higher (0.96 mg g^{-1}) than all other types under T_1. Total chlorophyll was lowest (0.45 mg g^{-1}) in MYD which was on par with Gangabondam and Kerasree S.F. Kerasree and Keraganga hybrids were on par with WCT. Under T_2, Gangabondam recorded the highest mean (0.96 mg g^{-1}) and had shown significant variation from other types; Kerasree S.F which was on par with MYD recorded the lowest mean (0.37 mg g^{-1}). WCT was on par with Kerasree and Keraganga was on par with its S.F hybrid.

For all varieties except Keraganga under T_1, total chlorophyll decreased in second month and increased in the third month .Under T_2, for all varieties total chlorophyll decreased in second month and increased in the third month

Carotenoids

In both the treatments, during first month, variation among coconut varieties for the coconut types were non-significant. During the second month as the treatments progressed, WCT was significantly higher than other varieties with a mean of (0.12 mg g^{-1}) and Gangabondam with the lowest mean (0.04 mg g^{-1}) was on par with MYD, under T_1. S.F hybrids were on par for the carotenoids under T_1. Under T_2, Kerasree which was on par with WCT and Gangabondam had shown the highest mean (0.06 mg

g^{-1}) and MYD which was on par with S.F hybrids, Keraganga and Gangabondam had shown the lowest mean value (0.03 mg g^{-1}). During third month, under T_1, Keraganga recorded highest mean of (0.40 mg g^{-1}) which varied significantly from all other varieties and lowest mean (0.18) was shown by MYD which was on par with Gangabondam. Under T_2, highest mean (0.30 mg g^{-1}) was given by Keraganga and lowest mean (0.16 mg g^{-1}) was given by Kerasree S.F.

For all coconut varieties carotenoid content had increased up to third month irrespective of the treatments.

4.4.2 Total Soluble Protein

Effect of water stress on total soluble protein is presented in Table 20. During first month under T_1, highest mean value for total soluble protein (5.5 mg g^{-1}) was shown by WCT which was on par with S.F hybrids and dwarfs. Under same treatment, Keraganga had shown the lowest mean value (2.5 mg g^{-1}), which varied significantly from other varieties. Under T_2, there was significant variation for protein content between varieties except WCT, Kerasree and Gangabondam. Highest mean value was shown by Keraganga S.F (5.35 mg $g^{-1)}$ and lowest mean value was shown by MYD (2.52 mg g^{-1}). Kerasree S.F varied non significantly from Keraganga S.F for the total soluble protein content under 50 % F.C.

During second month, S.F hybrids accumulated more of proteins in their leaf tissues in both the treatments. Under T_1, Kerasree S.F had the highest mean value (4.13 mg g^{-1}) and was on par with Keraganga S.F; whereas the Kerasree had the lowest mean value (1.3 mg g^{-1}). Dwarfs were on par and also shown no significant variation from Keraganga. Under T_2, S.F hybrid of Keraganga was significantly higher (4.5 mg g^{-1}) and that of Kerasree (1.45 mg g^{-1}) was significantly lower than other varieties. There was no significant variation between MYD, Gangabondam and Kerasree.

During the third month of treatment, S.F hybrid of Kerasree recorded highest mean (1.05 mg g^{-1}) with no significant difference from WCT under T_1. Keraganga was significantly lower than all other varieties (0.5 mg g^{-1}); and no significant variation was

noticed among dwarfs. Under T_2, WCT recorded highest treatment mean (0.876) with no significant variation from the hybrid varieties and Kerasree S.F. MYD recorded the lowest treatment mean (0.65 mg g^{-1}) with no significant variation from S.F hybrids and Gangabondam

Except Keraganga, all varieties had shown a decreasing trend for protein content up to third month under T_1. For Keraganga, protein content increased in second month and then decreased in third month. But under T_2, all the varieties got up with decreased protein content up to third month.

4.4.3 Free Amino Acid

Effect of water stress on free amino acid content in different coconut genotypes is shown in Table 21. In both the treatments, all coconut varieties had shown significant variation among themselves. MYD was highest in free amino acid content in leaves both under T_1 (4.88 mg g^{-1}) and T_2 (4.71 mg g^{-1}). Selectively fertilized hybrids were on the lower extreme under both situations where Kerasree S.F recorded the lowest mean value (1.69 mg g^{-1}) in T_1 and Keraganga S.F recorded the lowest mean value (1.1 mg g^{-1}) in T_2.

4.4.4 Reducing Sugar

Effect of water stress on reducing sugar content in different coconut genotypes is presented in Table 22. Highest mean value for sugar accumulation were shown by MYD under T_1 (36.07 mg g^{-1}) and WCT under T_2 (35.93 mg g^{-1}).MYD had shown significant variation from all other varieties in T_1, but WCT was on par with Gangabondam in T_2. Keraganga S.F had recorded the lowest mean value for sugar under T_1 (18.9 mg g^{-1}) and T_2 (12.5 mg g^{-1}) treatments; and it varied significantly from all other varieties under both the situations.

***Table 20. Effect of water stress on Total soluble protein*, mg g^{-1}**

Varieties & Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	2.55	4.80	2.53	1.95	0.50	0.85
Kerasree	4.30	4.67	1.30	1.85	0.80	0.82
MYD	4.55	2.52	2.90	1.85	0.71	0.65
Gangabondam	4.85	4.25	2.95	1.55	0.70	0.68
WCT	5.50	4.10	2.40	3.00	0.92	0.88
Kerasree S.F	5.07	5.30	4.13	1.45	1.05	0.75
Keraganga S.F	5.09	5.35	3.73	4.50	0.83	0.68
CD (0.05)	0.967	0.638	0.458	0.358	0.146	0.147

Table 21. Effect of water stress on Free amino acid, mg g^{-1}

Varieties &Hybrids	**100% F.C**	**50% F.C**
Keraganga	4.06	4.65
Kerasree	2.49	2.69
MYD	4.88	4.71
Gangabondam	4.63	4.08
WCT	2.71	2.71
Kerasree S.F	1.69	2.74
Keraganga S.F	1.93	1.10
C.D (0.05)	0.215	0.161

Table 22.Effect of water stress on Reducing sugar mg g^{-1}

Varieties &Hybrids	**100% F.C**	**50% F.C**
Keraganga	31.05	32.73
Kerasree	22.73	26.93
MYD	36.07	32.40
Gangabondam	32.80	35.73
WCT	33.60	35.93
Kerasree S.F	32.10	28.00
Keraganga S.F	18.90	12.50
C.D (0.05)	1.136	1.968

4.4.5 Starch

Highest accumulation of Starch under treatment T_1 was shown by Keraganga with a mean value (6.16 mg g^{-1}) and the lowest mean was given by Kerasree S.F (3.06 mg g^{-1}) as depicted in Table 23. Under T_1, Keraganga was on par with Gangabondam and showed significant variation from rest of the varieties; also Kerasree and its parents were on par under T_1. Under T2, WCT had shown the highest mean value (36.6 mg g^{-1}) and MYD had shown the lowest mean value (3.69 mg g^{-1}). All the varieties under T_2 had shown significant variation in between themselves.

4.4.6 Phenol

Under T_1, except for S.F hybrids, significant variation was noticed among varieties. Highest treatment mean was shown by WCT (60.33 mg g^{-1}) and lowest treatment mean was shown by Kerasree as shown in Table 24. Unlike T_1, S.F hybrids varied significantly under T_2. Keraganga S.F recorded the highest mean value (43.60 mg g^{-1}) and Kerasree S.F was on par with Keraganga which recorded the lowest mean value (31.03 mg g^{-1}). Dwarfs and hybrids had shown significant difference among each other.

4.4.7 Ascorbic acid

Table 25 shows the ascorbic acid content found in coconut genotypes under water stress. Content of ascorbic acid was highest in WCT under T1 with a mean of (0.25 mg 100 g^{-1}) and was lowest in Kerasree with a mean of (0.14 mg 100 g^{-1}). WCT varied significantly from all other varieties. Keraganga, S.F hybrids, and the dwarfs were on par with each other for the ascorbic acid content of leaves. Under T_2, highest mean was shown by Keraganga (0.29 mg 100 g^{-1}) and the lowest mean was shown by Kerasree (0.12 mg 100 g^{-1}). Keraganga was on par with MYD under T_2, and also the S.F hybrids and GB were on par.

Table 23. Effect of water stress on Starch content, mg g^{-1}

Varieties & Hybrids	100% F.C	50% F.C
Keraganga	6.19	30.15
Kerasree	4.69	32.55
MYD	4.78	3.69
Gangabondam	5.99	16.68
WCT	5.18	36.60
Kerasree S.F	3.06	24.28
Keraganga S.F	4.17	21.49
C.D (0.05)	0.501	2.213

Table 24. Effect of water stress on Phenol content, mg g^{-1}

Varieties &Hybrids	100% F.C	50% F.C
Keraganga	54.37	31.03
Kerasree	18.33	36.87
MYD	32.53	41.87
Gangabondam	47.20	35.60
WCT	60.33	41.33
Kerasree S.F	26.90	32.20
Keraganga S.F	28.07	43.60
C.D (0.05)	1.207	3.524

Table 25. Effect of water stress on Ascorbic acid content, mg 100 g^{-1}

Varieties &Hybrids	100% F.C	50% F.C
Keraganga	0.17	0.29
Kerasree	0.14	0.12
MYD	0.18	0.27
Gangabondam	0.20	0.19
WCT	0.25	0.26
Kerasree S.F	0.18	0.18
Keraganga S.F	0.17	0.19
C.D (0.05)	0.040	0.033

4.5 STRESS TOLERANCE PARAMETERS

4.5.1 **Proline Content**

Proline accumulation upon water stress in different coconut genotypes is presented in Table 26.

Proline accumulation was highest among dwarfs under T_2 during first month with a mean value of (10.33 µ moles g^{-1}) for Gangabondam. MYD had shown no significant variation from Gangabondam and WCT but varied significantly from all other varieties under T_2. Lowest mean (0.53 µ moles g^{-1}) under T_2 was given by Kerasree S.F which was significantly lower than all other types. MYD recorded the highest mean (4.39 µ moles g^{-1}) for proline under T_1 and unlike in T_2, MYD varied significantly from Gangabondam. Lowest mean (1.28 µ moles g^{-1}) under T_1 was shown by Keraganga which was on par with Kerasree; and S.F hybrids and WCT were also on par under T_1.

During second month, highest proline accumulation under T_1 was noticed in MYD (349.95 µ moles g^{-1}) which was significantly higher than all other varieties. Gangabondam which recorded lowest proline accumulation under T_1 that had no significant variation from Keraganga and WCT. Under T_2, WCT scored the highest mean value (316.51 µ moles g^{-1}) and Gangabondam holded the lowest treatment mean (59.45 µ moles g^{-1}) for proline accumulation. Both WCT and Gangabondam had shown significant variation from all other genotypes.

During the third month of treatment, Kerasree had the highest mean (297.26 µ moles g^{-1}) value and Keraganga had the lowest mean value (111.98 µ moles g^{-1}) under T_1. All the varieties significantly varied among themselves for proline accumulation under 100 % F.C. Under 50 % F.C, highest mean (325.73 µ moles g^{-1}) was given by Kerasree S.F and the lowest mean (41.56 µ moles g^{-1}) was given by Keraganga. In both the treatments, varieties had exhibited significant variation among themselves.

4.5.2 Enzymatic antioxidants

4.5.2.1 Peroxidase

Effect of water stress on peroxidase activity is depicted in Table 27. During first month, peroxidase was highest in S.F hybrid of Kerasree with mean of (1.14 activity g^{-1} min^{-1}) which was on par with Gangabondam and lowest in Keraganga (0.53 activity g^{-1} min^{-1}) which was on par with WCT under T_1. Under T_2, Kerasree S.F was significantly higher than rest of the varieties with a mean of (2.80 activity g^{-1} min^{-1}) and the lowest mean (0.77 activity g^{-1} min^{-1}) was shown by Keraganga which was on par with all other varieties except Kerasree and its S.F hybrid.

During second month of treatment, under T_1, Kerasree recorded highest mean (1.67 activity g^{-1} min^{-1}) and other varieties varied significantly from the highest mean and the lowest mean (0.38 activity g^{-1} min^{-1}) was shown by MYD which was on par with Gangabondam. Under T_2, Keraganga was significantly higher than rest of the varieties with a mean of (7.33 activity g^{-1} min^{-1}) and the lowest mean (0.27 activity g^{-1} min^{-1}) was shown by WCT which was on par with MYD.

During third month, S.F hybrid of Keraganga exhibited highest treatment mean (1.07 activity g^{-1} min^{-1}) and its normal hybrid exhibited the lowest mean (0.18 activity g^{-1} min^{-1}) value under T_1. All other varieties varied significantly from highest and lowest mean. Under T_2, the dwarfs holded the extremes, where Gangabondam had the highest mean (1.95 activity g^{-1} min^{-1}) and MYD had the lowest mean (0.28 activity g^{-1} min^{-1}) for peroxidase activity. S.F hybrids were on par with each other for their peroxidase activity and had shown significant variation from highest and lowest means.

Under T_1, all the varieties had shown a reduced peroxidase activity in the third month except MYD and Keraganga S.F. Under T_2, except Gangabondam and WCT which had shown an increased activity all other varieties including S.F hybrids had shown a decreased peroxidase activity in the third month

Table 26. Effect of water stress on Proline content, μ moles g^{-1}

Varieties &Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	1.28	5.64	19.39	97.91	111.98	41.56
Kerasree	1.41	3.46	201.45	131.02	297.26	281.67
MYD	4.39	9.47	349.95	209.23	202.10	161.83
Gangabondam	2.42	10.33	12.97	59.45	258.99	295.53
WCT	4.02	9.15	21.45	316.51	125.83	227.42
Kerasree S.F	4.00	0.53	33.77	195.99	141.99	325.73
Keraganga S.F	3.92	1.78	124.68	277.56	235.50	189.00
C.D (0.05)	0.344	1.014	13.150	7.404	13.955	24.339

Table 27. Effect of water stress on activity of Peroxidase, activity g^{-1} min^{-1}

Varieties &Hybrids	First month		Second month		Third month	
	100% F.C	**50 % F.C**	**100% F.C**	**50 % F.C**	**100% F.C**	**50 % F.C**
Keraganga	0.53	0.77	1.00	7.33	0.18	0.98
Kerasree	0.87	2.03	1.67	0.63	0.57	0.50
MYD	0.80	0.78	0.38	0.50	0.47	0.28
Gangabondam	1.12	0.83	0.58	0.63	0.42	1.95
WCT	0.63	1.08	0.88	0.27	0.62	0.78
Kerasree S.F	1.14	2.80	1.25	2.35	0.37	0.68
Keraganga S.F	0.77	1.13	0.73	1.10	1.07	0.57
C.D (0.05)	0.106	0.469	0.210	0.513	0.101	0.161

Table 28. Effect of water stress on activity of Catalase and Superoxide dismutase, activity g^{-1} min^{-1}

Varieties &Hybrids	**Catalase activity**		**SOD activity**	
	100% F.C	**50% F.C**	**100% F.C**	**50% F.C**
Keraganga	0.55	0.13	0.21	4.35
Kerasree	0.13	0.37	0.42	5.21
MYD	0.21	0.74	0.12	2.79
Gangabondam	1.03	0.15	5.61	2.68
WCT	0.48	0.26	2.93	3.64
Kerasree S.F	0.39	0.48	3.78	4.03
Keraganga S.F	0.31	0.46	5.28	4.38
C.D value(0.05)	.066	.042	0.137	0.354

4.5.2.2 Catalase

Activity of catalase varied significantly among all the varieties under T_1 as presented in Table 28. Highest mean was shown by Gangabondam (1.03 activity g^{-1} min^{-1}) and lowest mean was shown by Kerasree (0.13 activity $g^{-1}min^{-1}$). Under T_2, MYD was significantly higher than other coconut varieties with a mean of (0.74 activity $g^{-1}min^{-1}$) and Keraganga was significantly lower than rest of the varieties with a mean of (0.13 activity g^{-1} min^{-1}) except for Gangabondam. S.F hybrids were on par under T_2, and had shown significant difference from all other genotypes.

4.5.2.3 Superoxide Dismutase

Performance of dwarfs for SOD activity was different under T_1 and was almost similar under T_2. In T_1, Gangabondam recorded highest mean (5.61 activity $g^{-1}min^{-1}$) and MYD recorded lowest mean (0.12 activity $g^{-1}min^{-1}$); Gangabondam had shown significant variation from all other varieties whereas as MYD was on par with Keraganga and had shown significant difference from other types. Under T_2, Kerasree was significantly higher than all other varieties with a mean (5.21 activity $g^{-1}min^{-1}$) and Gangabondam was significantly lower than other varieties with a mean (2.68 activity $g^{-1}min^{-1}$) and was on par with Gangabondam. Table 28 also shows activity of SOD under water stress in coconut varieties and hybrids.

4.6 GENETIC VARIATION FOR CARBON ISOTOPE DISCRIMINATION

Table 29 shows the discrimination values (Δ) for stable isotope of carbon in thirty coconut genotypes. Among thirty genotypes evaluated, New guinea had the lowest discrimination value of 18.35 per mil and the type NCD had the highest discrimination value of 21.07 per mil. WCT which is generally considered as drought tolerant had a discrimination value of 19.29 per mil.

Table 29. Carbon isotope discrimination values (Δ) of 30 coconut genotypes, per mil

Sl No	Genotypes	Δ
1	New guinea	18.35
2	Andaman ordinary	18.61
3	Anandaganga	18.8
4	Godavari	19.08
5	Lakshaganga	19.11
6	Keraganga	19.11
7	Navasi	19.17
8	Tanjore	19.2
9	Java	19.27
10	WCT	19.29
11	COD	19.34
12	Philippines	19.45
13	Soubhagya	19.59
14	Thembli	19.65
15	Ayiramkachi	19.67
16	Komadan	19.68
17	Bombay G	19.72
18	Kappadam	19.75
19	Andaman Ranguchen	19.79
20	Lakshadweep micro	20.01
21	Sanramon	20.11
22	MYD	20.15
23	Cochin China	20.21
24	Fiji	20.34
25	Bensahybrid	20.37
26	Kerasree	20.37
27	Lakshadweep ordinary	20.42
28	Bansanda	20.49
29	Jamaica	20.6
30	NCD	21.07

Discussion

5. DISCUSSION

The water deficit environment is reported as a key factor that limit plant growth and development prior to the loss of productivity, especially of crop species (Reddy *et al*., 2004; Blum, 2005; Neumann,2008). 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 (Chaves *et al*., 2003; Costa e Silva *et al*., 2004). 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). These mechanisms can include: (i) drought escape by rapid development, which allows plants to finish their cycle before severe water stress; (ii) drought avoidance by, for instance, increasing water uptake and reducing transpiration rate by the reduction of stomatal conductance and leaf area; (iii) drought tolerance by maintaining tissue turgor during water stress via osmotic adjustment, which allows plants to maintain growth under water stress; and (iv) resisting severe stress through survival mechanisms (Izanloo *et al.*, 2008). All the plants are not equally capable in withstanding water stress and their response to the stress also varies.

