COMPARATIVE ASSESSMENT OF WHOLE PLANT WATER USE EFFICIENCY (WUE) OF COCONUT SEEDLINGS (*COCOS NUCIFERA*) TO DROUGHT TOLERANCE.

By

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(2012-20-120)

THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Comparative assessment of whole plant Water Use Efficiency (WUE) of coconut seedlings (*Cocos nucifera*) to drought tolerance"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled "**Comparative assessment of whole plant Water Use Efficiency (WUE) of coconut seedlings (*Cocos nucifera*) to drought tolerance**" is a record of research work done independently by Mr. Athul Boby C 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 him.

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We, the undersigned members of the Advisory committee of **Mr. Athul Boby C,** a candidate for the degree of B.Sc.-M.Sc. (Integrated) Climate Change Adaptation, agree that the thesis entitled **"Comparative assessment of whole plant Water Use Efficiency (WUE) of coconut seedlings (*Cocos nucifera*) to drought tolerance"** may be submitted by Mr. Athul Boby C in partial fulfillment of the requirement for the degree.

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Athul Boby C

Vellanikkara

The best time to plant a tree was twenty years ago. The second best time is now.

- A Chinese proverb

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Abbreviations

ASM	Available Soil Moisture
CGD	Chowghat Green Dwarf
MYD	Malayan Yellow Dwarf
KP	Kalpa Pratibha
KT	Kalpatharu
IPCC	Intergovernmental Panel on Climate Change
WUE	Water Use Efficiency
SOD	Super Oxide Dismutase
PPO	Poly Phenol Oxidase
POD	Peroxidase
CD	Critical Difference
mg	Milligram
g	Gram
f.wt	Fresh Weight
µg	Microgram
CRD	Completely Randomized design
ACCER	Academy of Climate Change Education and Research
KAU	Kerala Agricultural University
CPCRI	Central Plantation Crop Research Institute
ICAR	Indian Council of Agricultural Research
T_{max}	Maximum temperature
T_{min}	Minimum temperature
UNFCC	United Nations Framework Convention on Clima Change
No.	Number

Chapter 1

Introduction

The coconut tree (*Cocos nucifera*) is a member of the family Arecaceae (palm family) and the only species of the genus*Cocos*. Coconut can grow upto 30 m tall, with pinnate leaves 4–6 m long, and pinnae 60–90 cm long; old leaves break away cleanly, leaving the trunk smooth. Coconuts are generally classified into two general types: tall and dwarf. In India, the coconut palm is a major plantation crop which is grown in approximately 2 M ha. Coconut is extensively grown in the southern states of India and has a profound influence on both the culture and the rural economy of more than 10 million peoples. At an international level, coconut palms are grown in more than 86 countries around the world (Between 20^0N and 20^0S latitude) and the total production of coconut is around 61 billion nuts per annum (Asian Pacific Coconut Community, 2010). Top seven producers of coconut in the world are Indonesia, Philippines, India, Brazil, Sri Lanka, Thailand and Mexico (FAOSTAT, 2014). A mean annual temperature of 27^0C with a diurnal variation of 5^0C to 7^0C is required for optimum growth and maximum yield for coconut (Child 1964). A well distributed rainfall of about 2000 mm with a relative humidity of more than 60% is desirable for the coconut. Under drip irrigation, 40 liters of water per day is required. Coconut is salinity resistant and is grown in many soil types of India such as laterite, alluvial, red sandy loam, coastal sandy and reclaimed soils with a P^H ranging from 5.2 to 8.0.

The threat of climate change is projected to be more in coastal tract and hilly areas of India where plantation crop like coconut is the predominant crop which provides sustenance to more than 10 million people and contributes Rs. 83000 million annually to the Gross Domestic Product (GDP) of the country. Coconut is grown between 20° N and 20° S latitude. It can be grown even at 26° N latitude but the temperature is the main limitation. The optimum weather conditions for good growth and nut yield in coconut are well distributed annual rainfall between 130 and 230 cm, mean annual temperature of 27 °C, abundant sunlight ranging from 250 to 350 Wm^{-2} with at least 120

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hours per month of sun shine period. Since, it is humid tropical crop it grows well above 60 per cent humidity.