5.1 GROWTH PARAMETERS

Plant responses to water scarcity are complex, involving deleterious and /or adaptive changes, and under field conditions these responses can be synergistically or antagonistically modified by the superimposition of other stresses. Some of the differences among varieties can be traced to different capacities for water acquisition and transport rather than to drastic differences in metabolism at a given water status. Plants that stop growth to survive in a stressful environment could be considered tolerant, but such plants produce fewer grains, leaves or fruit, thus reducing their economic worth (DaMatta, 2004).

Although water stress affects most of the functions of plant growth, this effect depends on the level of water stress, the length of time to which the plant is subjected to water stress and the genotype of plant species. Growth involves both cell growth and development. These processes are very sensitive to water deficit because of their dependence upon turgor. Cell expansion and enlargement is one of the most sensitive processes affected by a change in plant water status (Begg and Turner, 1976).

Studies by various researchers has confirmed that water stress lead to growth reduction, which was reflected in plant height, leaf area, dry weight, and other growth functions (Kriedemann and Barrs,1981). It is clear from the results obtained in this study, that different levels of water stress have affected the growth of coconut genotypes differently, which is a clear indication of their different tolerance level under different degrees of stress. In the present study, drastic decrease was noticed for plant height under 50 % field capacity .Similar findings were reported by Boutraa *et al.* (2010) in wheat cultivars under water stress situation. As that of plant height, phyllochron is also useful parameter to estimate performance of different crops under water stress situation, as number of leaves matters for the available photosynthetic area for biomass production. Drought stress increased phyllocron compared to optimum irrigated condition. Phyllochron of coconut seedlings as studied in the present experiment increased under the stress situation compared to the fully irrigated situation irrespective of the genotypes (Figure 1). These results coincide with the work of Gholinezhad *et al* (2012) in sunflower. Albert and Carberry (1993) and Mc Cullough *et al.* (1994) reported that the phyllochron was increased by decreasing soil moisture.

With regard to the effect of water stress on total leaf area and specific leaf area, the results were obviously influenced by varietal variation. A marked decrease in leaf area were noted in WCT, Gangabondam and selectively fertilized hybrids. Findings of Gomez *et al.* (2010) confirm the results of present study. Similar variation of wheat genotypes in response to water stress was reported by Foulkes *et al.* (2007). Reduction in SLA noticed in WCT, Gangabondam and S.F hybrids underline the adaptive nature

of these coconut genotypes under water stress (50 % F.C) to reduce water loss from evaporative surfaces. Similar SLA response to water stress had been reported for two legume species (Villagra and Cavagnaro 2006) and Eucalyptus globules (Coopman *et al*. 2008). The impact of water stress on leaf growth can be explained as a method of adaptation to the conditions of water shortage to limit the rate of transpiration (Lu *et al*.,1998), in order to maintain the water supply in the soil around plant roots to increases the chance of survival of the plant (Passioura ,2002). The mechanism, by which plant leaf area is reduced under water stress, is through the reduction of cell elongation, which leads to the reduction of cell size and therefore the reduction of leaf area (Schuppler *et al*., 1998).

Serving as interfaces between plant and the soil, roots are much more exposed to drought stress than the upper plant parts. Therefore, the root system can be as affected, or even more affected, than the aerial parts of the plant for drought stress (Franco *et al*., 2011). Drought-tolerant genotypes in general extracted more soil moisture from the entire soil profile compared to the susceptible types, despite minor variations. Present study reports higher root biomass under water deficit situation (Figure.2) .This agrees with the results of Franco *et al* (2008) where he found increased branching of the roots, root surface area and total root length of *Silene vulgaris* plants under moderate drought-stress. This can minimize localized water depletion around roots, thus minimizing resistance to water transport to the root system (Franco *et al*., 2006). The root growth of coconut genotypes may shift to deeper sites in response to dehydration of superficial soil layers. Tall genotypes showed greater ability to produce deeper roots under water stress (Cintra *et al*., 1993) and found to have better ability to extract soil moisture from entire soil profile. Moreover, in present experiment S.F hybrids was most successful in producing maximum roots under 50 % F.C which might be one of its drought tolerant mechanisms.

Shoot growth is very sensitive to water-limited conditions. Shoot growth can be so sensitive to soil drying that substantial inhibition can occur before the

development of decreased water potentials in the aerial plant parts (Saab & Sharp 1989; Gowing, Davies & Jones 1990). Acclimatory changes in the root : shoot ratio or the temporary accumulation of reserves in the stem (Rodrigues et al., 1995) under water deficit are accompanied by alterations in carbon and nitrogen metabolism, the fine regulation of which is still largely unknown (Pinheiro *et al*., 2001). The root: shoot ratio of unstressed plants from this study was similar to those found by Benjamin *et al* (2014) in *Zea mays* in which shoot growth was proportionately more affected than root growth, leading to an increased root: shoot ratio as stress increased. In perennial plants like coconut when leaves have to withstand drought, the dissipation of excitation energy at the chloroplast level through processes other than photosynthetic C-metabolism is an important defense mechanism, which is accompanied by down-regulation of photochemistry and, in the longer term, of photosynthetic capacity and growth.

Water stress have a negative influence on carbon assimilation and biomass accumulation (Bohnert and Sheveleva, 1998). Nevertheless, carbon assimilation at the whole plant level always decreases as a consequence of limitations to CO_2 diffusion in the leaf, diversion of carbon allocation to non-photosynthetic organs and defense molecules, or changes in leaf biochemistry that result in the down-regulation of photosynthesis. Current study on coconut seedlings had reported a substantiate decrease in total dry matter production in stressed plants compared to the unstressed plants (Figure 2). Similar findings were given by Seng K.H (2014) in tomato plants, Ohashi *et al* (1999) in legumes, Nautiyal *et al* (2002) in groundnut and Sundaravalli *et al* (2005) in Albizzia seedlings where they found considerable decrease in biomass production under water stress situations. Decreased total DW may be due to the considerable decrease in plant growth, photosynthesis and canopy structure as indicated by leaf senescence during drought stress in *Abelmoschus esculentum* (Bhatt and Rao 2005).

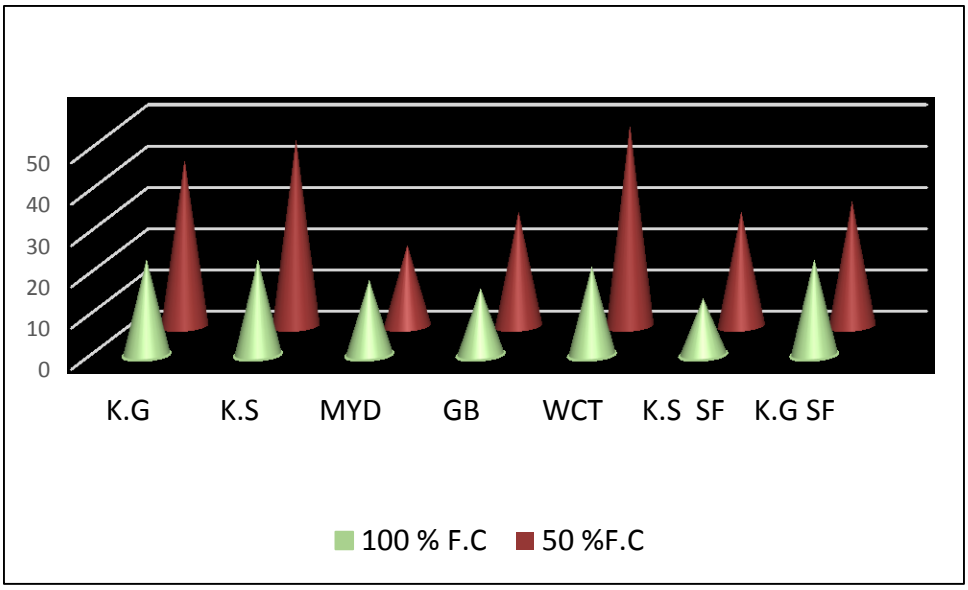

Fig. 1 Effect of water stress on Phyllochron of coconut, number of days

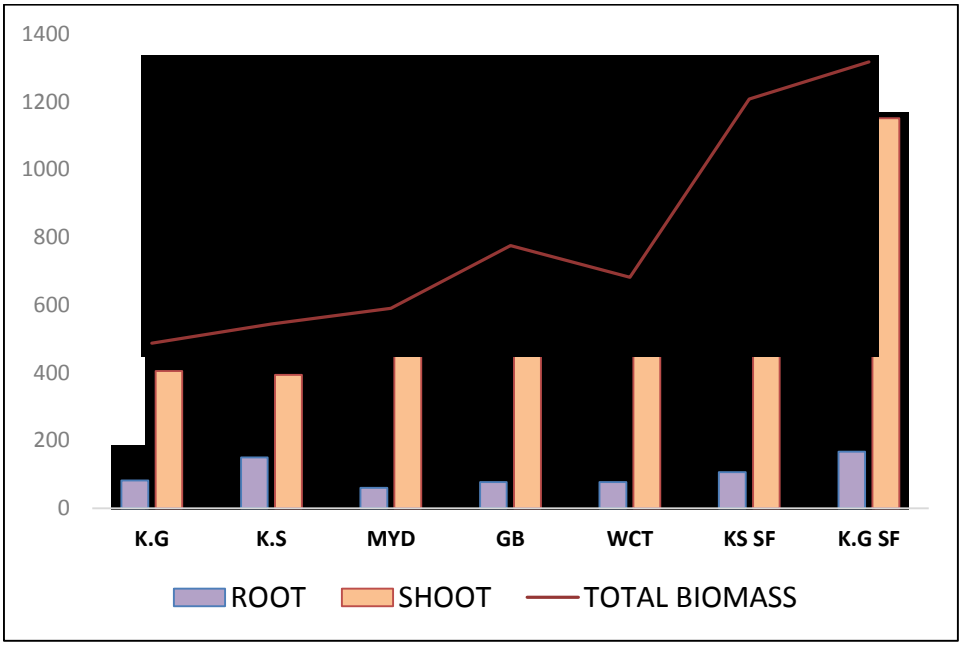

Fig.2 Effect of water stress on Root, Shoot and Total Biomass, g

Growth analyses are considered an appropriate first step for gaining understanding of the physiological basis of growth (Lambers, 1998). A commonly used ecological concept considers growth processes as the intrinsic rate of increase of the initial growth: the relative growth rate (RGR), which is related to the carbon economy of plants and integrates physiological processes, morphology, biomass allocation and leaf composition in one formula (Poorter and van der Werf, 1998). The RGR and its contributing component, the net assimilation rate (NAR), were compared to analyze adaptation mechanisms of plants. A large body of literature depicts the relationship between RGR and the photosynthesis and growth influencing factors: light (intensity, irradiance), nutrient supply, CO2 level, and soil moisture (Antunez *et al*., 2001; Poorter, 1989; Poorter and de Jong, 1999; Shipley, 2002). Relative growth rate and net assimilation rate as investigated in the current study reveals a marked decrease in all coconut genotypes under water deficit situation. These results are similar to the findings of Prado *et al.* (2001) and Gomez *et al.* (2007, 2008) where they reported impairment of carbon assimilation rate in tall and dwarf palms respectively in response to soil water deficit. Such responses can be explained by reduction in gaseous exchange and growth of expanding tissues by reduced cellular expansion.

5.2 PHYSIOLOGICAL PARAMETERS

By being environmentally controlled gateways into the plants controlling CO_2 uptake and transpiration stomata are central determinants of photosynthesis, cooling and nutrient uptake (Farooq *et al*., 2009). To be able to balance CO_2 uptake and water transpiration through stomatal movement is therefore an important response to changes in the environmental conditions. All the coconut varieties included in the current experiment exhibited a wide range of values for stomatal frequency and stomatal index. Manthriratna & Sambasivam (1974) also reported that stomatal densities of varieties and forms of the coconut palms could be a varietal characteristic. Over 3 months of study, stomatal frequencies among varieties decreased within a range of 2-26% under stress situation (Figure 3 a). Selectively fertilized hybrids in particular reported a lesser

stomatal frequency under water deficit situation. These results agree with the findings of Solangi *et al.* (2010) where they conducted stomatal studies of different coconut varieties and later inferred that less stomatal frequency may be used as a parameter to screen the variety for drought tolerance in coconut. Decreased stomatal conductance reported in the present experiment for all the coconut genotypes under water deficit treatment is stated by the decreased stomatal frequency (Figure 3 b). These results are similar to the findings of Jayasekara *et al.* (1996), where they reported lower level of stomatal conductance of fifteen year old coconut palms under soil water deficit situations. The model proposed by Cowan (1982) suggests that variations in stomatal conductance in response to changes in environmental variables could be explained in terms of maximizing assimilation and minimizing transpiration. The decrease in stomatal conductance and concurrent net photosynthesis during mid-day closure of stomata is a regulatory mechanism imposed in response to the short- term stress condition.

Lowered stomatal conductance under water limited situation due to partial stomatal closure gives an indirect clue on reduced rate of transpiration as both water vapour and gaseous exchange occurs through the same pivotal gate in epidermis *i.e.* stomata. The rate of transpiration is directly related to the gradient of water vapour concentration in the intercellular spaces of the leaf and the ambient air. Reduction in leaf area as an early response to water deficit allow plants to reduce transpiration thus increasing water use efficiencies (WUE) (Xu and Zhou, 2005; Monclus *et al.*, 2006; Aguirrezabal *et al.*, 2006). In this experiment, lower values of cumulative water transpired and reduced rate of transpiration under 50 % F.C compared to that under 100 % F.C by selectively fertilized hybrids reveals their water use efficient nature. However, dwarf genotypes like MYD and Gangabondam consumed comparatively large quantities of water with a higher transpiration rate. High E in dwarf genotypes resulted in elevated consumption of water as compared to other varieties and hybrids. Tall varieties, in contrast, show a more conservative water use (Voleti *et al*., 1993).

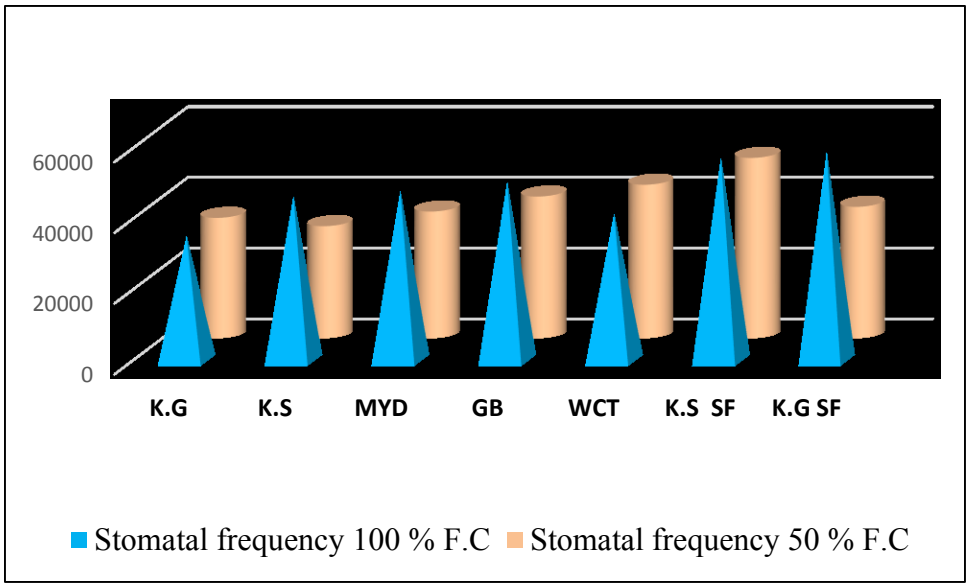

Fig. 3 a) Effect on water stress Stomatal frequency, number of stomata cm^{-2}

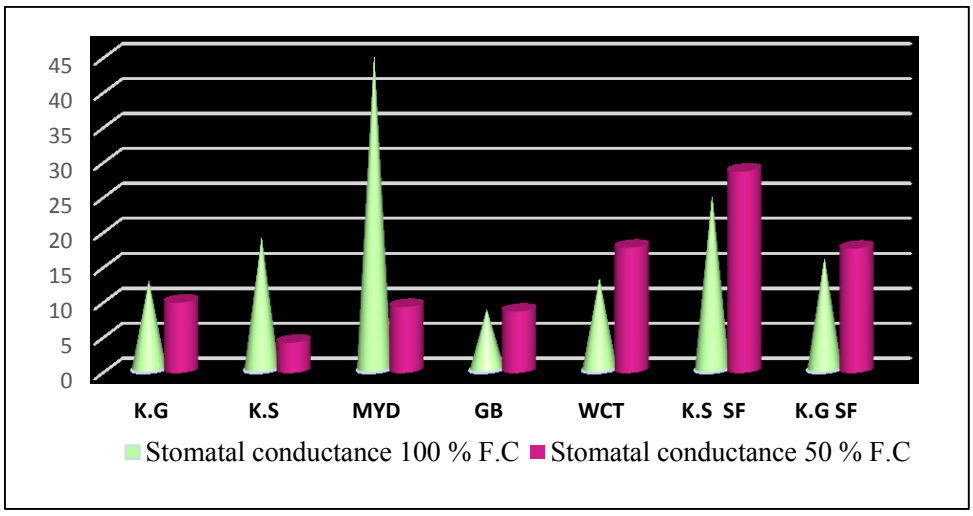

Fig .3 b) Effect on water stress on Stomatal conductance, milli moles m^{-2} s^{-1}

Kasturi Bai *et al.* (1997) observed that West African Tall (WAT) behaves relatively better than the hybrids under drought conditions, due to lower gs (0.10 mol m^{-2} s^{-1}) and, as a consequence, improved tissue water conservation.

The ion leakage is an indicator of cell membrane stability and integrity, which is commonly considered as one of the best physiological components of drought tolerance in plants (Kocheva *et al*., 2004; Xu *et al*., 2008). 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 (Repellin *et al*., 1994). Membrane integrity status of coconut cultivars in present study is expressed as percentage leakage of solutes which on lower level upon water stress reveals a drought tolerant and water use efficient character. In this experiment, dwarf variety Malayan Yellow Dwarf shows highest membrane integrity under stress compared to other genotypes. These results are justified by works of Gomez *et al* (2010), where they reported higher membrane stability in drought tolerant Brazilian green dwarf coconut genotypes subjected to drought stress.

Water potential in a plant, which is the energy-level of water, is controlled by the availability of water from the soil, the demand of water imposed by the atmosphere and the resistance to water movement within the plant. Maintenance of a higher leaf water potential under stress conditions is a desirable trait, as that would enable tissues to maintain favourable metabolic activities to withstand desiccation. Genotypic variation in plant recovery from dehydration, as a measure of tolerance, was positively correlated with plant water status (i.e. RWC) retained during desiccation. Among different coconut genotypes studied, Kerasree S.F with its highest relative water content in its leaves in water stress situation proved to be water use efficient (Figure 4). Also, the Kerasree S.F transpired lowest volume of water among the coconut genotypes which also helped to maintain a sufficient leaf water status under stress situation. As reported by Rajagopal and Kasturi Bai (2002), maintenance of high leaf water status is the basis of drought tolerance mechanisms in coconut.

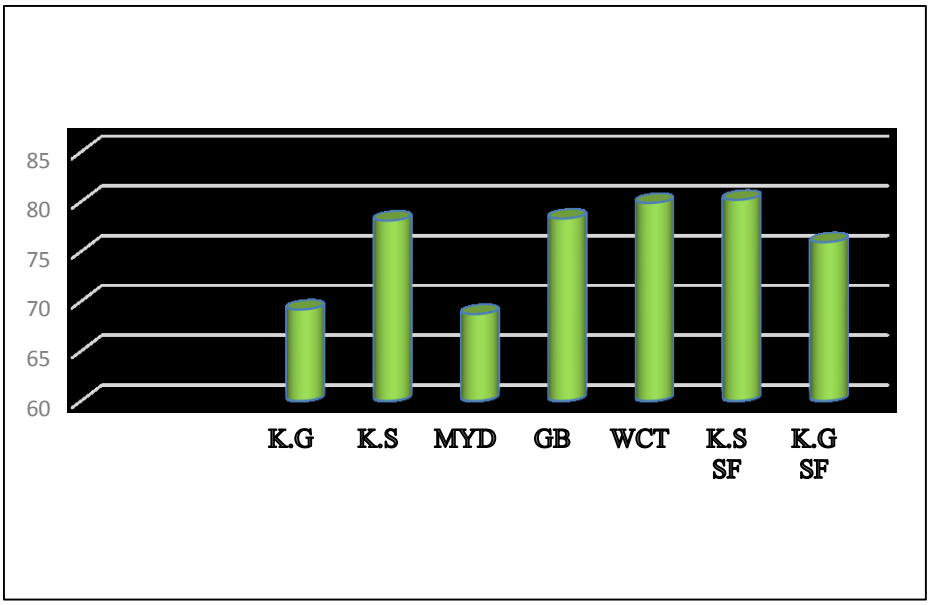

Fig 4. Effect of water stress on Relative water content, percentage

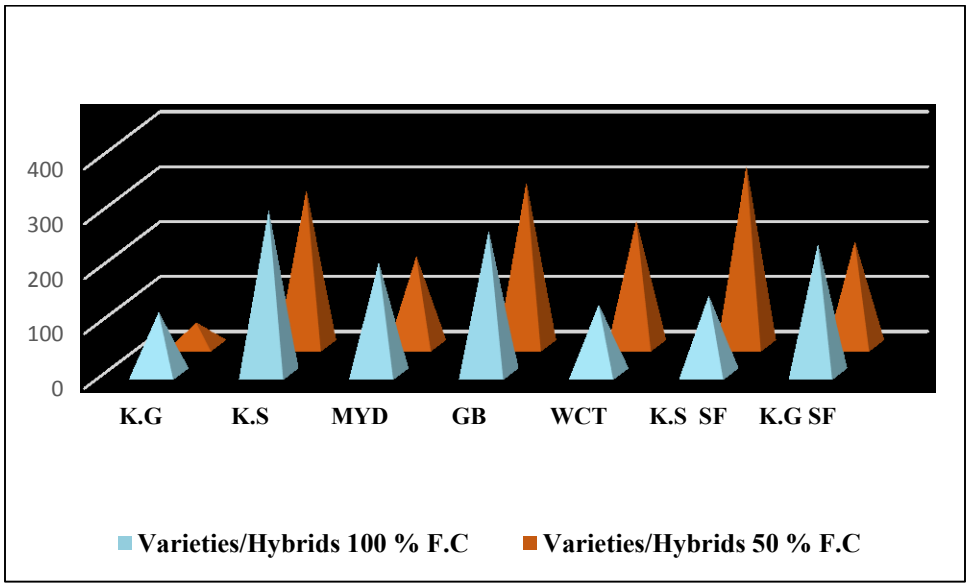

Fig 5. Effect of water stress on Proline accumulation, μ moles g^{-1}

As that of membrane integrity, chlorophyll stability index is also found to be correlated with drought tolerance (Kaloyereas, 1958). High values of CSI as observed in current study for certain coconut genotypes indicate that their leaf chlorophyll content is less affected by water stress of 50 % F.C and hence proves to be water use efficient. Similar high values of CSI was reported in drought tolerant chickpea genotypes by Rahbarian *et al.* (2011),when they studied the effect of drought stress in tolerant and susceptible chickpea genotypes.

Formation of a wax layer on the leaf surface is an adaptive mechanism to withstand water deficit situations. Kurup *et al* (1993) observed a clear indication of a negative relationship between the epicuticular wax content (EWC) of leaves and transpiration rate. In the present study, under water deficit situation all coconut varieties reported a markable increase in wax deposition up to second month. This result coincides with the findings of Rajagopal *et al* (1988) which specifies 32% increase deposition of epicuticular wax in coconut palms subjected to severe water stress as compared to the well watered palms. However, during the third month (Dec-Jan), reduction in wax deposition might be due to the favorable weather experienced in the experimental area. This observation can be justified by the reports from Sutter & Langhans (1979, 1982) which mention that low light intensity and high relative humidity can result in reduced wax deposition

5.3 BIOCHEMICAL PARAMETERS

Coconut leaves have highly efficient systems that protect cell membranes and their intracellular components which forms the basis of imparting drought tolerance under drought stress. Photosynthesis, the most complex process involved in the synthesis of glucose in all plant species is greatly influenced by chlorophyll composition and any alteration in composition and quantity of these pigments from the desired level under stress readily affects the photosynthetic rate of the plant. Here in this study, a decline in total chlorophyll content was noted in all the coconut varieties under water limited situation. This is in agreement with the results of

Madhusudhan and Sudhakar (2014), where they reported decreased chlorophyll content in groundnut cultivars under water stress. The decrease of chlorophyll under water stress may be also due to decreased rate of its synthesis or enhanced chlorophyllase activity (Drazkiewicz, 1994). Vyas (2001) found chlorophylls are closely associated with drought tolerance and suggested that these parameters as biochemical markers for the identification of drought tolerant genotype in cluster bean.

Water stress has a profound effect upon plant metabolism, and results in a reduction in protein synthesis. According to Martignone *et al.* (1987), soluble protein content was the first nitrogenous compound affected under stress conditions, which at severity got denatured and lost its activity and also decreased the photosynthetic efficiency. The results of the present study revealed a drastic decrease of 95-97 % in soluble protein content of coconut seedlings under moderate water stress situation. Similar results were 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. The reduction in quantity of soluble proteins observed in present experiment can be related to the reduced rate of protein biosynthesis and increased breakdown of protein under limited environment (Ranieri *et al.,* 1989; Good and Zaplachinski, 1994; Bolarin *et al*., 1995). As suggested by earlier workers, protein degradation might be the result of increased activity of protease or other catabolic enzymes, which were activated under drought stress, or due to fragmentation of proteins due to toxic effects of reactive oxygen species resulting in reduced protein content (Davies 1987).

Proline accumulation in leaves of drought-stressed plants has been abundantly documented (Pagter *et al.,* 2005; Türkan *et al*., 2005). In the current study, remarkable increase in proline accumulation was seen in all coconut seedlings under water stress irrespective of varieties (Figure 5). This is in agreement with the findings of Jayasekara *et al.* (1993) which reported high levels of proline in leaves of tolerant coconut genotypes during the dry season. However, proline accumulation noted in Kerasree S.F

was sufficiently higher to maintain lowest osmotic potential among all genotypes, and thereby retaining highest leaf water status under water stress situation. Proline accumulation due to water stress results from a stimulated synthesis, inhibited degradation or an impaired incorporation of proline into proteins (Heuer, 1999) and conferring drought tolerance than in acting as a simple osmolyte (Szabados and Savoure, 2009). It may protect proteins structure by maintaining their structural stability (Rajendrakumar *et al*., 1994), act as free radical scavenger (Reddy *et al.,* 2004), as well as, be involved in the recycling of NADPH + H+ via its synthesis from glutamate (Hare and Cress,1997). Proline has been demonstrated to confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than increasing osmotic adjustment (Vendruscolo *et al.,* 2007).

Apart from proline as discussed, other osmolytes studied under this experiment that includes free amino acids, starch, sugar and phenol which had also presented a wide variation between two different water regimes. All kinds of compatible solutes increased under water deficit treatment irrespective of the seedling genotypes. Similar results of heightened accumulation of free amino acids in coconut in response to drought stress is well documented by Kasturi Bai and Rajagopal (2000). Strogonov (1964) attributed that the accumulations of amino acids may be due to the hydrolysis of proteins. Increased free amino acid may be related to decreased protein concentration under 50 % field capacity .Moreover, elevated starch and sugar accumulation noticed in this study under the water stress situation might be the part of osmotic adjustment in coconut seedlings which is similar to the findings of Lakmini *et al* (2006) in coconut and El-Sharkawy and Cock (1987) in Cassava.

Water stress is inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly O^{2-} and H_2O_2 in chloroplasts, mitochondria, and peroxisomes. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy.

POX is a major enzyme scavenging H_2O_2 in chloroplasts produced through dismutation of O_2-catalyzed by superoxide dismutase. In current experiment, reasonable increase in catalase and peroxidase activity was noticed in water use efficient genotypes like Kerasree S.F which might have helped these hybrids to recover from oxidative stress under 50 % F.C (Figure 6). According to Sudhakar *et al.* (2001), increase in CAT and POX activity is supposed to be an adaptive trait possibly helping to overcome the damage to the tissue metabolism by reducing toxic levels of H_2O_2 produced during cell metabolism and protection against oxidative stress.

The ability of plants to overcome oxidative stress partly relies on the induction of SOD activity and subsequently on the up regulation of other downstream antioxidant enzymes (Alscher *et al.* 2002). In our experiment, the results showed significantly enhanced SOD activity in coconut seedlings exposed to water stress (Figure 7). According to this fact that SOD processing is known to be substrate inducible (Tsang *et al*., 1991), an increase in the SOD activity may be attributed to the increased production of active oxygen species as substrate that lead to increased expression of genes encoding SOD. Our results are consistent with other studies reporting the increased SOD activity in response to drought stress in sunflower (Gunes et al. 2008), poplar (Xiao *et al.,* 2008), cowpea (Manivannan *et al*. 2007), liquorice (Pan *et al*., 2006), and pea (Malecka *et al*., 2001) which plants use to overcome oxidative stresses (Foyer & Noctor, 2003).

5.4 WATER USE EFFICIENCY & CARBON ISOTOPE DISCRIMINATION

Zhang *et al* (2004) defines WUE as the ratio of biomass yield to crop evapotranspiration, and is expressed in units of kilograms per cubic meter of water. As discussed, screening for water use efficient coconut palms relies on biometric (specific leaf area, biomass accumulation etc.), physiological (transpiration rate, relative water content, stomatal behavior etc.) and biochemical (osmotic adjustment, antioxidants etc.) analyses.

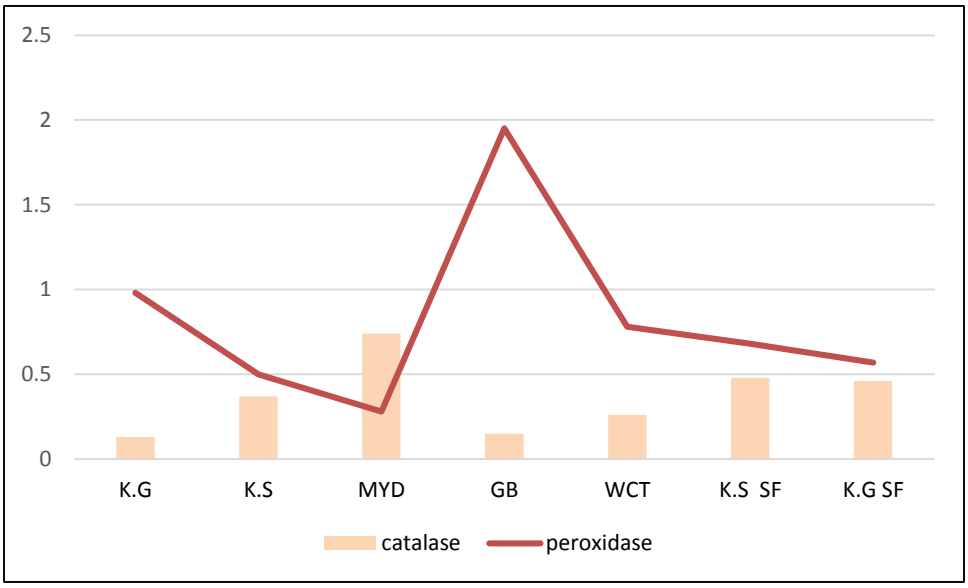

Fig 6. Activity of Catalase and Peroxidase under water stress,activity $g^{-1}min^{-1}$

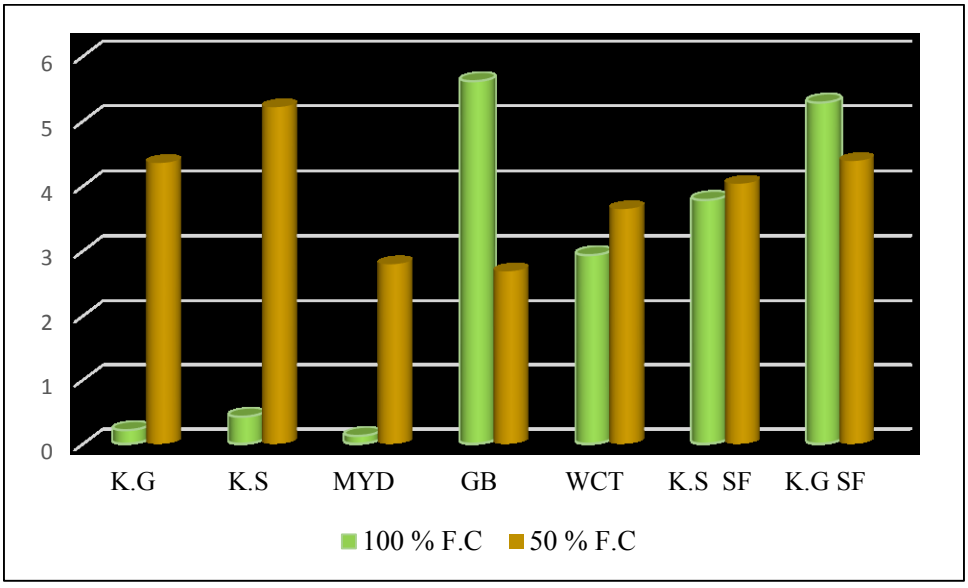

Fig 7. Effect of water stress on SOD activity, activity $g^{-1}min^{-1}$

Work on drought tolerance in coconut at the Central Plantation Crops Research Institute (CPCRI), Kasargod indicated variability for WUE, dry matter production and yield in coconut cultivars (Rajagopal *et al*., 1989; Kasturi Bai *et al*., 1996). Thus, it should be possible to identify high WUE types with a capacity for high biomass production in order to develop water use efficient coconut types, i.e. with drought tolerance and high yield. Different genotypes of coconut used in the present study which included tall, dwarfs and hybrids performed in varied pattern under both treatments in accumulating biomass and transpiration ;thereby exhibiting a markable variation in water use efficiency. Gomes *et al*., (2002) reported that WUE has been shown to vary among varieties and also among ecotypes of the same variety in coconut. Estimation of WUE for selectively fertilized hybrids in this study revealed a reliable value compared to tall and dwarfs (Figure 8). This may be attributed to the increased biomass accumulation and relative decrease in water transpired. However, WCT which is generally considered as drought tolerant, had less dry matter accumulation under stress when compared to S.F hybrids. Plate 5 shows a comparison of total dry matter accumulated in S.F hybrids with that of WCT.

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 (Hubick *et al*., 1986, Martin and Thorstenson 1988, Johnson *et al*., 1990). In current experiment for evaluating water use efficiency through stable isotope discrimination, Kerasree S.F which reports lowest discrimination value for carbon isotope can be considered as the most water use efficient. This coincides with the gravimetric data for WUE which also reveals Kerasree S.F as the best water use variety under 50 % F.C.