Coconut has been considered as extravagant in water consumption. Daily water requirement is estimated as high as 120 L by an adult coconut depending on soil moisture content and evaporative demand of the atmosphere. Mean *E* varied from 0.09 to 1.52 L day^{-1} m^{-2} leaf area in 3.5-year-old dwarf coconut palms, as estimated by measurements of xylem sap flux density. Water deficit stress affects coconut production in almost all coconut growing countries, since it is mainly a rainfed plantation. Hence, the productivity is low in these areas by ~50 per cent of irrigated gardens. Coconut faces summer dry spells each year apart from the frequent occurrence of drought. Coconut plantation during the last 3 to 4 years is facing the severe threat of climate change in Karnataka, Tamil Nadu, Kerala and Andhra Pradesh which are the major coconut growing states. Lakhs of coconut trees were withered during the summer months of 2013 and 2014 in south interior Karnataka due to scanty rainfall. Almost similar fatality happened in some districts of Tamil Nadu. During the summer of 2016 vast tracts of coconut withered in Northern Kerala due to extended drought. Though some trees recover with the arrival of monsoon but the production will be affected at least for 3 years. Being perennial in nature,coconut palm had a long duration from the initiation of inflorescence primordia to nut maturity (~44 months) with longer pre-fertilization period (~32 months) than post-fertilization (12 months) period. Hence, the impact of drought occurring at any of the critical stages of the development of inflorescence affects nut yield not only in current year but also in next three years to follow, thus makes the problem more severe.

Many different approaches have been followed to assess the physiological responses of coconut to sub-optimal water supply such as field scale measurements of transpiration, growth and yield, leaf level responses of container grown plants and accumulation of osmolytes that may confer drought tolerance. Tolerance or resistance to drought usually implies some improvement or maintenance of metabolic process that enable a plant to regulate cell water status and maintain leaf turgor under

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stressful conditions. One way to achieve this is by partially closing the stomata, which restricts transpiration and water loss, but may also decrease carbon assimilation. While some studies have focused on finding or developing specific drought tolerant genotypes (defined as having improved yield under drought conditions), others have identified genotypes with greater whole plant water use efficiency. Mathematically, whole plant water use efficiency (WUE) is simply the ratio of accumulated plant biomass to the amount of water used. Yield is defined as the product of the ratio of nut mass to total plant mass (harvest index), the amount of water transpired by the crop and the crop transpiration (or water use) efficiency. Therefore, plants with high WUE either maintain or match nut yield compared to other genotypes under the same drought conditions.

In coconut earlier photosynthesis and transpiration was measured under water deficit stress to calculate the intrinsic water use efficiency. However, there is no report on the whole plant water use efficiency. Hence, a study was conducted at ICAR-CPCRI, Kasaragod involving two dwarf (Malayan Yellow Dwarf (MYD) and Chowghat Green Dwarf (CGD) and two tall varieties of coconut seedlings (Kalpatharu (KT) and Kalpa Pratibha (KP)) with the following objectives

Objective

To determine the whole plant WUE of tall and dwarf coconut genotypes and to phenotype the contrasting WUE genotypes for drought tolerance.

Chapter 2

REVIEW OF LITERATURE

2.1 Growing Conditions of Coconut

Of all the cultivated trees in the world, the coconut has the widest geographical range (Ghai and Wadhi, 1983). Coconut is grown between 20°N longitude and 20°S latitude (Woodroof, 1970;Persley, 1992).The mean annual temperature for optimum growth and maximum yield has been found to be 27°C with a diurnal variation of 5°C to 7°C, abundant sunlight ranging from 250 to 350 Wm^{-2} with at least 120 hours of sun shine period per month. Also, a well distributed rainfall of about 2000 mm and above 60 per cent humidity is ideal for the proper growth and higher yield (Child, 1964). However, coconut generally requires 40 liters of water per day (Rajagopal and Kasturi-bai, 1999). It can tolerate a wide range of soil conditions, the major soil types that support coconut in India are laterite, alluvial, red sandy loam, coastal sandy and reclaimed soils with a pH ranging from 5.2 to 8.0.

2.2 Climate Change and Its Impact on Coconut

Each of the last three decades has been successively warmer at the Earth's surface than any preceding decade since 1850. The period from 1983 to 2012 was very likely the warmest 30-year period of the last 800 years in the Northern Hemisphere, where such assessment is possible (high confidence) and likely the warmest 30-year period of the last 1400 years (medium confidence) (IPCC, 2014). Thus, there is enough scientific evidence to conclude that the change in climate had been occurring since the dawn of industrialization. The change in climate has also affected the precipitation. There is also increase in the intensity or frequency of many ecosystem disturbances like drought (medium confidence) and impact of various climate related extremes such as drought reveal that several ecosystems and human systems are significantly vulnerable (very high confidence) (IPCC,2014).