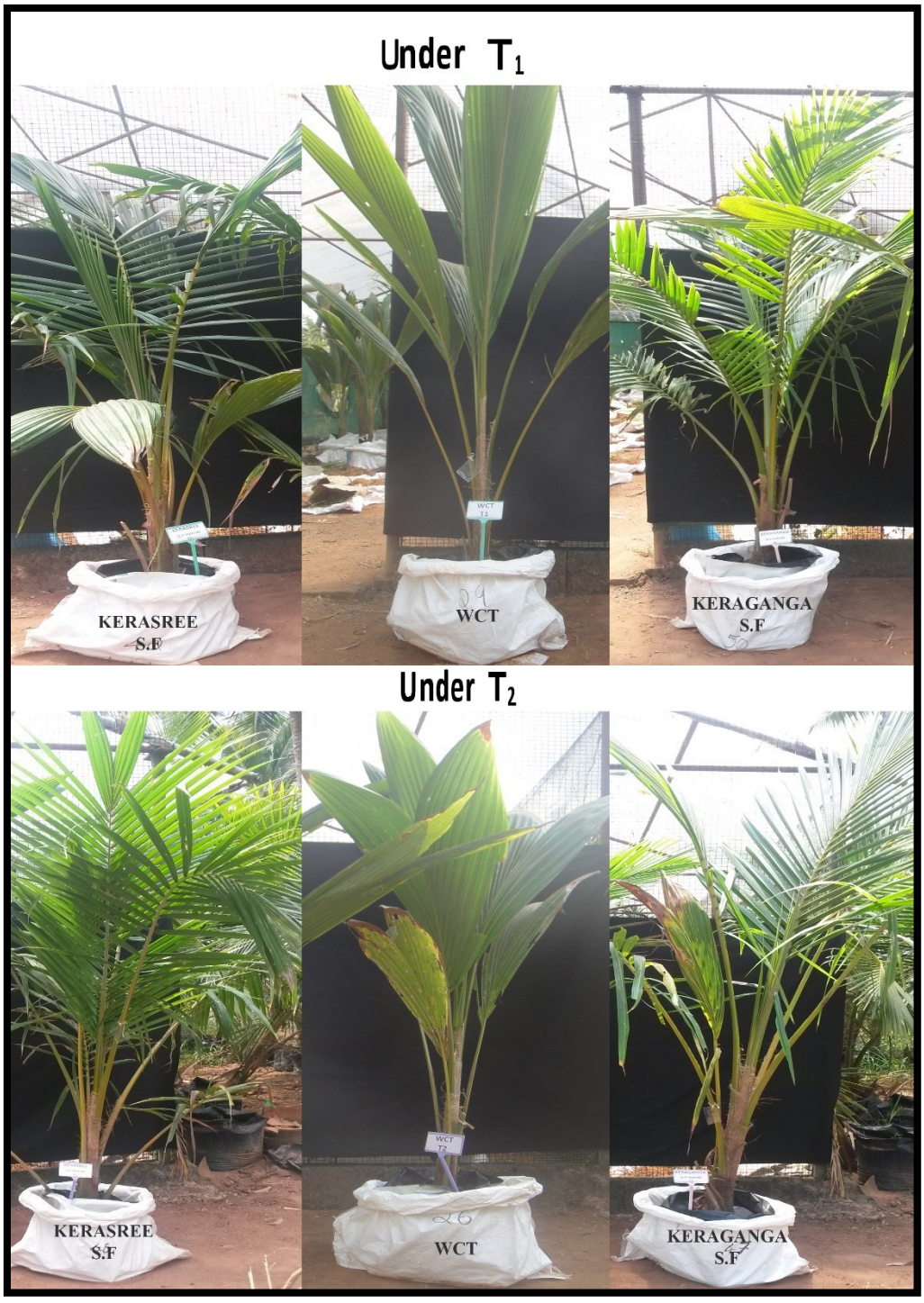

Plate 5. Comparison of biomass accumulation of S.F hybrids and WCT under T_1 and T_2

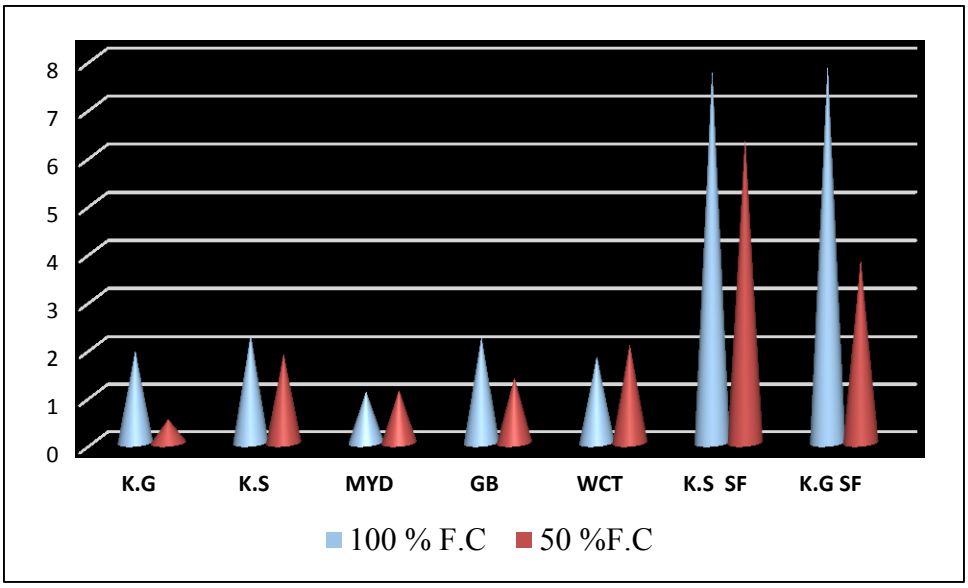

Fig 8. Effect of water stress on water use efficiency, g kg^{-1}

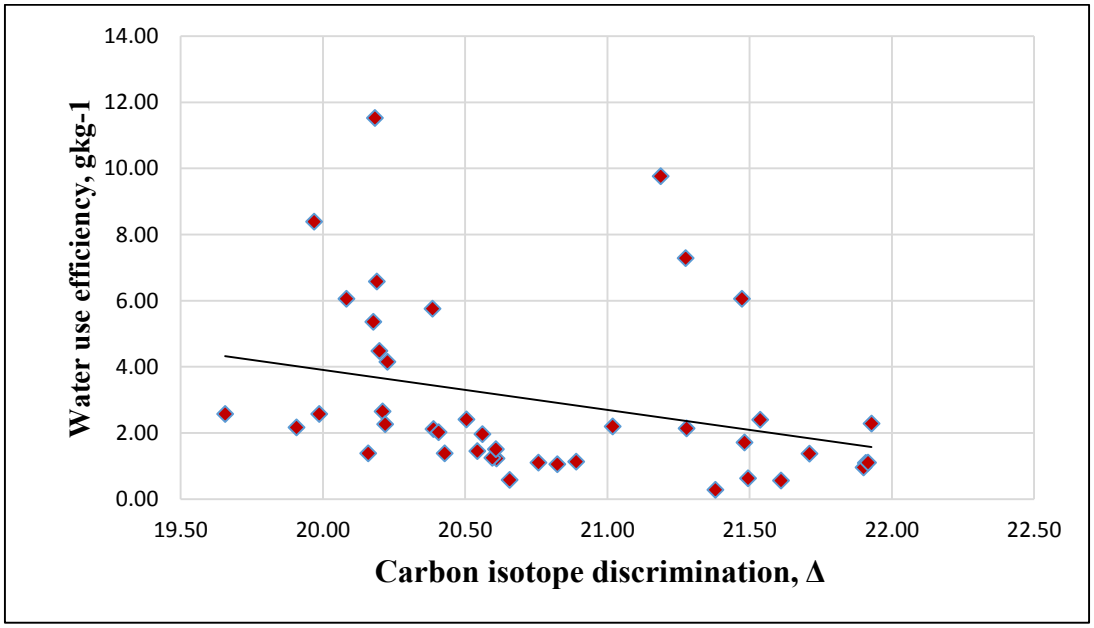

Fig 9. Relation between Water use efficiency and Carbon isotope discrimination

Similar results were previously reported by Adiredjo *et al* (2014) in sunflower and Li C (1999) in *Eucalyptus microtheca.* Lower values of Δ13C under soil water deficit (Farquhar and Richards, 1984; Ehdaie *et al.,* 1991; Condon *et al*., 1992: Merah *et al*., 1999), can be mainly due to the stomatal closure, and thereby causing *ci/ca* to be lower.

Low delta is reasonably correlated with high TE (Hall *et al.,* 1994). Delta was therefore taken to represent crop WUE (Condon *et al*., 2002).From the data obtained for WUE and delta in the current experiment, a correlation study indicates a negative relation in coconut for these parameters under stress which is presented in Figure 9. Negative correlation between WUE and carbon isotope discrimination was reported in sunflower (Lauteri *et al*., 1993) and *Eucalyptus microtheca* (Li C.1999).

Summary

6. SUMMARY

The coconut palm known as *Kalpa vriksha* is of great antiquity in India. Every part of the tree is useful to man and it provides livelihood for millions of people in the country. The palm is amenable to both plantation and homestead management and it can be either a major crop or a minor one in a homestead garden of mixed crops. With coverage of 8.2 lakh ha, coconut occupies 38 per cent of the net cropped area and provides livelihood to over 3.5 million families in Kerala. At present, coconut production in Kerala is descending due to various biotic and abiotic stresses which is one of the major concerns of coconut growers.

Drought is the second most important factor reducing the productivity of coconut next to dreaded root (wilt) disease. Adverse effect of drought at any given time persists for the subsequent two to three years in coconut. This assumes greater significance in Northern Kerala, where there will be regular drought from Dec-May every year. Since 90 per cent of the area under coconut is rainfed in the state, any attempt to enhance the water stress tolerance of the crop will improve the productivity. Only way towards increasing coconut production in drought affected areas is through development of drought tolerant varieties and hybrids. This study is an attempt to evaluate various coconut genotypes for water use efficiency through a novel technique of stable isotope discrimination with emphasis to selectively fertilized coconut hybrids of Kerasree and Keraganga. In the present programme, efforts were made to analyze any relation between gravimetric data of water use efficiency and carbon isotope discrimination in coconut. Moreover, various water stress tolerance mechanisms working in coconut under moderate water stress were studied to evaluate performance of selectively fertilized coconut hybrids. The salient findings of the study are summarized below.

Among all genotypes evaluated, selectively fertilized hybrids of Kerasree and Keraganga were significantly superior for total dry matter accumulation under water deficit situation, besides transpiring sufficiently large volume of water. An average of 14 % increase in total dry matter was noted for S.F hybrids which was the chief reason

behind their water use efficient behavior. Though there was significant reduction for net assimilation rate in all coconut genotypes under moisture deficient regime, least reduction of 39 % was noticeable for Kerasree S.F.

Situation of moderate water stress caused a significant fall in leaf water status of all coconut genotypes under evaluation. However this decrease in relative water content were lowest for Kerasree (1%) and Kerasree S.F (2%) compared to control; and Keraganga and Keraganga S.F failed to retain a higher leaf water status under water stress compared to Kerasree genotypes with a decline of 18 % and 14% respectively with respect to control. Percentage leakage of solutes which defines the integrity of membrane system under stress situation was significantly lowest in Kerasree S.F (2 %) when compared to Keraganga (69 %) and MYD (39 %) whose membrane systems were highly unstable. In all coconut types studied, it was noticed that a water stress of 50 % F.C was low enough to cause any significant damage to chlorophyll and this is evident from the chlorophyll stability index range of 0.1-0.7 % under 50 % field capacity.

Water use efficiency being the function of total dry matter accumulated and cumulative water transpired, S.F hybrids proved to be the most water use efficient compared to other varieties and hybrids on account of maximum dry matter accumulation. Results of carbon isotope discrimination was negatively correlated with gravimetric data of WUE so that genotype with highest water use efficiency (Kerasree S.F) marks the lowest discrimination value for C^{13}.

Reduction in total chlorophyll content under 50 % F.C was however high in S.F hybrids compared to normal hybrids and dwarfs. Under water deficit treatment, GB and WCT have maintained a high metabolic efficiency by preventing much degradation of total soluble protein and as such a least reduction of 2 % and 4 % were noticed respectively. An increase of 129 % in concentration of proline under water stress was noted in selectively fertilized hybrid of Kerasree which was highly remarkable. Also the activity of enzymatic antioxidants like catalase and superoxide dismutase were also noticeable in all coconut genotypes especially S.F hybrids under stress situation.

Genetic variation for ^{13}C discrimination was studied in 31 coconut genotypes that includes Talls, Dwarfs and Hybrids and the data revealed a discrimination range of (18.37-21.07).

Water use efficiency generally estimated through the method of gravimetry which is quite laboursome. Among the coconut varieties evaluated for WUE, Kerasree S.F was found to be the most water use efficient variety under water deficit treatment, which coincides with its lowest value for C^{13} discrimination. Hence the study revealed that stable isotope discrimination can be used as an alternative for gravimetric method to screen out water use efficient genotypes.

Invitro pollen selection followed by selective fertilization is novel and cost effective approach for the development of drought tolerant varieties and hybrids. This technique is characterized by artificially imposing the desired selective pressure during pollination and fertilization so that the pollen grains which are tolerant to selection pressure only will germinate and fertilize the ovule. Present study proves the efficacy of selective fertilization, which offers great prospects for drought tolerance breeding in perennials.

Future line of work.

Carbon isotope discrimination has thus proved to be useful in determining water use efficiency of coconut genotypes. Besides the isotope of carbon, oxygen isotope can also be assessed for the discrimination process under water deficit situation. This will give a detailed in view of processes underlying discrimination.

Present study was focused only on evaluating seedlings for water use efficiency under moderate water stress without considering any yield attributes. Projecting water use efficiency evaluation through stable isotope discrimination with selectively fertilized hybrids of high yielding parents rather than considering drought tolerant traits alone will give a resourceful data for coconut breeding programme.

Coconut palm is considered to be drought tolerant to moderate water stress up to 6 months. A water stress of 50 % F.C for three months was not high enough to clearly understand the drought tolerance mechanisms working in coconut. Hence, evaluating for water use efficiency under severe water stress situation of 25 % F.C can give a clear understanding of water stress tolerance mechanisms in coconut besides expressing full potential for water use efficiency by tolerant genotypes.

References

Acar, O., Turkan, I., Zdemir, F.O. 2001. Superoxide dismutase and peroxidase activities in drought sensitive and resistant barley (*Hordeum vulgare* L.) varieties. *Acta Physiologiae Plantarum*. 3: 351–356.

Adiredjo, A. L., Navaud, O., Muños, S., Langlade, N. B., Lamaze, T., and Grieu, P. 2014. Genetic control of water use efficiency and leaf carbon isotope discrimination in sunflower (*Helianthus annuus* L.) subjected to two drought scenarios. *PLoS one.* 9(7): e101218.

Aguirrezabal, L., Bouchier-Combaud, S., Radziejwoski, A., Dauzat, M., Cookson, S.J., Granier, C. 2006. Plasticity to soil water deficit in *Arabidopsis thaliana*: dissection of leaf development into underlying growth dynamic and cellular variables reveals invisible phenotypes. *Plant Cell Environ*. 29:2216–2227.

Ahmed, C.B., Rouina, B.B., Sensoy, S., Boukhris, M. and Abdallah, F.B. 2009. Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. *Environ. Exp. Bot*. 67: 345-352.

Alavi-Samani, S.M., Pirbalouti, A.G., Kachouei, M.A., and Hamedi, B. 2013.The influence of reduced irrigation on herbage, essential oil yield and quality of *Thymus vulgaris* and *Thymus daenensis*. *J. Herb Drugs*. 4(3): 109-113.

Albert, D.G. and Carberry, P.S. 1993. The influence of water deficit prior to tassel initiation on maize growth, development and yield. *Field Crop Res.* 31: 55-59.

Alcazar, R., Cuevas, J.C., Patron, M., Altabella, T., and Tiburcio, A.F. 2006. Abscisic acid modulates polyamine metabolism under water stress in *Arabidopsis thaliana*. *Physiol Plantarum*. 128: 448–455.

Almeselmani, M., Deshmukh, P.S., Sairam, R.K., Kushwaha, S.R, Singh, T.P. 2006. Protective role of antioxidant enzymes under high temperature stress. *Plant Sci* .171: 382–388.

Alscher, R.G., Erturk, N., Heath, L.S. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress. *J. Exp. Bot*. 53:1331–1341.

Alvarez, S., Navarro, A., Banon, S. and Sánchez-Blanco, M. J. 2009. Regulated deficit irrigation in potted Dianthus plants: effects of severe and moderate water stress on growth and physiological responses. *Sci Hortic*. 122: 579-585.

Amos, B., and WaIters, D.T. 2006. Maize root biomass and net rhizodeposited carbon: An analysis of the literature. *Soil Sci. Soc. Am. J.* 70: 1489-1503.

Ananthi, K., Vijayaraghavan, H., Karuppaiya, M., and Anand, T. 2013. Drought induced changes in chlorophyll stability index, relative water content and yield of cotton genotypes. *Insight Botany*. 3: 1-5.

Antunez, I., Retamosa, E.C., Villar, R. 2001. Relative growth rate in phylogenetically related deciduous and evergreen woody species. *Oecol*. 128:172–180.

Araus, J.L., Villegas, D., Aparicio, N., Garcia Del Moral, L.F., El Hani, S., Rharrabti, Y., Ferrio, J.P., Royo, C. 2003. Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. *Crop. Sci*. 43: 170–180.

Arora A., Sairam, R.K., Sriuastava, G.C. 2002. Oxidative stress and antioxidative system in plants. *Curr. Sci.* 82: 1227–1238.

Asha, S. and Rao, K.N. 2002. Effect of simulated water logging on the levels of amino acids in groundnut at the time of sowing. *Indian J. Plant Physiol.* 7(3): 288-291.

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora* .199: 361–376

Ashraf, M. and Foolad, M.R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot*. 59(2): 207–216

Ashraf, M. and Mahmood, S. 1990. Response of four Brassica species to drought stress. Environ. Exp. Biol. 30: 93-100.

Azevedo, P.V., da Silva, B.B., da Silva, V.P.R. 2003. Water requirements of irrigated mango orchards in northeast Brazil. *Agric*. *Water Manage.* 58 (3): 241–254.

Babu, R.C, Zhang, J.X., Blum, A., Ho, D.T.H., Wu, R., and Nguyen, H.T. 2004. HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. *Plant Sci.* 166: 855–862.

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Lopes, J.I., Goncalves, B.C., Ferreira, T.C., Correia, C.M., 2007. Physiological behaviour, oxidative damage and antioxidative protection of olive trees grown under different irrigation regimes. *Plant Soil.* 292: 1–12.

Baker, J. T., Pinter, P. J., Reginato, R. J and Kanemasu, E. T. 1986. Effects of temperature on leaf appearance in spring and winter wheat cultivars. *Agron. J.* 78: 605-613.

Barrera-Figueroa, B., Pena Castro, J., Acosta-Gallegos, J.A., Medrano, R.R., Cazares, B.X. 2007. Isolation of dehydration responsive genes in a drought tolerant common bean cultivar and expression of group 3 late embryogenesis abundant mRNA in tolerant and susceptible cultivars of bean. *Funct Plant Biol*. 34: 368-381.

Bartels, D. and Sunkar, R. 2005. Drought and salt tolerance in plants. *Crit. Rev. Plant Sci*. 24: 23–58.

Bates, L., Waldren, R.P., Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*. 39: 205-207.

Bayoumi, T.Y, Eid, M. and Metwali, E.M. 2008. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *Afr. J. Biotechnol.* 7: 2341–2352.

Beattie, G. A. and Marcell, L.M. 2002. Effect of alterations in cuticular wax biosynthesis on the physicochemical properties and topography of maize leaf surfaces. *Plant Cell Environ*. 25: 1–16.

Begg J.E and N.C. Turner, 1976, Crop water deficits. *Adv. Agron*., 28:161-217.

Ben Ahmed, C., Ben Rouina, B., Sensoy, S., Boukhris, M., Ben Abdallah, F. 2009.Changes in gas exchange, proline accumulation and antioxidative enzyme

activities in three olive cultivars under contrasting water availability regimes. *Environ. Exp. Bot*. 67: 345–352.

Benjamin, J.G., Nielsen, D.C., Vigil, M.F., Mikha, M.M., and Calderon, F. 2014. Water deficit stress effects on corn (*Zea mays*, L.) root: shoot ratio. *Open. J. Soil Sci*. 4: 151-160

Bhatt, R. M. and Srinivasarao, N. K. 2005.Influence of pod load on response of okra to water stress. *Indian J. Plant Physiol*. 10: 54–59.

Bilger, W., Johnsen, T., and Schreiber, U. 2001. UV-excited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants. *J. Exp. Bot*. 52: 2007–2014.

Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regul*. 20: 135-148.

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential- are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* 56: 1159-1168.

Blum, A. and Ebercon, A. 1981, Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci*. 21: 43–47.

Blum, A., Shpiler, L., Golan, A. and Mayer, J. 1989. Yield stability and canopy temperature of wheat genotypes under drought stress. *Field Crop Res*. 22(41): 289-286.

Bohnert, H. J. and Sheveleva, E. 1998. Plant stress adaptations making metabolism move. *Curr. Opin. Plant Biol.* 1: 267—274.

Bolarín, M.C., Santa Cruz, A., Cayuela, E. and Peréz-Alfocea, F. 1995. Short-term solute changes in leaves and roots of cultivated and wild tomato seedlings under salìnity. *J. Plant Physiol*. 147: 463-468.

Bor, M., Ozdemir, F., and Turkan, I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet (*Beta vulgaris* L.) and wild beet (*Beta maritima* L.). *Plant Sci.* 164: 77–84.

Boughalleb, F. and Hajlaoui, H. 2011. Physiological and anatomical changes induced by drought in two olive cultivars (cv. Zalmati and Chemlali). *Acta Physiol. Plant*. 33: 53-65.

Boutraa, T., Akhkha, A., Al-Shoaibi, A. A., and Alheliji, A.M. 2010. Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabia. *J. Taibah Univ. Sci*. 3: 39-48.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72: 248-254

Bray, E. A. 2002. Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. *Plant Cell Environ*. 25: 153–161.

Brugnoli, E. and Farquhar, G.D. 2000. Photosynthetic fractionation of carbon isotopes. In: Leegood, R.C., Sharkey, T.D., and von Caemmerer, S. (ed.), Advances in photosynthesis: physiology and metabolism. Dordrecht, The Netherlands, Kluwer Academic Publishers, pp. 399–434

Brunel, Simpson, J. H., Herezeg, A., Whitehead, R. and Walkcr, G. 1992. Stable isotope composition of watcr vapor as an indicator of transpiration fluxes from rice crops. *Water Rcsour Res.* 28 (5). 1407 -1416

Cao, H., Sun, C., Sao, H., and Lei, T. 2011. Effects of low temperature and drought on the physiological and growth changes in oil palm seedlings. *Afr. J. Biotechnol*. 10: 2630-2637.

Carter, G.A. 1991. Primary and secondary effects of water content on the spectral reflectance of leaves. *Am. J. Bot*. 78: 916–924.

Castro-Diez, P., Puyravaud, J.P., and Cornelissen, J.H.C. 2000. Leaf structure and anatomy as related to leaf mass per area variation in seedlings of a wide range of woody plant species and types. *Oecol*. 4: 476486.

Cerovic, Z.G, Ounis, A., Cartelat, A., Latouche, G., Goulas, Y., Meyer, S., and Moya, I. 2002. The use of chlorophyll fluorescence excitation spectra for the non-

destructive in situ assessment of UV-absorbing compounds in leaves. *Plant Cell Environ*. 25: 1663–1676.

Ceulemans, R., Praet, L.V., and Jiang, X.N. 1995. Effects of CO2 enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus)*. *New Phytol* .131: 99–107.

Chaitanya, K.Y., Sundar, D. Jutur, P.P., and Reddy, A.R .2003. Water stress effects on photosynthesis in different mulberry cultivars. *Plant Growth Regul*. 40(1): 75-80.

Chandra Babu, R. , Zhang, J., Blum, A., David Ho, T. H., Wu, R. and Nguyen, H.T. 2004. HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. *Plant Sci.* 166: 855–862.

Chaum, S., Takabe, T., and Kirdmanee, C. 2010. Osmotic potential, photosynthetic abilities and growth characters of oil palm (*Elaeis guineensis* Jacq.) seedlings in responses to polyethylene glycol induced water deficit. *Afri. J. Biotechnol*. 9: 6509-6516.

Chaum, S., Takabe, T., and Kirdmanee, C. 2012. Physio biochemical responses of oil palm (*Elaeis guineensis* Jacq.) seedlings to mannitol and polyethylene glycol induced iso-osmotic stress. *Plant Prod Sci*. 15: 65-72.

Chaum, S., Yamada, N., Takabe, T., Kirdmanee C (2011) Mannitol-induced water deficit stress in oil palm (*Elaeis guineensis* Jacq.) seedlings. *J. Oil Palm Res*. 23: 1194-1202.

Chaves, M. M., Maroco, and J.P. Pereira, J.S. 2003. Understanding plant responses to drought-from genes to the whole plant. *Funct. Plant Biol*. 30: 239–264.

Chaves, M.M. 1991. Effects of water deficits on carbon assimilation. *J .Exp. Bot*. 42: 1–16

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

Chen, T. and Creeb, K.H. 1991.Combined effects of drought and salt stress on growth, hydration and pigment composition of cotton. *Field crop Abst*. 44 (8):5819.

Choluj, D., Karwowska, R., Ciszewska, A., and Jasinska, M. 2008. Influence of long-term ´drought stress on osmolyte accumulation in sugar beet (*Beta vulgaris* L.) plants. *Acta Physiol. Plant*. 30: 679–687.

Cintra, F.L.D., Passos, E.E.M., and Leal, .LS. 1993. Evaluation of root system distribution in Tall coconut cultivars. *Oleagineux*. 48: 453-461.

Clarke, N.A., Hetschkun, H.M., and Thomas, T.H. 1996. Stress mechanisms in sugar beet. In: Fenwick, G.R, Hedley, C., Richards, R.L., and Khokhar, S. (eds), Agric Food Quality: An Interdisciplinary Approach, The Royal Society of Chemistry, Cambridge, pp. 75–78.

Condon A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D.2002. Improving intrinsic water-use efficiency and crop yield. *Crop Sci*. 42:122–131.

Condon, A.G., Farquhar, G.D., Richards, R.A. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. *Aust J Plant Physiol*. 17: 9–22.

Condon, A.G., Richards, R.A. and Farquhar, G.D. 1992. The effect of variation in soil water availability, vapour pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. *Aust. J. Agric. Res*. 43: 935-947.

Condon, A.G., Richards, R.A., Rebetzke, G.J., and Farquhar G.D. 2006.The application of carbon isotope discrimination in cereal improvement for water limited environments, Drought Adaptaion in Cereals, The Haworth Press.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D. 2004. Breeding for high water-use efficiency. *J. Exp. Bot*. 55: 2447–2460.

Coopman, R.E., Jara, J.C., Bravo, L.A., Saez, K.L., Mella, G.R., and Escobar, R. 2008. Changes in morpho-physiological attributes of *Eucalyptus globulus* plants in response to different drought hardening treatments. *Electron. J. Biotechn*. 11(2):1-10.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture — not by affecting ATP synthesis. *Trends Plant Sci.* 5: 187-188.

Costa e Silva, F., Shvaleva, A., Maroco, J.P., Almeida, M.H., Chaves, M.M., and Pereira, J.S. 2004. Responses to water stress in two *Eucalyptus globulus* clones differing in drought tolerance. *Tree Physiol*. 24(10): 1165-1172.

Cowan, L. R. 1982. Regulation of water use in relation to carbon gain in higher plants. In: Lange, O.L and J. Bewley, J.D (eds.), *Encyclopedia Plant Physiology* ,VoL 12B,Springer-Verlag, Berlin, , pp. 535-562.

Cramer, G.R., Ergu, A., Grimplet. J., Tillett, R.L., Tattersall, E.A.R, and Bohlman, M.C. 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Funct Integr Genomics*. 7: 111–134.

Cuntz, M., Ciais, P., Hoffmann, G., and Knorr, W. 2003. A comprehensive global three-dimensional model of d18O in atmospheric CO2: Validation of surface processes. *J. Geophys. Res.* 108(D17). 4527p.

Cuy, R.D., Fogel, M., and Berry, J.A. 1993. Photosynthetic fractionation of the stable isotopes of oxygen and carbon. *Plant Physiol*. 101: 37-47.

DaMatta, F.M. 2004. Exploring drought tolerance in coffee: a physiological approach with some insights for plant breeding. *Braz. J. Plant Physiol*. 16: 1–6.

Davies, K.J.A .1987. Protein damage and degradation by oxygen radicals. General aspects. *J. Biochem. Chem*. 262: 9895-9901.

De Costa, W.A.J.M. 2001. Plant water relations: Principles and applications. Printing unit, University of Peradeniya, Sri Lanka.

Dekov, I., Tsonev, T. and Yordanov, I. 2000. Effects of water stress and high temperature stress on the structure and activity of photosynthetic apparatus of *Zea mays* and *Helianthus annuus. Photosynthetica* 38: 361-36.

Dellai, J., Trentin, G., Bisognin, D., and Streck, N. 2005. Phyllochron in different densities of potato plants. *Cienc. Rural*. 35 (6): 1269-1274.

Demming-Adams, B., Adams, W.W. 1993. The xanthophyll cycle, protein turnover, and the high-light tolerance of sun-acclimated leaves. *Plant Physiol*. 103: 1413-1420.

Dias, M. C and Bruggemann, W. 2010. Limitations of photosynthesis in *Phaseolus vulgaris* under drought stress: gas exchange, chlorophyll fluorescence and Calvin cycle enzymes. *Photosynthetica*, 48: 96-102.

Dingkuhn, M., Farquhar, G.D., De D, S.K., O'Toole, J.C., Datta, S.K. 1991. Discrimination of ^{13}C among upland rice having different water use efficiencies. *Aust. J. Agri. Res*. 42: 1123–1131.

Djakovic, T. and Jovanovic, Z. 2002. Effect of N deficiency on leaf growth and cell wall peroxidase activity in contrasting maize genotypes. *Plant Cell Environ*. 25:265-272.

Dole, M., Lane, G., Rudd, D., and Zaukelies, D. A. 1954. Isotopic composition of atmospheric oxygen and nitrogen. *Geochim. Cosmochim. Ac*. 6: 65-78.

Doorenbos, J. and Pruitt, W.O.1977. Crop water requirements. Irrigation and Drainage Paper 24, FAO, Rome.

Drazkiewicz, M. 1994. Chlorophyllase: Occurrence, functions, mechanism of action, effects of external and internal factors. *Photosynthetica*. 30: 321 331.

Ehdaie, B., Hall , A.E., Farquhar, G.D., Nguyen, H.T., Waines, J.G. Water-use efficiency and carbon isotope discrimination in wheat. 1991. *Crop Sci*. 31: 1282-1288.

El Hafid, R., Smith, D.H, Karrou, M., and Samir, K. 1998. Morphological attributes associated with early-season drought tolerance in spring durum wheat in a mediterranean environment. *Euphytica.* 101. 273-282.

El-Juhany, L. I., and Aref, I.M. 2005. Interactive effects of low water supply and high salt concentration on the growth and dry matter partitioning of *Conocarpus erectus* seedlings. *Saudi J. Biol. Sci.* 12(2): 147-157.

El-Juhany, L.I., and Aref, I. M. 1999. Growth and dry matter partitioning of *Leucaena leucocephala* (Lam.) de Wit. trees as affected by water stress. *Alexandria J. Agric. Res.* 44(2): 237-259.

El-Sharkawy, M. A. and Cock, J. H., 1987. Response of cassava to water stress. *Plant Soil.* 100: 345-36.

Elsheery, N.I., and Cao, K.F. 2008. Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. *Acta Physiol. Plant*. 30: 769–777.

El-Tayeb, M.A. and Hassanein, A.M. 2000. Germination, seedling growth, some organic solutes and peroxidase expression of different *Vicia faba* lines as influenced by water stress. *Acta Agron. Hung*. 48: 11-20.

Escalante, F., Gamboa, M.R., Lecher, J.G., Arroyo, G, A., Zizumbo, D., Oropeza, C., and Peña-Rodríguez, L.M .2002. Major components from epicuticular wax of Cocos nucifera. *Rev. Soc. Quim. Mex.* 46:247–250.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra S.M.A. 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev*. 299: 185-212.

Farquhar, G. D., Condon, A. G. and Masle, J. 1994. Use of carbon and oxygen isotope composition and mineral ash content in breeding for improved rice production under favourable, irrigated conditions. In: Cassman, K. G. (ed.), *Breaking the yield barrie*r, Manila, The Philippines: IRRI, pp. 95–101.

Farquhar, G., and Lloyd, J. 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Ehleringer, J.R., Hall, A.E., and Farquhar, G.D. (eds), Stable Isotopes and Plant Carbon-Water Relations. Academic Press, San Diego. pp 47–70.

Farquhar, G., Barbour, M.M., and Henry, B.K. 1998. Interpretation of oxygen isotope composition of leaf material. In: Griffiths, H. (ed.), Stable Isotopes: Integration of Biological, Ecological and Geochemical Processes. BIOS Scientific Publishers, Oxford, pp. 27–62

Farquhar, G.D. and Richards, R.A. 1984. Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. *Aust. J. Plant Physiol*. 11: 539–552.

Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 40: 503–537.

Farquhar, G.D., O'Leary, M.H and Berry, J.A. 1982. On relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol*. 9:121–137.

Foulkes, M.J., Sylvester-Bradley, R., Weightman, R., and Snape, J.W. 2007. Identifying physiological traits associated with improved drought resistance in winter wheat. Field *Crops Res*. 103:11–24.

Foyer, C., and Noctor, G. 2003. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant*. 119: 355–364.

Franco, J. A., Arreola, J., Vicente, M. J. and Martínez-Sánchez, J. J. 2008. Nursery irrigation regimes affect the seedling characteristics of Silene vulgaris as they relate to potential performance following transplanting into semi-arid conditions. *J. Hortic. Sci Biotechn*. 83: 15-22.

Franco, J. A., Martínez-Sánchez, J. J., Fernández, J. A. and Banon, S. 2006. Selection and nursery production of ornamental plants for landscaping and xero gardening in semi-arid environments. *J. Hortic. Sci. Biotechn*. 81: 3-17.

Franco, J.A., Bañón, S., Vicente, M.J., Miralles, J. and Martínez-Sánchez, J.J. 2011. Root development in horticultural plants grown under abiotic stress conditions – a review. *J. Hortic .Sci. Biotechn*. 86: 543-556.

Fu, J. and Huang, B. 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.* 45: 105-114.

Galmes, J., Cifre, J.J., Medrano, H., Flexas, J. 2005. Modulation of relative growth rate and its components by water stress in Mediterranean species with different growth forms. *Oecol*. 145: 21–31.

Galmés, J., Medrano, H., and Flexas, J. 2007. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytol*. 175: 81-93.

Garcia-Plazaola, J.I, Becerril, J.M. 2000. Effects of drought on photoprotective mechanisms in European beech (*Fagus sylvatica* L.) seedlings from different provenances. *Trees.* 14: 485–490.

Gaspar, T., Penel, C., Hagege, D., and Greppin, H. 1991. Peroxidases in plant growth, differentiation, and development processes. In: Łobarzewski, J., Greppin, H., Penel, C., and Gaspar, T. (eds.), *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases*. University M. Curie Skłodowska, Lublin, pp 249–280.

Gholinezhad, E., Aynaband, A., Hassanzade, G.A., Noormohamadi, G., and Bernousi, I. 2012. Effect of drought stress on grain yield, quality traits, phyllochron and leaf appearance rate of sunflower hybrid Iroflor at different levels of nitrogen and plant population. *Am-Euras. J. Agric. & Environ. Sci*. 12(3): 306-314.

Gigon, A., Matos, A., Laffray, D., Zuily-fodil, Y., and Pham-Thi, A. 2004. Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (Ecotype Columbia). *Ann. Bot*. 94: 345–351.

Gomes, E.P., Oliva, M.A., Mielke, M.S., Almeida, A.A.F. and Aquino, L.A. 2010. Osmotic adjustment, proline accumulation and cell membrane stability in leaves of Cocos nucifera submitted to drought stress. *Sci. Hort.* 126: 379-384.

Gomes, F. P., Mielke, M. S., Almeida, A-AF and Muniz, W. S. 2002. Leaf gas exchange in two dwarf coconut genotypes in the southeast of Bahia State, Brazil. *Coconut Res. Dev*. 18:37–55.

Gomes, F.P. 2006. Gas exchange in green dwarf coconut subjected to water stress cycles. PhD thesis. Lush, Federal University of Viçosa.

Gomes, F.P., Oliva, M.A., Mielke, M.S., Almeida, A-A.F, Leite, H.G, and Aquino, L.A. 2007. Photosynthetic limitations in leaves of young Brazilian Green Dwarf coconut (Cocos nucifera L. 'nana') palm under well-watered conditions or recovering from drought stress. *Environ. Exp*. *Bot*. in press:

Gomes, F.P., Oliva, M.A., Mielke, M.S., Almeida, A-A.F., Leite, H.G. 2006. Photosynthetic irradiance-response in leaves of dwarf coconut palm (*Cocos nucifera* L. 'nana', Arecaceae): Comparison of three models. *Sci. Hort*.109:101-105.

Gomes, F.P., Oliva, M.A., Mielke, M.S., Almeida, A-A.F., Leite, H.G., Aquino, L.A., 2008. Photosynthetic limitations in leaves of young Brazilian Green Dwarf coconut (*Cocos nucifera* L. 'nana') palm under well-watered conditions or recovering from drought stress. *Environ. Exp. Bot*. 62: 195–204.

Gomes, F.P., Oliva, M.A., Mielke, M.S., Almeida, A.F., and Aquino, L.A.2010. Osmotic adjustment, proline accumulation and cell membrane stability in leaves of Cocos nucifera submitted to drought stress. *Sci Hort.* 126(3): 379—384.

Gomes, F.P., Prado, C.H.B.A., 2010. Coconut palm: ecophysiology under water deficit conditions. In: Damatta, F.M. (ed.), *Ecophysiology of Tropical Tree Crops*. Nova Science Publishers Inc., New York, pp. 151–179.

Gomes, F.P.; Prado, C.H.B.A. 2007. Ecophysiology of coconut palm under water stress. *Braz. J. Plant Physiol*. 19(4): 377-391.

Gomes, M.S. and Rangasamy, P. 2002. Correlation and path analysis of yield and physiological characters in drought resistance rice. *Int. J. Mendel*, 19: 33-34

Gong, H., Zhu, X., Chen, K., Wang, S., and Zhang, C. 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci.*169:313–321.

Good, A., and Zaplachinski, S. 1994. The effect of drought stress on free amino acids accumulation and protein synthesis in *Brassica napus*. *Physiol. Plantarum*. 90: 9-14.

Gowing, D.J.G., Davies, W.J. and Jones, H.G. 1990. A positive root-sourced signal as an indicator of soil drying in apple, *Malus x domestica* Borkh. *J. Exp. Bot*. 41: 1535–1540.

Greenway, H., and Munns, R. 1980. Mechanism of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol*. 31: 149-190.

Gregory, P.J. 2006. Roots and the Physico-Chemical Environment. In: Gregory, P.J., (ed.), *Plant Roots: Growth, Activity, and Interaction with Soils*. Blackwell Publishing, Oxford, 131-173.

Gucci, R., Massai, R., Xiloyannis, C., and Flore, J.A .1996. The effect of drought and vapour pressure deficit on gas exchange of young kiwi fruit (*Actinia deliciosa* var. *deliciosa*) vines. *Ann. Bot*. 77: 605-613.