Plantation agriculture is one of the high priority sectors where the impacts of climate change exceed the tolerance limit, with implications for the livelihoods of millions of people who are dependent on this sector as well as contribution of coconut to the national GDP (Ranasinghe and Thimothias 2012). As coconut is perennial in nature, a seedling of coconut would experience the increased CO_2 concentration, temperature, changed rainfall pattern as it ages in the next 50 years of its economic yield producing lifespan (Nareshkumar and Aggarwal, 2013). It is reported that amount of rainfall and length of dry spells largely influences the coconut productivity in different agro-climatic zones of India (Nareshkumar et al., 2008). Minimum temperatures above 10 °C enhance flowering nevertheless, temperature greater than 10 °C for one month cause nut fall (Nareshkumar et al., 2008). The coconut simulation models predicted that yield will decline in the east coast region by about 2 percent in 2020, 8 percent in 2050 and 31 percent in 2080. The yield will increase in states such as Kerala, Tamil Nadu and Karnataka and is projected to decline in Andhra Pradesh, Orissa and Gujarat (Nareshkumar et al., 2008).Coconut is mainly grown as a rainfed crop and the productivity is about 50% less in these areas than in irrigated gardens. Coconut faces summer dry spells each year apart from the frequent occurrence of drought years. Being perennial in nature, coconut palm had a long duration from the initiation of inflorescence primordia to nut maturity (about 44 months) with longer pre-fertilization period (about 32 months) than post-fertilization period (12 months). Hence, the impact of drought occurrence during any of these critical stages of development of the inflorescence results in a yield loss (Rajagopal et al., 1996; Rajagopal 2000). This reduction in yield can be observed for a further three more years, making the problem more severe (Nareshkumar et al., 2002). In worst affected areas, at least a period of four years is required to recover completely. Any water deficit during this recovery period hampers the recovery rate of coconut. Water deficit at early stages of coconut development can even lead to seedling mortality. Hence development of cultivars or hybrid with high drought tolerance capacity and stable yields is essential. As coconut is predominantly grown as a rainfed crop, drought stress affects coconut production in almost all countries where it is grown. To sum up drought tolerance in coconut is the cumulative effect of several inductive

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morphological, anatomical, Physiological and biochemical mechanism (Rajagopal V and Kasturi-bai. 2002)

2.3 Water Use Efficiency

Water use efficiency (WUE) is widely used to evaluate the plant adaptation to limited water supply (Araus et al., 2002; Moghaddam et al., 2013). WUE may be estimated as the ratio between net photosynthesis (Pn) and transpiration (E), which is known as instantaneous water use efficiency (physiological index) (Mediavilla et al., 2002; Polley, 2002), as the ratio between Pn and stomatal conductance (gs), which is known as intrinsic water use efficiency (physiological index) (Boyer, 1996; Pascual et al., 2013) and as the ratio of dry matter accumulation over time to the amount of water transpired which is identified as biomass/yield water use efficiency (agronomic index). Reduction in fresh weight due to drought stress has also been reported earlier for a number of plant species (Taylor and Ashcroft, 1972; Petropoulos et al., 2008; Álvarez et al., 2011; Cerekovi ˇ c′ et al., 2014). Studies in maize, pistachio, wheat, sorghum, ground nut, Aleppo pine, cup Plant, lucrenegrass and legumes (Hebbar et al., 1994; Esmaeilpour et al., 2016; Hai-dong et al., 2016; Tolk et al., 2015; Choury et al., 2016; Hassan et al., 2017; Schoo., et al., 2017) have shown that under drought conditions, the WUE of drought resistant varieties increases, thus making higher WUE of a variety a desirable trait for countering the drought. Studies have also shown that in an irrigated field having different soil texture, plants of same species and variety grown in sand predominant soil texture tends to show more WUE (Tolk and Howell, 2015). WUE also increases on the type of irrigation as drip irrigation has been demonstrated to show the highest WUE (Mahmoud and Ahmed, 2016). During water stresses conditions, the plants tend to close their stomata to decrease the water loss through transpiration, increasing their stomatal resistance. This is achieved by release of abscisic acid (ABA) which itself is regulated with the help of enzymes like 9-*cis*-epoxycarotenoid dioxygenase 3 *(NCED3).*In plants, during drought, mutations in *nced3* gene encoding *NCED3* enzyme reduced the drought tolerance, while the overexpression of *NCED3* caused enhanced drought tolerance and/or increased WUE in several plant species (Luchi et al., 2001; Tung et al., 2008). Although instantaneous WUE did not show any significant difference, it was 13% higher in