Guerfel, M., Baccouri, O., Boujnah, D., Chaïbi, W. and Zarrouk, M.2009. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars. *Sci. Hort.* 119: 257-263.

Gunes, A., Pilbeam, D.J., Inal, A. and Coban, S. 2008. Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms, and lipid peroxidation. *Commun. Soil Sci. Plant Anal*. 39: 1885-1903.

Guo, Z., Ou, W., Lu, S. and Zhong, Q. 2006. Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. *Plant Physiol. Biochem*. 44: 828-836.

Hall, A.E., Richards, R.A., Condon, A.G., Wright, G.C. and Farquhar, G.D. 1994. Carbon isotope discrimination and plant breeding. In: Janick, J. (ed.), Plant Breeding Reviews. New York, Wiley, pp. 81-113.

Hare, P.D., and Cress, W.A. 1997. Metabolic implications of stress induced proline accumulation in plants. *Plant Growth Reg*. 21: 79–102.

Harris, L.J. and S.N. Ray, 1935. Determination of plasma ascorbic acid by 2, 6-dichlorophenol indophenol titration. *Lancet.* 1: 462.

Hatfield, J. L., Sauer, T. J. and Prueger, J. H. 2001. Managing soils to achieve greater water use efficiency: a review. *Agron. J*. 93: 271–280.

Hayatu, M. and Mukhtar, F.B. 2010. Physiological responses of some drought resistant cowpea genotypes (*Vigna unguiculata* (l.) .Walp) to water stress. Bayero J. *Pure Appl. Sci*. 3(2): 69 – 75

Hendry, G., Houghton, J.D., and Brown, S.B. 1987. The degradation of chlorophyll a biological enigma. *New Phytol*. 107: 255–302.

Hessini, K., Martínez, J.P., Gandour, M., Albouchi, A., Soltani, A., and Abdelly, C. 2009. Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in *Spartina alterniflora*. *Environ. Exp. Bot*. 67: 312–319.

Heuer, B., 1999. Osmoregulatory role of proline in plants exposed to environmental stressed. In: Pessarakli, M. (Ed.), *Handbook of Plant and Crop Stress*. Marcel Dekker, New York, pp. 675–695.

Horner, J.D. 1990. Nonlinear effects of water deficits on foliar tannin concentration. *Biochem System Ecol*. 18: 211-213.

Hubick, K.T., Farquhar, G.D., and Shorter, R. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Aust. J. Plant Physiol.* 13: 803-816

Hura, T., Grzesiak, S., Hura, K., Grzesiak, M.T, and Rzepka, A. 2006. Differences in the physiological state between triticale and maize plants during drought stress and followed rehydration expressed by the leaf gas exchange and spectrofluorimetric methods. *Acta. Physiol. Plant.* 28: 433–443

Hussain, M., Malik, M.A., Farooq, M., Ashraf, M.Y., Cheema, M.A. 2008. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* 194: 193-199.

Ibrahim, L. 1995. Effects of nitrogen supply, water stress and interaction between water and nitrogen on assimilate partitioning in poplar. Ph D thesis, University of Aberdeen, UK.

Ingram J., and Bartels, D. 1996. The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 47: 377–403.

IRHO-CIRAD., 1992. Coconut-Study of yield factors. *Oleagineux*. 47:324-337.

Iturriaga, G., Suarez, R. and Nova-Franco, B. 2009. Trehalose Metabolism: From Osmoprotection to Signaling. *Int. J. Mol.Sci.* 10: 3793-3810.

Iyer, S., and Caplan, A. 1998. Products of proline catabolism can induce osmotically regulated genes in rice. *Plant Physiol*. 116: 203–211.

Izanloo, A., Condon, A.G., Langridge, P., Tester, M., and Schnurbusch, T. 2008. Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *J. Exp. Bot*. 59(12): 3327-3346.

Jaleel, C.A., Gopi, R., Sankar, B., Gomathinayagam, M., Panneerselvam, R. 2008. Differential responses in water use efficiency in two varieties of Catharanthus roseus under drought stress. Comp Rend Biol. 331: 42-47.

Jaleel, C.A., Manivannan, P., Wahid, A., Farooq, M., Somasundaram, R. and Panneerselvam, R. 2009. Drought stress in plants: A review on morphological characteristics and pigments composition. Int. J. Agric. Biol. 11: 100–105.

Jayasekara, C., Nainanayake, N.P.A.D., and Jayasekara. K.S. 1996. Photosynthetic characteristics and productivity of the coconut palm. *Cocos*. 11: 7–20.

Jayasekara, K.S., Ranasinghe, C.S., and Mathes, D.T. 1993. Screening for high yield and drought tolerance in coconut. In: Nair, M.K., Khan, H.H., Gopalasundaran, P., Bhaskara Rao, E.V.V. (eds.), *Advances in Coconut Research and Development*, Oxford & IBH Publishing Co Pvt. Ltd., New Delhi, pp. 209–218.

Jenks, M. A., Tuttle H. A., Rashotte, A. M., and Feldmann, K. A. 1996. Mutants in Arabidopsis altered in epicuticular waxes and leaf morphology. Plant Physiol. 1101(1): 377–385.

Jenks, M.A. 2002. Critical issues with the plant cuticle's function in drought tolerance. In: Wood, A.J. (ed.), *Biochemical and Molecular Responses of Plants to the Environment*. Research Signpost, Kerala, India, pp. 97–127.

Jenks, M.A., and Ashworth, E.N. 1999. Plant Epicuticular Waxes: function production and genetics. In: Janick J (ed.), *Horticultural Reviews*. Vol. 23.

Jeyakumar, P., Kavino, M., Kumar, N. 2007. Physiological performance of papaya cultivars under abiotic stress conditions. *Acta Hort*. 740:209-214.

Jharna, D.E., Chowdhury, B.L.D., Haque, M.A., Bhuiyan, M.R.H. and Husain, M.M. (2003). A preliminary investigation of the proline content and nitrate reductase activity of some groundnut genotypes as index of screening for drought tolerance. *Bangladesh J. Agril. Res.* 28(2): 283-290.

Johnson, D.A., Asay, K.H., Tieszen, L.L., Ehleringer, J.R., and Jefferson, P.G. 1990. Carbon isotope discrimination: potential in screening cool-season grasses for water-limited environments. *Crop Sci*. 2: 338-343.

Jones, H. G. 2004. What is water use efficiency?. In: Bacon, M. A. (ed.), *Water Use Efficiency in Plant Biology*, Blackwell Publishing, Oxford, UK, pp. 27–41.

Kakkar, P., Das, B, and Viswanathan, P.N.1984. A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.* 21:130-132

Kala, S. and Godara, A. K. 2011. Effect of moisture stress on leaf total proteins, proline and free amino acid content in commercial cultivars of *Ziziphus mauritiana*. *J. Sci. Res*. 55:65-69.

Kaloyereas S.A. 1958. A new method of determining drought resistance. *Plant Physiol*. 33: 232-233

Kasturi Bai, K. V., and Rajagopal, V. 2000. Osmotic adjustment as a mechanism for drought tolerance in coconut (*Cocos nucifera* L.). *Indian J. Plant Physiol*. 5: 320–323.

Kasturi Bai, K. V., Rajagopal, V., Chempakam, B., and Naresh Kumar, S. 2005. Inductive response of protective enzymes in coconut (*Cocos nucifera* L.) subjected to stress. *Ind. J. Hort*. 62: 363–366.

Kasturi Bai, K.V, Rajagopal, V., Balasimha, D., Gopalasudaram, P. 1997. Water relations, gas exchange and dry matter production of coconut (*Cocos nucifera* L.) under irrigated and non-irrigated conditions. *Coconut Res. Develop*. 13: 45-58.

Kasturi Bai, K.V., Rajagopal, V., 2004. Is stress-induced proline accumulation an indicator of leaf water status in coconut? *J. Plantation Crops* 32 (Suppl.): 1–4.

Kasturi Bai, K.V., Rajagopal, V., Prabha, C.D., Ratnambal, M.J., George M.V. 1996. Evaluation of coconut cultivars and hybrids for dry matter production. *J. Plant. Crops.* 24:23-28.

Kasturi Bai, K.V.,and Rajagopal, V. 1999. Impact of drought on nut yield. In: Rajagopal, V., and Ramadasan,A. (eds) *„Advances in Plant Physiology and Biochemistry of Coconut Palms*. Asian and Pacific Coconut Community, Publication. Jakarta

Kaya, M.D, Okeu, G., Atak, M., Çıkılı, Y., and Kolsarıcı, O. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Euro. J. Agron.* 24: 291–295.

Kerstiens, G. 1996. Cuticular water permeability and its physiological significance. *J. Exp. Bot*. 47:1813–1832

Khalil, C., Boufous, E., and Mousadik, A.E. 2011. Diversity of salt tolerance during germination in *Medicago ciliaris* (L.) and *Medicago polymorpha* (L.). *Atlas J. Plant Biol.* 1: 6–12.

Khanna-Chopra, R., and Selote, D.S. 2007. Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than -susceptible wheat cultivar under field conditions. *Environ. Exp. Bot*. 60: 276–283.

Kishor, P.B.K, Sangama, S, Amrutha, R.N, Laxmi, P.S, Naidu, K.R, Rao, K.S. 2005. Regulation of proline biosynthesis degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Curr Sci.* 47(1): 285-293.

Knight, H. and Knight, M.R. 2001. Abiotic stress signalling pathways: specificity and cross-talk. *Trends Plant Sci*. 6: 262–267.

Kocheva, K., Lambrev, P., Georgiev, G., Goltsev, V., Karabaliev, M. 2004. Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress. *Bioelectrochemistry*. 63: 121–124.

Kolarovic, J., Popovic, M., Mikov, M., Mitic, R., and Gvozdenovic, L. 2009. Protective effects of celery juice in treatments with Doxorubicin. *Molecules*. l4 (4):1627 - l638.

Kondo, M., Murty, M.V.R. and Aragones, D.V. 2000. Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. *Soil Sci. Plant Nutr*. 46: 721-732

Konwar, P.B. 2009. Characterization of some selected rice cultivars under moisture stress condition. M.Sc thesis Assam Agricultural University. Jorhat.

Kranner, I., Beckett, R.P., Wornik, S. Zorn, M. and Pfeifhofer, H.W. 2002. Revival of resurrection plant correlates with antioxidant status. *Plant J*. 31: 13-24.

Kraus, T.E., Pauls, K.P. and Fletcher, R.A. 1995. Paclobutrazol and hardening-induced thermo tolerance of Wheat: Are heat shock proteins involved?. *Plant Cell Physiol*. 26:59-67.

Kriedemann, P.E., Barrs, H.D., and Kozlowski, T.T. (eds). 1981. *Water Deficits and Plant Growth*. Academic Press, New York , pp. 325–416.

Kumar, R. and Singh, S.B. 1996 – Changes in biochemical constituents of sunflower leaves in relation to Alternaria blight development. *Phytopathol. News*. 26: 234–236.

Kumar, U. M., Devendra, R. Ramaswamy, Rao, N. R.C., Ashok, Stephen, R., Gangadhara G.C., Hussain, A.I.S. and Wright, G. C. 1998. Measurement of transpiration efficiency under field conditions in grain legume crops. *Plant Physiol. Biochem*. 25 (1): 67-75.

Kurup, V.V.G.K., Voleti, S.R, and Rajagopal, V. 1993. Influence of weather variables on the content and composition of leaf surface wax in coconut. *J. Plant. Crops*. 2:71-80.

Kusaka, M., Lalusin, A.G., and Fujimura, T. 2005. The maintenance of growth and turgor in pearl millet (*Pennisetum glaucum* [L.] Leeke) cultivars with different root structures and osmo-regulation under drought stress. *Plant Sci*. 168: 1–14.

Lakmini, W.G.D., Nainanayake, N.P.A.D., and De Costa, W.A.J.M. 2006. Biochemical changes of four different coconut (*Cocos nucifera* L.) forms under moisture stress condition. *J. Agric. Sci*. 2 (3): 1-7

Lambers, H., Chapin III, F.S., and Pons, T.L. 1998. *Plant Physiological Ecology*. Springer-Verlag, New York.

Lauteri, M., Brugnoli, E., and Spaccino, L. 1993.Carbon isotope discrimination in leaf soluble sugars and in whole-plant dry matter in *Helianthus annuus* L. grown under different water conditions. In: Ehleringer J.R, et al., (eds), *Stable Isotopes*

and Plant Carbon – Water Relations. San Diego. Academic Press, inc. pp.93–108.

Lawlor, D.W., and Cornic, G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ*. 25: 275–294.

Lecoeur, J., Wery, J., Turc, O., and Tardieu F. 1995. Expansion of pea leaves subjected to short water-deficit: cell number and cell-size are sensitive to stress at different periods of leaf development. *J. Exp. Bot*. 46: 1093–1101.

Lee, X., Smith, R. and Williams, J. 2006. Water vapour O^{18}/O^{16} isotope ratio in surface air in New England, USA. *Tellus B : Chem. Phys. Meteorol.* 58: 293–304.

Lei, Y.B., Yin, C.Y., and Li, C.Y. 2006. Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. *Physiol. Plant*. 127: 182–191.

Li, C. 1999. Carbon isotope composition, water-use efficiency and biomass productivity of Eucalyptus microtheca populations under different water supplies. *Plant Soil*. 214: 165–171.

Li, J.X., Wang, X.Q, Watson, M.B, and Assmann, S.M. 2000. Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. *Sci.* 287: 300–303

Liu, S., Liu, J., Cao, J., Bai, C, and Shi, R. 2006. Stomatal distribution and character analysis of leaf epidermis of jujube under drought stress. *J. Anhui Agric Sci*. 34: 1315–1318.

Lu, Z.M., Percy, R. G., Qualset, C. O. and Zeiger, E. 1998.Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures. *J. Exp. Bot.* 49, 453–460.

Luck, H. 1974. Catalase in methods of enzymatic analysis. Bergmeycr, J. and Grabi, M. Academic Press. NewYork. Pp 885-890.

Ludlow, M.M., and Muchow, R.C. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. *Adv. Agron*. 43:107–153.

Luhova, L., Lebeda, A., and Hederrova, D., Pec, P .2003. Activities of amine oxidase, peroxidase and catalase in seedling of *Pisum sativum* L. under different light conditions. *Plant Soil Environ*. 49:151–157.

Luna, C.M., Gabriela, M., Pastori, Driscoll, S., Groten, K., Bernard, S. and Foyer, C.H. 2004. Drought controls on H2O2 accumulation, catalase (CAT) activity and CAT gene expression in wheat. *J. Exp. Bot*. 56 (411): 417–423.

Mabrouk, A., El-Sharkawy and Cock, J.H. 1987. Response of cassava to water stress. *P1ant. Soil*. 100:345-360

Madhan Mohan, M., Lakshmi Narayanan, S., and Ibrahim, S.M. 2000. Chlorophyll Stability Index (CSI): Its impact on salt tolerance in rice. *Int. Rice Res. Notes*, 25: 38-39.

Madhusudan, K.V., and Sudhakar, C. 2014. Effect of water deficit stress on growth and chlorophyll pigments in two cultivars of groundnut. *Paripex Ind. J. Res.* 3(4): 8-9.

Mafakheri, A. Siosemardeh, A., Bahramnejad, B., Struik, P.C. and Sohrabi, Y. 2011. Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars. *Aust. J. Crop Sci*. 5: 1255—1260.

Maherali, H., Reid, C.D, Polley, H.W., Johnson, H.B, and Jachson, R.B. 2002. Stomatal acclimation over a sub ambient to elevated CO_2 gradient in a C3/C4 grassland. Plant. Cell. Environ. 25:557–566.

Makhmudov, S.A. 1983. A study on chlorophyll formation in wheat leaves under moisture stress. *Field Crop Abstr*. 39: 1753.

Malecka, A., Jarmuszkiewicz, W. and Tomaszewska, B.2001. Antioxidative defense to lead stress in sub cellular compartments of pea root cells. *Acta Biochim*. Polon. 48: 687-698.

Manivannan, P., Abdul Jaleel, C., Kishorekumar, A., Sankar, B., Somasundaram, R., Sridharan, R., and Panneerselvam R. 2007. Changes in antioxidant metabolism of *Vigna unguiculata* (L.) .Walp. By propiconazole under water deficit stress. *Colloids Surf. B*. 57: 69–74.

Manthriratina, M.A.P.P. and S. Sambasivam. 1974. Stomatal density in varieties and from of the coconut. *Ceylon Cocon Q*. 25: 105-108.

Maricle, B.R., Cobos, D.R and Campbell, C.S. 2008. Biophysical and morphological leaf adaptations to drought and salinity in salt marsh grasses. *Environ. Exp Bot*. 60: 458-467.

Martignone, R.A., Guiamot, J.J. and Nakayama, F. 1987. Nitrogen partitioning and leaf senescence in soybean as related to nitrogen supply. *Field Crops Res*. 17: 17-20.

Martin, B., and Thorstenson, Y.R. 1988. Stable carbon isotope composition (d 13C), water use efficiency and biomass productivity of Lycopersicon esculentum, Lycopersicon pennellii, and the F1 hybrid. *Plant Physiol*. 88: 213–217.

Martinez, C.A., Loureiro, M.E., Oliva, M.A., and Maestri M.2001. Differential responses of superoxide dismutase in freezing resistant Solanum curtilobum and freezing sensitive Solanum tuberosum subjected to oxidative and water stress. *Plant Sci*.160: 505–515.

Martinez, J.P., Kinet, J.M., Bajji, M., Lutts, S. 2005. NaCl alleviates polyethylene glycol induced water stress in the halophyte species *Atriplex halimus* L. *J. Exp Bot*. 56(419):2421–2431

Martínez, J.P., Silva, H., Ledent, J.F., and Pinto, M. 2007. Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.). *Eur.J. Agron*. 26: 30–38.

Mathew, S. and T.E. Abraham, 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food Chem*. 94: 520-528.

Mayr, U., Funfgelder, S., Treutter, D., Feucht, W., 1995. Induction of phenol accumulation by pesticides under the control of environmental factors. In: Manka M. (ed.). *Environmental Biotic Factors in Integrated Plant Disease Control.* Polish Phytopathological Society, Varsaw, Poland. 399-402

Mc Cready, R. M., Guggolz, J., Silviera, V., and Owens, H. S. 1950. Determination of starch and amylose in vegetables. *Anal.Chem*. 22:1156.

Mc Cullough, D.E., Girardin, P.H., Mihajlovic, M., Agvilera, A. and Tollenaar, M. 1994. Influence of Nitrogen supply on development and dry matter accumulation of an old new maize hybrid. *Can. J. Plant and Soil. Sci*. 74: 461-466.

Medrano, H., Escalona, J.M., Bota, J., Gulías, J., and Flexas, J. 2002. Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann. Bot,* 89(7): 895-905.

Meenakumari, Sain-Dass, Vimala, T., and Arora, P. 2004. Physiological parameters governing drought tolerance in maize. *Indian J. Plant Physiol*. 9: 203-207

Merah, O., Deleens, E., and Monneveux, P. 1999. Grain yield, carbon isotope discrimination, mineral and silicon content in durum wheat under different precipitation regimes. *Physiol. Plantarum.* 107: 387-394.

Meyer, S., Cartelat, A., Moya, I., and Cerovic, Z.G. 2003. UV-induced blue-green and far-red fluorescence along wheat leaves: a potential signature of leaf ageing. *J. Exp. Bot.* 54: 757–769.

Mittova, V., Volokita, M., Guy M., and Tal M. 2000. Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative Lycopersicon pennellii. *Physiol. Plantarum*.110: 42–51.

Moghadam, P.A., Imani, A.A., Shahbazi, H. and Rjabalizadeh, K. 2013. A study on superoxide dismutase change trends in reproductive stages under drought stress in lentil genotypes. Ann. Biol. Res. 4 (4):220-223

Moller, I.M., Jensen, P.E., Hansson, A. 2007. Oxidative modifications to cellular components in plants. *Ann. Rev. Plant Biol.* 58: 459–481.

Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., Delay, D., Petit, J-M.l., Barbaroux, C., Thiec, D., Brechet, C., Brignolas, F. 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoids*, *Populus nigra*. *New Phytol.* 169: 765–777.

Montalvo-Hernández, L., Piedra-Ibarra, E., Gómez-Silva, L., Lira-Carmona, R., Acosta-Gallegos, J.A, Vazquez-Medrano, J., Xoconostle-Cázares, B., and Ruíz-Medrano, R. 2008. Differential accumulation of mRNAs in drought-tolerant and susceptible common bean cultivars in response to water deficit. *New Phytol* .177: 102–113.

Montero-Tavera, V., Ruiz-Medrano, R., and Xoconostle-Cazares, B. 2008. Systemic nature of drought-tolerance in common bean. *Plant Signal. Behav*. 3: 663-666.

Moore, M. and Stein, W.H. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem*. 176:367-388.

Morales, F., Cartelat, A. A, lvarez-Fernandez, A., Moya, I., Cerovic, Z.G. 2005. Time-resolved spectral studies of blue-green fluorescence of artichoke (*Cynara cardunculus* L. Var. *Scolymus*) leaves: identification of chlorogenic acid as one of the major fluorophores and agemediated changes. *J. Agric. Food Chem*. 53: 9668–9678.

Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Klucas, R.V., and Aparicio-Trejo P. 1994. Drought induces oxidative stress in pea plants. *Plantarum*. 194: 346–352.

Müller, P., Li, X.P., and Niyogi, K.K. 2001. Non-photochemical quenching. A response to excess light energy. *Plant Physiol*. 125: 1558-1566.

Munns, R. and Termaat, A. 1986. Whole plant responses to salinity. *Aust. J. Plant Physiol*. 13: 143-166.

Murray, D. B. 1977. Coconut palm. In: Alvim, P. de T. and Kozlowski, T.T (Eds.). *Ecophysiology of Tropical Crops*. London: Academic Press, pp. 383–407.

Myers, R.J.K., Foale, M. A. and Done, A. A. 1984. Response of grain sorghum to varying irrigation frequency in the Ord irrigation area. II. Evapotranspiration water-use efficiency. *Aust. J. Agri. Res.* 35(1). DOI: 10.1071/AR9840031

Namirembe, S., Brook, R.M., and Ong, C.K. 2008. Manipulating phenology and water relations in *Senna spectabilis* in a water limited environment in Kenya. *Agrofor. Syst*. 75:197–210.

Natarajan, N. and S. Kumaravelu. 1993. Screening for drought resistance rice. *Oryza*. 30: 251-253.

Nautiyal, P.C., Ravindra, V. and Joshi, Y.C. 2002. Dry matter partitioning and water use efficiency under water-deficit during various growth stages in groundnut. *Indian J. Plant Physiol*. 7: 135–139.

Navabpour, S., Morris, K., Allen, R., Harrison, E., A-H-Mackerness, S., Buchanan-Wollaston, V. 2003. Expression of senescence enhanced genes in response to oxidative stress. *J Exp Bot.* 54: 2285–2292.

Navari-izzo, F., Quartacci, M. F. and Izzo, R. 1990. Water-stress induced changes in protein and free amino acids in field-grown maize and sunflower. *Plant Physiol and Biochem*. 28: 531-537.

Navas, M.L., and Garnier, E. 2002. Plasticity of whole plant and leaf traits in Rubia peregrine in response to light, nutrient and water availability, *Acta Oecol*. 23:375-383.

Neumann, P.M. 2008. Coping mechanisms for crop plants in drought-prone environments. *Ann Bot*. 101:901-907.

Nilson, S.E., and Assmann, S.M. 2007. The control of transpiration. Insights from Arabidopsis. *Plant Physiol*. 143: 19–27.

Nobel, P.S. 1991. Physicochemical and Environmental Plant Physiology. Academic Press, San Diego, CA, 635 p.

Noctor, G., Veljovic-Jovanovic, S., and Foyer, C.H. 2000. Peroxide Processing In: *Photosynthesis: Antioxidant Coupling and Redox Signalling*. Philosophical Transactions of the Royal Society of London. 355: 1465–1475

Nogueira, L. C, Nogueira, L. R. Q, Miranda, F. R. 1998. Irrigation of coconut. In: Ferreira, J. M. S., Warwick, D. R. N, and Siqueira, L. A (eds), *The Coconut cultivation in Brazil.* Embrapa, Brasília .pp.159-187.

Nonami, H. 1998. Plant water relations and control of cell elongation at low water potentials. *J. Plant Res.* 111: 373–382.

O'Leary, M. H. 1988. Carbon isotopes in photosynthesis. *Biosci.* 38:325--336.

Ohashi, Y., Saneoka, H., Matsumoto, K., Ogata, S. Gnanasiri, S., Premachandra, and Fujita, K. 199. Comparison of Water Stress Effects on Growth, Leaf Water Status, and Nitrogen Fixation Activity. In: *Tropical Pasture Legumes Siratro and Desmodium with Soybean*, Soil Sci. Plant Nutr. 45 (4): 795-802.

Omasa, K. and Takayama, K. 2003. Simultaneous measurement of stomatal conductance, non-photochemical quenching, and photochemical yield of photosystem II in intact leaves by thermal and chlorophyll fluorescence imaging. *Plant Cell Physiol*. 44: 1290-1300.

Ozdemir, F., Bor, M., Demiral, T., and Turkan, I. 2004. Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (Oryza sativa L.) under salinity stress. *Plant Growth Regul*. 42: 203–211

Ozkur, O., Ozdemir, F., Bor, M., Turkan, I., 2009. Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* .Desf to drought. *Environ. Exp. Bot*. 66: 487–492.

Ozturk, Z.N., Talamé, V., Deyholos, M., Michalowski, C.B, Galbraith, D.W., Gozukirmizi, N., Tuberosa. R., and Bohnert, H.J. 2002. Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. *Plant Mol. Biol.* 48:551-573.

Pagter, M., Bragato, C., and Brix, H. 2005. Tolerance and physiological responses of Phragmites australis to water déficit. *Aquat. Bot.* 81: 285–299.

Pan, Y., Wu, L.J., and Yu, Z.L. 2006. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis* Fisch). *Plant Growth Regul*. 49:157–165.

Parida, A.K., Dagaonkar, V.S., Phalak, M.S., Aurangabadkar, L.P. 2008. Differential responses of the enzymes involved in proline biosynthesis and degradation in drought tolerant cotton genotypes during drought stress and recovery. *Acta Physiol. Plant*. 30: 619-627

Passioura, J. B. 2002 Environmental biology and crop improvement. *Funct. Plant Biol.* 29: 537–546.

Passioura, J.B. 2007. The drought environment: physical, biological and agricultural perspectives. *J. Exp. Bot*. 58: 113-117.

Passos EEM, Silva JV (1990) Fonctionnement des stomates de cocotier (*Cocos nucifera*) au champ. *Can.J. Bot.* 68:458-460.

Passos, C.D., Passos, E.E.M., and Prado, C.H.B.A. 2005. Seasonal behavior of water potential and gas exchange of four varieties of coconut. Rev. Bras. Frutic. 27:248-254.

Passos, E.E.M., Passos, C.D., and Aragao, W.M. 2003. Gas exchanges in dwarf coconut genotypes under water stress conditions. PhD thesis, Rural Federal University of Amazonas,

Passos, E.E.M., Prado, C.H.B.A, Leal, M.L.S. 1999. Stomatal conductance, leaf water potential and issuance of leaves and flowers in three genotypes of dwarf coconut. *Agrotropica.* 11:147-152.

Patakas, A., and Noitsakis, B. 2001. Leaf age effects on solute accumulation in water-stressed grapevines. *Plant Physiol*. 158: 63-69

Perez-Perez, J.G., Robles, J.M., Tovar, J.C., and Botia, P. 2009. Response to drought and salt stress of lemon 'Fino 49' under field conditions: water relations, osmotic adjustment and gas exchange. *Sci. Hortic*, 122: 83–90.

Peuke, A.D., Gessler, A., and Rennenberg, H. 2006. The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. *Plant Cell Environ*. 29: 823–835.

Philips, J.R., Oliver, M.J. and Bartels, D. 2002. Molecular genetics of desiccation and tolerant systems. In: Black, M. and Pritchard, H.W. (ed.*), Desiccation and Survival in Plants: Drying Without Dying.*CAB International, pp. 319-342

Pinheiro, H.A., DaMatta, F.M., Chaves, A.R.M, Loureiro, M.E., Ducatti, C.2005. Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. *Ann. Bot*. 96: 101-108.

Pinheiro, R.G., Rao, M.V., Palyath, G., Murr, D.P., and Fletcher, R.A. 2001. Changes in the activities of antioxidant enzymes and their relationship to genetic and paclobutrazol - induced chilling tolerance of maize seedlings. *Plant Physiol*.114 (2):695-704

Poorter, H., 1989. Interspecific variation in relative growth rate: on ecological causes and physiological consequences. In: Lambers, H. (ed.), *Causes and Consequences of Variation in Growth Rate and Productivity in Higher Plants.* SPB Academic Publishing, The Hague, The Netherlands, pp. 45–68.

Poorter, H., de Jong, R., 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytol.* 143: 163–176

Poorter, H., Van der Werf, A, .1998. Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. In: Lambers, H., Poorter H, and Vuuren M van (eds), *Inherent Variation In Plant Growth: Physiological Mechanisms and Ecological Consequences.* Backhuys, Leiden, pp 309–336

Prado, C.H.B.A, Passos E.E.M, and Moraes, J.A.P.V. 2001. Photosynthesis and water relations of six tall genotypes of *Cocos nucifera* in wet and dry seasons. *South Afr. J. Bot*. 67:169-176.

Premachandra, G.S., Saneoka, H., Fujita, K., and Ogata, S.1993. Water-stress and potassium fertilization in field-grown maize (Zea-mays L.) - effects on leaf water relations and leaf rolling. *J. Agron.Crop.Sci. 170* (3): 195-201

Purseglove, J. W. 1972. *Tropical Crops: Monocotyledons*. London: Longman.

Purushotham, M.G., Patil, T.G., Prasad, S.N. 1998. Development of in vitro PEG stress tolerant cell lines in two groundnut genotypes. *Indian J. Plant Physiol*. 3:49-51.

Rahbarian, R., Khavari-nejad, R., Ganjeali, Ali. Bagheri, A., and Najafi, F. 2011. Drought stress effects on photosynthesis, chlorophyll fluorescence and water relations in tolerant and susceptible chickpea (Cicer arietinum l.) genotypes. *Acta Biol. Cracov. Bo.t* 53(1): 47–56.

Rai, V.K., and Sharma, U.D. 1991.Amino acids can modulate ABA induced stomatal closure, stomatal resistance and K+ fluxes in Vicia faba leaves.-Beitr. *Biol. Pflanz*. 66: 393–405.

Rajagopal, V., and Kasturi Bai, K.V. 2002. Drought tolerance mechanism in coconut. *Burot. Bull.* 17:21-22.

Rajagopal, V., Kasturi Bai, K.V. and Shivashankar, S. 1988. Physiological and biochemical criteria for breeding for drought tolerance in coconut. In: *Proc. Nat. Symp. Coconut Breeding and Management* (Sailas. E.G.. Arvindakshan, M. and Jose. A.-eds), Kerala Agril. Univ., Vellanikkara (India), 23—26 Nov., 1988. pp. 136—143.

Rajagopal, V., Kasturi Bai, K.V., and Voleti, S.R. 1990. Screening of coconut genotypes for drought tolerance. *Oleagineux* 45:215-223.

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

Rajendrakumar, C.S.V., Reddy, B.V.B., and Reddy, A.R. 1994. Proline-protein interactions: protection of structural and functional integrity of M4 lactate dehydrogenase. *Biochem. Biophys Res. Commun*. 2:957–963.

Ramanjulu, S. and Bartels, D.2002. Drought- and desiccation-induced modulation of gene expression in plants. *Plant Cell Environ*. 25: 141–151.

Ramesh Babu, V., Murthy, P.S.S. and Sankara Reddi. G.H.S.1982. Water status and avoidance of radiation interception by pigeon pea. *Plant Physiol. Biochem*. 9: 103-112.

Ranasinghe, C.S., Nainanayake, A., Gunawardena, M., de Saram, P.S.A. and Jayathilake, A. 2003. Report of the Plant Physiology Division In: Jayasekara, C, Everard, J.M.D.T, Appuhamy, P.H.A.N, Idirisinghe, I.M.S.K., (eds). *Report for 2003*. Coconut Research Institute, Srilanka, pp. 181-183.

Ranieri, A, R. Bernardi, P. Lanese, and G.F. Soldatini. 1989. Changes in free amino acid content and protein pattern of maize seedlings under water stress. *Env. Exp. Bot*. 29:351-35.

Rebetzke, G.J., Condon, A.G., Richards, R.A. and Farquhar G.D. 2002. Selection for reduced carbon-isotope discrimination increases aerial biomass and grain yield of rainfèd bread wheat. *Crop Sci.* 42: 739-745.

Rebetzke, G.J., Richards, R.A., Condon, A.G. and Farquhar, G.D. 2006. Inheritance of carbon isotope discrimination in bread wheat (*Triticum aestivum* L.). *Euphytica*.1 -1 0.

Reddy, A.R., Chaitanya, K.V., and Vivekanandan, M. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol*. 161:1189-1202.

Reddy, K.P., Subhani, S.M., Khan, P.A., Kumar, K.B. 1995. Effect of light and benzyl adenine and dark- treated graving rice (*Oryza sativa*) leaves - changes in peroxidases activity. Plant Cell Physiol. 26: 987- 994.

Regier, N., Streb, S., Cocozza, C., Schaub, M., Cherubini, P., Zeeman, S.C., Rrey, B., 2009. Drought tolerance of two black poplar (*Populus nigra* L.) clones: contribution of carbohydrates and oxidative stress defence. *Plant Cell Environ*. 32:1724–1736.

Repellin, A., Daniel, C., Zuily-Fodil, Y., 1994. Merits of physiological tests for characterizing the performance of different coconut varieties subjected to drought. *Oleagineux.* 49: 155–168.

Reynolds, M., and Tuberosa, R. 2008. Translational research impacting on crop productivity in drought-prone environments. *Curr. Opin. Plant Biol*. 11, 171–179.

Riederer, M., and Schreiber, L. 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. *J Exp Bot* .52:2023–2032

Rodrigues, M.L., Pacheco, C.A., Chaves, M.M. 1995. Soil-plant relations, root distribution and biomass partitioning in *Lupinus albus* L. under drought conditions. *J. Exp. Bot*. 46: 947—956.

Rodriguez, D.J., Romero-Garcia, J., Rodriguez-Garcia, R., and Angulo-Sanchez, J. L. 2002. Characterization of proteins from sunflower leaves and seeds: Relationship of biomass and seed yield. In: Janick, J., and Whipkey, A. (eds), *Trends in new crops and new uses.* ASHS Press, Alexandria, VA, pp. 143-149.

Saab, I.N. and Sharp, R.E. 1989. Non-hydraulic signals from maize roots in drying soil: Inhibition of leaf elongation but not stomatal conductance. *Planta*. 179: 466–474.

Sairam, R. K., Kumutha, D., Ezhilmathi, K., Deshmukh, P. S., and Srivastava, G. C. 2008. Physiology and biochemistry of waterlogging tolerance in plants. *Biol. Plantarum*. 52: 401-412

Sairam, R.K, Deshmukh, P.S, and Shukla, D.S. 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J. Agron Crop Sci.* 178: 171–178.

Sairam, R.K., Shukla, D.S. and Deshmukh, P.S. 1996. Effect of homobrassinolide seed treatment on germination, α-amylase activity and yield of wheat under moisture stress condition. *Ind J. Plant Physiol*. 1: 141-144.

Samuel, D., Kumar, T.K.S., Ganesh, G., Jayaraman, G., Yang, P.W., Chang, M.M., Trivedi,V.D., Wang, S.L., Hwang, K.C., Chang, D.K., and Yu, C., 2000. Proline inhibits aggregation during protein refolding. *Protein Sci.* 9: 344–352.

Sánchez, F.J., Manzanares, M., de Andres, E.F., Tenorio, J.L., Ayerbe, L., 1998. Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. *Field Crop Res*. 59, 225–235.

Sanchez-Blanco, P., Rodríguez, M.A., Morales, M.F., Ortuño, A., and Torrecillas. 2002. Comparative growth and water relations of Cistus albidus and Cistus monspeliensis plants during water deficit conditions and recovery. *Plant Sci*. 162:107-113.

Sayar, R., Kemira, H., Kameli, A., Mosbahi, M., 2008. Physiological tests as predictive appreciation for drought tolerance in durum wheat (*Triticum durum* Desf.). *Agron. Res*. 6: 79–90.

Scandalios, J.G., Guan, L. and Polidoros, A.N. 1997. Catalases in plants: Gene structure, properties, regulation, and expression. In: Scandalios, J.G (ed.) *Oxidative Stress and Molecular Biology of Antioxidant Defenses*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 343-406.

Schuppler U., He, P.H., John, P.C.L., Munns, R. 1998. Effects of water stress on cell division and cell-division-cycle-2-like cell-cycle kinase activity in wheat leaves. *Plant Physiol.* 117:667–678.

Seel, W.E, Hendry, G.A.F., Lee, and J.A. 1992. The combined effect of desiccation and irradiance on mosses from xeric and hydric habitats. *J Exp Bot* .43: 1023-1030

Seki, M., Umezawa, T., Urano, K., and Shinozaki, K. 2007. Regulatory metabolic network in drought stress responses. *Curr. Opin. Plant Biol.* 10, 296–302.