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unirrigated palms. Higher WUE under unirrigated condition has been reported in wheat (Aggarwal and Sinha, 1983) and in coconut (Rajagopal et al., 1989). Higher WUE associated with higher stomatal resistance was reported by Fischer and Turner (1978). Efficiency of dry matter production has been found to be a heritable trait (Ramadasan et al, 1985). Higher dry matter (DM) production characteristics of some of the drought tolerant types implies the higher stomatal regulatory mechanism of these types leading to higher water use efficiency (WUE) (Kasturi-bai et al.,1996). Generally, the physiological WUE-Pn/Transpiration rate was low during the post monsoon period and the physiological WUE increased as summer approached. However, palms grown at Kidu and Veppankulam regions showed a reverse trend. Irrigated palms and palms provided with water conservation measures maintained relatively high physiological WUE compared to rainfed palms during summer months. However, palms grown at Veppankulam maintained the physiological WUE from post monsoon till summer. Overall, the observations showed that there are three types of physiological WUE responses from the palms – Low physiological WUE during post-monsoon period and high Physiological WUE during summer (in Ratnagiri and Ambajipeta); high physiological WUE during post monsoon and low physiological WUE due to reduction in the Pn rate during summer and maintenance of the transpiration rate (in Kidu); due to reduction of both Pn and transpiration rate, maintenance of Physiological WUE from the post monsoon period throughout the summer period (in Veppankulam). Drip irrigation in coconut palms also has shown improved physiological WUE (Nareshkumar et al., 2002). WUE has been shown to vary among varieties and also among ecotypes of the same variety (Passos et al., 1999; Prado et al., 2001; Gomes et al., 2002). In this study we have measured the whole plant water use efficiency of coconut genotypes across different moisture regimes.

2.4 Morphological parameters

To cope with drought stress, plants respond with complex physiological and biochemical changes that influence their growth and morphology. Relatively rapid physiological changes may be followed by alterations in shoot and root growth,

morphology, and anatomy that affect plant functioning in a longer time scale (Nosalewicz et al., 2016)

In a study conducted by (Zaher-Ara et al., 2016) on ten different citrus plants, drought stress significantly decreased the length, dry weight and fresh weight of both the root and shoot along with decrease in the total tissue water content and total number of seed germination. The degree of the drought effects also had a positive correlation with the intensity of the drought applied to the plants. Water availability is the key factor for dry matter production in plant. Low water availability decreases water and nutrient uptake, photosynthetic rate and translocation of photo assimilates. An experiment conducted by Nahar et al. (2011) with the four tomato genotypes proved the reduction in dry matter production under water deficit condition, similar to those reported by Aragon (1988).

Understanding the control processes of dry matter production will help to understand the adaptive mechanism of plants to water stressed or water limited environments (Smith, 1989). The importance of studies on dry matter production for the improvement of coconut productivity has been highlighted by Foale (1993). Annual Dry matter production varied greatly between unirrigated and irrigated conditions. The dry matter production of the reproductive parts of the palms are affected more than the vegetative parts (Kasturi-bai et al., 1997). Kasturi-bai et al. (1996) observed that hybrids produced higher Reproductive dry mass than vegetative dry mass when compared to tall varieties in unirrigated palms than in irrigated palms. The fact that vegetative dry mass production did not show any significant difference between the irrigated and unirrigated palms reveal that the partitioning of dry mass towards yield is affected more by moisture stress than the vegetative parts. Higher vegetative dry mass production at the expense of reproductive dry matter production during water deficit condition in coconut palms has been also observed by Rajagopal et al. (1989). Reproductive dry mass production mainly

depends on the dry weight of the nut and the partitioning of dry matter towards its components – husk, shell and copra (Kasturi-bai et al., 1996).