Seng, K.H. 2014. The effects of drought, waterlogging and heat stress on tomatoes (*Solanum lycopersicon* L.). Ph.D thesis, Lincoln University, New Zealand, 289 p.

Shao, H.B., Chu, L.Y., Shao, M.A., Abdul Jaleel, C., and Hong-Mei, M. 2008. Higher plant antioxidants and redox signaling under environmental stresses. *Comp. Rend. Biol*. 331: 433–441.

Shao, H.B., Chuc, L.Y., Wu, G., Zhang, J.H., Lua, Z.H., and Hug, Y.C. 2007. Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. *Colloids Surfaces B*. 54:143-149

Sharma, S.S., Dietz, K.J. 2006. The significance of amino acids and amino-acid derived molecules in plant responses and adaptation to heavy stress. *J. Exp Bot*. 57(4): 711-726

Shinozaki, K., Yamaguchi-Shinozaki, K. 2007. Gene networks involved in drought stress response and tolerance. *J. Exp. Bot*. 58: 221–227.

Shipley, B., 2002. Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. *Funct. Ecol.* 16, 682–689.

Shiriga, K., Sharma, R., Kumar, K., Yadav, S., Hossain, F., and Thirunavukkarasu, N. 2014. Expression pattern of superoxide dismutase under drought stress in maize. *Int. J. Innovative Res. Sci. Eng. Technol*. 3:11333–11337.

Shivasankar S., Nagaraja, K.V., Volati, S.R. and Kasturi Bai, K.V. .1993. Biochemical changes and leaf water status of coconut genotypes differing in drought

tolerance. In: Nair, M.K. , Khan, H.H., Gopalasundaran, P. and Bhaskara Rao, E.U.V (eds*)., Advances in Coconut Research and Development*, pp. 253-254.

Shivashankar, S., Kasturi Bai, K.V., Rajagopal, V. 1991. Leaf water potential, stomatal resistance and activity of enzymes during development of moisture stress in the coconut palm. *Trop. Agric*. 68:106-10.

Siddique M.R.B., Hamid, A., and Islam M.S. 2000. Drought stress effects on water relations of wheat. *Bot. Bull. Acad*. *Sin* 41: 35–39.

Sikuku, P.A., Netondo, G.W. Onyango, J.C., and Musyimi, D.M. 2010. Chlorophyll fluorescence, protein and chlorophyll content of three nerica rainfed rice varieties under varying irrigation regimes. *J. Agric. Biol. Sci*. 5:19-25

Silva Junior, C.D, Passos, E.E.M., Gheyi, H.R 2002. Saline water application development and physiological behavior of the coconut. *Rev. Bras. Eng. Agric. Amb*. 6:39-44.

Silva, E.C., Silva, M.F.A., Nogueira, R.J.M.C., and Albuquerque, M.B. 2010. Growth, evaluation and water relations of Erythrina velutina seedlings in response to drought stress. Braz. J. Plant Physiol. 22(4):225-233

Silva, V.P.R. 2004. On climate variability in Northeast of Brazil. *J Arid Environ*. 58, 575-596.

Silva, W., Ferreira, L.R., Silva, A.A., and Firmino, L.E. 2000. Rate of transpiration eucalyptus seedlings in response to water level in the soil and living with brachiaria. *Pesq. Agropec. Bras*. 35:923-928.

Singh, D.K., Sale, P.W.G, Pallaghy, C.K., Singh, V. 2000. Role of proline and leaf expansion rate in the recovery of stressed white clover with increased phosphorus concentration. *New Phytol.* 146(2): 261-269.

Sircelj, H., Tausz, M., Grill, D., and Batic, F. 2007. Detecting different levels of drought stress in apple trees (*Malus domestica* Borkh.) with selected biochemical and physiological parameters. *Sci. Hortic*. 113: 362-369.

Slama, I., Messedi, D., Ghnaya, T., Savoure, A. and Abdelly, C. 2006. Effects of water deficit on growth and proline metabolism in Sesuvium portulacastrum. *Environ. Exp. Bot*. 56: 231-238

Solangi, A.H., Arain, M.A and Iqbal, M.Z.2010. Stomatal studies of coconut (*Cocos nucifera* L.) varieties at coastal area of Pakistan. *Pak. J. Bot*. 42(5): 3015-3021.

Solomon, K.F. and Labuschagne, M.T. 2004. Inheritance of evapotranspiration and transpiration efficiencies in diallel F1 hybrids of durum wheat (*Triticum turgidum* L. *var. durum*). Euphytica 136: 69—79.

Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem*. 195: 19-23.

Sousa, S.M. 2006. Seasonal behavior of seven genotypes of coconut tree (*Cocos nucifera* L.) in overnight ecological conditions. PhD thesis .Rural Federal University of Amazonas, State of Pará. Manaus.

Souza, R.P., Machado, E.C.,Silva, J.A.B., Lagoa, A.M.M.A., and Silveira, J.A.G. 2004.Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environ. Exp. Bot*. 51(1): 45–56.

Spence, R.D., Wu, H., Sharpe, P.J.H., and Clark, K, G. 1986. Water stress effects on guard cell anatomy and the mechanical advantage of the epidermal cells. *Plant. Cell .Environ*. 9: 197–202.

Sreenivasulu, N., Grimm, B., Wobus, U., and Weschke W. 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt sensitive seedlings of foxtail millet (*Setaria italica*). *Physiol. Plantarum*. 109: 435–442.

Srivastava, A., Strasser, R.J. 1996. Stress and stress management of land plants during a regular day. *J. Plant Physiol*., 148:445-455.

Strogonov, B.P. 1964. Physiological Basis of Salt Tolerance of Plant. Acad Sci USSR, Davey and Co., New York, USA.

Sudhakar, C., Lakshmi, A., and Giridarakumar, S. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.* 161: 613-619.

Sundaravalli, V., Paliwal, K. and Ruckmani, A. 2005. Effect of water stress on photosynthesis, protein content and nitrate reductases activity of Albizzia seedlings. *J. Plant Biol*. 32: 13-17.

Sundaresan, S., Sudhakaran, P.R. 2006. Water stress-induced alterations in the proline metabolism of drought-susceptible and -tolerant cassava (*Manihot esculenta*) cultivars. *Physiol. Plant*. 94: 635-642.

Surendar, K.K., Devi, D.D., Ravi, I., Jeyakumar, P. and Velayudham, K. 2013. Physiological and biochemical behavior in banana cultivars and hybrids under water deficit. *Afr. J. Agric. Res.* 8(31): 4198-4208

Sutter, E., and Lanç1ans, R.W. 1979. Epicuticular wax formation on carnation plantlets regenerated from shoot tip culture. *J. Am. Soc. Hortic. Sci.* 104:493-496.

Sutter, E., and Langhans, R.W. 1982 Formation of epicuticular wax and its effect on water loss in cabbage plants regenerated from shoot-tip culture. *Can. J. Bot*. 60: 2896-2902.

Szabados, L., and Savouré, A. 2009. Proline: a multifunctional amino acid. *Trend Plant Sci*. 15:89-97.

Taiz, L., and Zeiger, E. 2006. Plant Physiology, Sinauer Associates Inc.

Taiz, L., and Zeiger, E. 2010. Plant Physiology (5th ed.). Sinauer Associates, Inc.

Tan, Y., Zongsuo, L., Hongbo, S., and Feng, D. 2006. Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of Radix astragali at seedling stage, *Coll Surf B Biointerfaces*. 49: 60-65.

Thimann, K.V .1980.The senescence of leaves. In: Thimann, K.V. (ed.), *Senescence in Plants.* CRC Press, Boca Raton, FL, pp .85-115.

Tsang, E.W.T., Bowler, C., Herouart, D., Van Camp, W., Villarroel, R., Genetello, C., Van Montagu, M. and Inzé, D. 1991. Differential regulation of superoxide dismutases in plants exposed to environmental stress. *The Plant Cell.* 3:783–792.

Tsukaya, H .2006. Genetics, cell cycle and cell expansion in organogenesis in plants. *J Plant Res* .119: 1–4.

Türkan, I., Bor, M., Özdemir, F., and Koca, H., 2005. Differential responses of lipid peroxidation and antioxidants in leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive P. vulgaris L. subjected to polyethylene glycol mediated water stress. *Plant Sci*. 168: 223–231.

Van Heerden, P.R.D., Swanepoel, J.W., and Kruger, G.H.K. 2007. Modulation of photosynthesis by drought in two desert scrub species exhibiting C3-mode CO2 assimilation. Environ. Exp. Bot. 61:124-136.

Vaughn, B.H., Ferretti, D.F., Miller, J., and White, J.W.C. 2004. Stable isotope measurements of atmospheric CO2 and CH4. In: de Groot PA, (ed.) *Handbook of Stable Isotope Analytical Techniques*. Amsterdam, the Netherlands: Elsevier, 272–304.

Vendruscolo, E.C.G., Schuster, I., Pileggi, M., Scapim, C.A., Molinari, H.B.C., Marur, C.J., and Vieira, L.G.E. 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant. Physiol*. 164(10): 1367–1376.

Verslues, P.E., Agarwal, M., Katiyar-Agarwal, S., Zhu J., and Zhu, J.K. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J*. 45: 523-539.

Verslues, P.E., and Zhu, J.K. 2005. Before and beyond ABA: upstream sensing and internal signals that determine ABA accumulation and response under abiotic stress. *Biochem. Soc. Trans.* 33: 375–379.

Villadsen, D., Rung, J.H., and Nielsen, T.H. 2005. Osmotic stress changes carbohydrate partitioning and fructose-2, 6-bisphosphate metabolism in barley leaves. *Funct. Plant Biol.* 32: 1033–1043.

Villagra, P.E., and Cavagnaro, J.B. 2006 Water stress effects on the seedling growth of *Prosopis argentina* and *Prosopis alpataco*. *J. Arid Environ*. 64: 390–400

Volaire, F., Thomas, H., and Lelievre, F. 1998. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: I. Growth, death, water relations and solute content in herbage and stubble. *New Phytol.* 140:439-449

Voleti, S.R., Kasturi Bai, K.V., and Rajagopal, V. 1993. Water potential in the leaves of coconut (Cocos nucifera L.) under rainfed and irrigated conditions. In: Nair M.K, Khan H.H., Gopalasundaram, P., Bhaskara Rao, E.V.V (eds). *Advances in Coconut Research and Development.* Oxford & IBH Publishing, New Delhi. pp. 243-245

Vyas, S.P., Garg, B.K., Kathju, S. and Lahiri, A.N. 2001. Influence of potassium on water relations, photosynthesis, nitrogen metabolism and yield of cluster bean under soil moisture stress. *Indian J. Plant Physiol*. 6: 30-37.

Wachira, F., Ngetich, W., Omolo, J., and Mamati, G. 2002. Genotypex environment interactions for tea yields. *Euphytica*. 127: 289-296.

Wang, F.Z., Wang, Q.B., Kwon, S.Y., Kwak, S.S., and Su, W.A. 2005. Enhanced drought toIerance of transgenic rice plants expressing a pea manganese superoxide dismutase. J. *Plant Physiol*. 162:465-472.

Wang, Y.B., Holroyd, G., Hetherington, A.M., and Ng, C.K.Y. 2004. Seeing 'cool' and 'hot'-infrared thermography as a tool for non-invasive, high-throughput screening of Arabidopsis guard cell signalling mutants. *J. Exp. Bot.* 55:1187–1193.

Watanabe, S., Kojima, K., Ide, Y., and Satohiko, S. 2000. Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* in vitro. *Plant Cell Tiss Org*. 63(3): 199-206.

Way, H., Chapman, S., McIntyre, L., Casu, R., Xue, G.P., Manners, J., and Shorter, R. 2005. Identification of differentially expressed genes in wheat undergoing gradual water deficit stress using a subtractive hybridization approach. *Plant Sci*. 168: 661-670.

Williams, R. F. 1946. The physiology of plant growth with special reference to the concept of net assimilation rate. *Ann. Bot.* 10: 41 – 72.

Xiao, X., Xu, X., and Yang, F. 2008. Adaptive responses to progressive drought stress in two *Populus cathayan*a populations. *Silva Fenn*. 42: 705-719.

Xu, P.L., Guo, Y.K., Bai, J.G., Shang. L., and Wang, X.J. 2008. Effects of long-term chilling on ultrastructure and antioxidant activity in leaves of two cucumber cultivars under low light. *Physiol. Plantarum*. 132: 467–478.

Xu, Z., Zhou, G., and Shimizu, H. 2009. Are plant growth and photosynthesis limited by pre-drought following rewatering in grass?. *J. Exp. Bot.* 60: 3737-3749

Xu, Z.Z., and Zhou, G.S. 2005. Effects of water stress and nocturnal temperature on carbon allocation in the perennial grass, *Leymus chinensis*. *Physiol. Plantarum*. 123: 272–280.

Yadav, S.K., Lakshmi, N.J., Maheswari, M., Vanaja, M., and Venkateswarlu, B. 2005. Influence of water deficit at vegetative, anthesis and grain filling stages on water relation and grain yield in sorghum. *Indian J Plant Physiol*. 10: 20-24.

Yang, F., Hu, J., Li, J., Wu, X., and Qian, Y. 2009. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. *Plant Growth Regul.* 58, 131–136.

Yang, H.M., and Wang, G.X. 2001. Leaf stomatal densities and distribution in Triticum aestivum under drought and CO2 enrichment. *Acta Phytoecologica Sinica*. 25: 312–316.

Yildirim, A., Oktay, M., and Bilaloglu, V. 2001. The antioxidant activity of the leaves of *Cydonia vulgaris. Turk. J. Med. Sci.* 31:23-27.

Yogameenakshi, 2002. Genetic analysis for drought tolerance in rice. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Zhang, M., Duan, L., Tian, X., He, Z., Li, J., Wang, B., and Li, Z. 2007. Unicanazole-induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. *J. Plant Physiol*.

Zhang, M., Duan, L., Zhai, Z., Li, J., Tian, X., Wang, B., He, Z., and Li, Z. 2004. Effects of plant growth regulators on water deficit-induced yield loss in soybean. In: Proceedings of the 4th International Crop Science Congress Brisbane, Australia.

Zhang, Y.P., Wang, Z.M., Wu, Y.C., and Zhang, X. 2006. Stomatal characteristics of different green organs in wheat under different irrigation regimes. *Acta Agron Sin.* 32: 70–75.

Zhu, H.S. and Huang, P.S. 1994. Soil water stress and active oxygen metabolism in rice. *J. Nanjing Agric. Univ*. 17: 7-11.

Zhu, J. K. 2002. Salt and Drought Stress Signal Transduction in Plants. *Ann. Rev. Plant Phys. Plant Mol. Biol.* 53: 247-273.

Appendix

Table 1. Weather parameters of Rain out shelter during the experimental period (Oct 2014-Jan 2015)

Date	Temperature(o C)	Humidity (%)	Light intensity (lux)
Oct 9,2014	33.5	86	610.5
Oct 16,2014	32.5	85	523.5
Oct 23 ,2014	32.3	83	508.3
Oct 30,2014	31.1	86	491.5
Nov 6,2014	31.9	85	485.8
Nov 13,2014	30.2	79	482.4
Nov 20,2014	31	76	512.1
Nov 27,2014	30.4	74	510.3
Dec 4,2014	30.7	72	497.7
Dec 11,2014	29.9	72	444.1
Dec 18,2014	29.6	72	423.2
Dec 25,2014	28.6	71	313.7
Jan 1,2015	29.4	71	309.2
Jan 8,2015	29.1	71	310.3

Abstract of the thesis

"Evaluation of selectively fertilized coconut hybrids (*Cocos nucifera* L.) for water use efficiency through Stable Isotope Discrimination

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ABSTRACT OF THE THESIS

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ABSTRACT

A study entitled "Evaluation of selectively fertilized coconut hybrids (*Cocos nucifera* L.) for water use efficiency through stable isotope discrimination" was undertaken with an objective to evaluate the selectively fertilized coconut hybrids for water use efficiency, and to study the mechanism of water stress tolerance in coconut, and to estimate genetic variability in coconut for water use efficiency through stable isotope discrimination. A pot culture experiment was conducted for three months in Rain out shelter of the Department of Plant Physiology, where one year old coconut seedlings of seven coconut varieties and hybrids (Kerasree, Keraganga, Malayan Yellow Dwarf, Gangabondam, West Coast Tall and selectively fertilized hybrids of Kerasree and Keraganga) were grown and evaluated for water use efficiency through gravimetric method. The experiment was laid out in CRD with equal sets of seedlings under two treatments viz 100 % Field Capacity (T_1) and 50 % Field Capacity (T_2) with 3 replications.

Water transpired from individual seedlings were estimated daily through gravimetry. The transpiration loss was replenished by adding specific quantity of water to maintain the plants at respective soil moisture levels. Total dry matter accumulation was determined through the initial and final samplings. Physiological and biochemical analyses were done at monthly intervals. At the end of three months, leaf samples from experimental seedlings were collected and sent to Isotopic Ratio Mass Spectrophotometric (IRMS) facility, UAS Bangalore for stable isotope discrimination. Meanwhile, leaf samples from adult palms of 30 coconut genotypes were collected from RARS, Pilicode and sent for stable isotope discrimination.

Among all genotypes, Kerasree S.F exhibited highest WUE (6.3 g/kg) under T_2 by accumulating maximum dry matter (188.33 g) with a comparatively lower volume of cumulative transpired water. Results of carbon isotope discrimination was negatively correlated with gravimetric data of WUE so that genotype with highest water use efficiency (Kerasree S.F) marks the lowest discrimination value for C^{13}

(19.90). Decreased stomatal frequency and stomatal conductance up to 3 months in all coconut genotypes resulted in decreasing transpiration rate under 50 % F.C (T_2). Relative water content was decreasing over the period of study under T_2 for all varieties and highest tissue moisture content was maintained by Kerasree S.F (80.19 %). Wax deposition under water stress was highest in Kerasree (3.93 mg/cm^2). Highest membrane integrity and chlorophyll stability index (99.19 %) under T_2 was noticed in MYD. Pigment components viz chlorophyll a, chlorophyll b, total chlorophyll and carotenoids decreased with the extent of stress. Total soluble protein content decreased in all coconut types under T_2. Proline content and activity of enzymatic antioxidants were high in selectively fertilized hybrid of Kerasree.

Genetic variation for C^{13} discrimination was studied in 30 coconut genotypes that includes Talls, Dwarfs and Hybrids and the data revealed a discrimination range of (18.37-21.07).

Among the coconut genotypes evaluated for WUE, hybrid Kerasree S.F was found to be the most water use efficient genotype under water deficit treatment, which coincides with its lowest value for C^{13} discrimination. Hence the study revealed that stable isotope discrimination can be used as an alternative for gravimetric method to screen out water use efficient genotypes. Moreover, efficacy of stress tolerance screening technique like selective fertilization is once again proved, which offers great prospects for drought tolerance breeding in perennials.