Studies have shown that the average leaf number in the drought affected plants decrease with respect to control plants of the same variety and drought susceptible varieties shows higher decrease in number of leaves when exposed to drought than drought tolerant varieties (Jangid and Dwivedi., 2017). This result was also supported the observations made under previous projects of Ors and Suarez, 2017; Hussain et al. (2008); Bhatt and Rao (2005) and Sankar et al. (2007). Studies in cotton proved that cotton plants shed their leaves during water deficit period.Thus the functioning of PSII and the photosynthetic electron transport systems of cotton plants show a relatively high stability underwater deficit. This is due to the fact that the total number of PSII system in the whole plant decreases creating lesser ROS (Zhang et al., 2010; 2011). Senescence program is accelerated by biotic and abiotic stresses. Oxidative degradation in lipid, protein and DNA content by ROS stimulates ageing and reduces the life duration of the plant (Jangid and Dwivedi, 2016).

2.5 Physiological parameters

2.5.1 Photosynthesis rate

Coconut canopy intercepts 72% of incidental light (Moss 1992) and light saturation of photosynthesis occurs around 1400 mmol m^{-2} s^{-1}, typical to C3 species. It is well known that water deficit decreases photosynthetic activity due to stomatal closure and/or the reduced activity of photosynthetic enzymes (Lawlor and Cornic, 2002; Flexas et al., 2006) and/or from changes in photosynthetic metabolism (Lawlor, 2002). Many studies have shown that the decreased photosynthesis under water stress can be associated with the perturbations of the biochemical processes (Graan and Boyer, 1990; Lauer and Boyer, 1992). As drought sets in, the initial response of plants is to close the stomata thereby increase its stomatal resistance (Chaves, 1991). Although this process can conserve water by reducing evapo-transpiration, this process automatically reduces CO_2 exchange with the atmosphere thereby reducing the total photosynthesis rate (Pn). During extreme drought conditions, photosynthetic rate decrease further due to increased metabolic activities of enzymes like Rubisco along with higher stomatal resistance (Bota

et al., 2004). Also the effect of excess light (EL) can cause severe damage to plants. EL induces photo-oxidation, which results in the increased production of highly reactive oxygen intermediates that negatively affect biological molecules and, if severe, a significant decrease in plant productivity (Li et al., 2009). Water stress that induces a decrease in leaf water potential and in stomatal opening, leading to the down-regulation of photosynthesis-related genes and reduced availability of CO_2, has been known as one of the major factors for the reduced productivity observed in the EL induced stress (Osakabe and Osakabe, 2012).Net photosynthesis rates were high during post monsoon period. However, it declined as the summer approached. However, palms receiving irrigation retained their Pn rates of the post monsoon period even during summer periods compared to the rainfed palms whose Pn rates decreased. It must be also noted that in the irrigated palms, apart from the post monsoon Pn rates, the overall canopy Pn rates may be significantly high, thus contributing towards higher yield in these palms when compared to rainfed palms. Lower stomatal conductance in rainfed palms during summer months also contributed towards lower Pn rates in these palms. However, when irrigation was provided to these rainfed palms, the Pn rates increased and became almost equal to the Pn rates of post monsoon period (Naresh Kumar et. al ., 2006) Earlier studies have shown that the photosynthetic rates were low in rainfed coconut palms (Naresh Kumar et al.,2002). Adding soil moisture conservation techniques resulted in higher photosynthetic rates which ultimately resulted in higher nut yields (Naresh Kumar et al., 2006).The photosynthetic rates in irrigated palms were significantly higher than in rainfed palms. In rainfed palms all leaves had lower Pn rates. Younger leaves have higher Pn rates than in older leaves, thus optimizing the efficiency of light use (Lawlor 1995)

2.5.2 Chlorophyll Fluorescence

Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three facts: it can be used to drive photosynthesis, excess energy can be dissipated as heat or it can be re-emitted as light, chlorophyll fluorescence. Any increase in the efficiency of one will result a decrease in the yield of the other two (Maxwell and Johnson 2000) Chlorophyll fluorescence measurements is a useful technique for assessing the plant stress responses (Daymond and Hadley, 2004). Fluorescence can give information about

the plant's capacity to withstand various environmental stresses and the extent to which these stresses have damaged the photosynthetic apparatus. FV/Fm which has been used to quantify PS II efficiency (Kraus and Weis 1991) showed a strong linear relationship with LWP. Fv/Fm reduced upto 33% in coconut was observed, thus, showing irreparable damage to the PS II system. The observations clearly revealed that in coconut PS II is highly sensitive to soil water deficit and micrometeorological variables. Hence, proper maintenance of soil and leaf water status is highly crucial for protecting the photosynthetic apparatus and survival of coconut seedlings during summer months (Kasturi-bai et. al., 2006)

2.5.3 Stomatal Response

Plant responses to drought stress depend on timing, speed, severity and length of the drought event (Anjum et al., 2011; Chaves et al., 2002; Praba et al., 2009). Stomatal closure is one of the first responses to drought stress (Anyia and Herzog, 2004; Arndt et al., 2001).In response to a water deficit stress, ion- and water-transport systems across membranes function to control turgor pressure changes in guard cells and stimulate stomatal closure. Endogenous ABA (Abscisic Acid) is rapidly produced during drought, triggering a cascade of physiological responses, including stomatal closure. Studies in *Arabidopsis* have showed that this production of ABA is regulated by a signal transduction network - 9-*cis*-epoxycarotenoid dioxygenase3 *(NCED3)* which catalyzes a key step in ABA biosynthesis, and *NCED3* expression is rapidly induced by drought stress in a vascular tissues, thus eventually contributing to an increase in the stomatal resistance of the plants (luchi et al., 2001; Endo et al., 2008; Behnam et al., 2013). Increased stomatal and cuticular resistance is often considered as an important drought resistant character in variety selections (Farooq et al., 2012). Drought studies in wheat and beans have showed that stomatal resistance increase with increases in the drought intensity (Lu and Zhang, 1999). The stomatal resistance in coconut which was low during the wet months increased rapidly from January reaching a peak in March followed by a decline. Water loss from coconut palm is reduced in the dry season compared to wet season due to higher stomatal resistance (Kasturibai et al., 1988). Stomatal conductance in coconut was higher under irrigated as compared with the unirrigated conditions. The

stomatal conductance was higher in hybrids when compared to tall varieties in coconut (Kasturibai et al., 1977). Schulze and Hall (1982) showed that under drought situation, at ambient CO_2 concentration, stomata of C_3 plants generally decrease their aperture prior to the changes in the photosynthetic capacity. In *Nerium*sp., similar observations have been recorded (Gollan et al., 1985).

2.5.4 Chlorophyll Content

Chlorophyll content also decreases under drought stress, it may be because of reduction in activity of the enzymes involved in chlorophyll synthesis (Ashraf and Karim 1991), or may be due to increase in chlorophyll break down (Kaewsuksaeng, 2011). Plants can monitor chloroplast status by plastid-to-nucleus signals, as plastid-to-nucleus retrograde signaling. This signaling system can regulate the expression of genes that function in the chloroplast. The retrograde signaling plays an important role in regulating the chloroplast production processes and also in the adaptive responses to environmental stresses (Chan et al., 2010). Chlorophyll intermediates, such as Mg-protoporphyrin IX (Mg-Proto), control the expression of nuclear genes in plants exposed to EL conditions, acting as a retrograde signal. The *genomes uncoupled* (*gun*) mutants, *gun4* and *gun5*, exhibit impaired generation of Mg-Proto that has been shown to act as a signal to repress various light-harvesting Chl a/b-binding *LHCB* gene expression in *Arabidopsis*, thus reducing the chloroplast production during water stress, thus reducing total chlorophyll content of the plant (Mochizuki et al., 2001; Strand et al., 2003; Pontier et al., 2007).

2.5.5 Leaf water potential

Water potential (leaf) in a plant, which is the energy level of water, is controlled by the availability of water from the soil, the demand for water imposed by the atmosphere and the resistance to water movement within the plant (Dee Roo, 1969). The changes in water status of plants depend on the evaporative demand in the atmosphere. Significantly higher LWP was observed in the coconut palms with irrigation than in the palms without irrigation. The low LWP in the palms under no irrigation induced stomatal closure thus reducing further water loss by transpiration. As compared with unirrigated

condition, irrigated palms showed significantly higher transpiration rates. Hybrids have relatively higher transpirational loss of water under rainfed conditions as compared to tall varieties like WCT (Kasturibai et al., 1997)

2.6 Biochemical parameters

2.6.1 Total sugar

In drought affected plants, the amount of total soluble sugar significantly increased in relation to the decreased water potential provided by the drought within the shoot system (Zaher-Ara et al., 2016). This accumulation could be the result of a greater degree of conversion of starch into soluble sugars (Turner et al., 1978) and/ due to lower sugar utilization. During water stress, enzymes activity of acid invertase, neutral invertase, phosphate synthase and sucrose synthase showed an increasing trend (Ghate et al., 2016). Thus, drought stress usually leads to an increase in starch hydrolysis and reduction in sucrose translocation, with the maintenance and/or even build-up of concentrations of reducing sugars in leaf tissue (Bunce 1982; Chaves 1991; Campos et al., 1999; de Souza et al., 2005)

2.6.2 Protein Content

In a study conducted by Vartania et al. (1987), the total soluble protein content increased, resulting in the increased water potential of the plant. Water stress affects the protein levels in plants, but the results of different investigators are contradictory. Some authors have reported decreased protein levels in plants under water stress (Pierre and Savoure, 1990; Roy-Macauley et al., 1992). Others have found an absence of deleterious effects of drought on protein levels in plants (Todd and Basler, 1965). Increases in plant protein levels have also been reported (Singh and Rai, 1982). The protein content may increase due to two reasons. One reason could be due to some degree of biosynthesis of so called "stress proteins", i.e. heat shock proteins including dehydrins that accumulate in plants in response to environmental stresses (Close, 1996, 1997; Svenssonetal., 2002; Marian et al., 2003). The second reason could be due to the production of water soluble proteins thus increasing the osmotic potential of the plants and water soluble proteins like proline has ROS scavenging property too (Anjum et al., 2000)

2.6.3 Free Radical Scavenging Enzyme Activities

Superoxide Dismutase (SOD) Peroxidase (POD), Catalase (CAT) and polyphenol oxidase (PPO) are the major scavenging enzymes in plants whose purpose is to eliminate the threat posed by ROS. ROS production is one of the consequence of drought stress. Superoxide (O^{2-}), singlet oxygen (O^{2}), hydroxyl ions (OH^{-}), and hydrogen peroxide (H_2O_2) are accumulated in plant cell during the drought stress which have harmful effects on nucleic acids, proteins, and lipids (Smirnoff 1993).Co-operation among these enzymes is essential for the effective protection from ROS (Scebba et al., 1998).The limitation to CO_2 assimilation may lead to an imbalance between photochemical activity at photosystem II (PSII) and electron requirement for photosynthesis, leading to the plants being exposed to excess energy. Excess energy is potentially harmful to PSII reaction centers due to over reduction of the photosynthetic electron chain which inevitably increases the production of ROS (Asada, 1999). Plant has specific antioxidative defense mechanism to combat the effect of these toxic elements by producing antioxidants. Accumulation of superoxide dismutase (SOD) scavenges O_2- to H_2O_2 (Bowler et al., 1992), while peroxidase (POD) and catalase (CAT) convert H_2O_2 to H_2O at different cellular locations (Asada 1999).The recent investigations carried out in tomato (*Lycopersicon esculentum* Mill.) under drought stress (Jangid and Dwivedi, 2017) also supports this claim.

2.6.4 Epicuticular Wax

Cuticle, which covers the primary aerial surface of terrestrial plants, is thought to be a critical evolutionary adaptation that allowed the first plant to colonize land (Samuels et al., 2008). Wax content on the plant tissue plays an important role in the plant's response towards environmental stress like drought and water logging conditions. (Palmer 1992; Hwang et al., 2002). The cuticular layer is composed of cutin and wax and important to the land plants (Riederer 2006; Raffaele et al., 2008; Schreiber 2010). The epidermal wax can be divided into epicuticular wax and intracuticular wax, which are deposited outside of the cuticle and in the cuticular mixtures, respectively (Pollard et al., 2008; Yeats and Rose 2013; Go et al., 2014). The compositions of cuticular cutin and wax vary among species, organs and tissues. Cuticular wax is a mixture of lipids mainly

composed of very-long-chain fatty acids (VLCFAs), primary and secondary alcohols, aldehydes, ketone, alkanes and wax esters (Broun et al., 2004; Samuels et al., 2008) Cutin is vital elements for the regulation of epidermal permeability and non-stomatal water loss (Schreiber 2010; Burghardt and Riederer 2003), and play crucial roles in protecting plants against insects, pathogens, UV light, and frost (Fiebig et al., 2000). In addition, it was reported that the wax content is associated with pollen fertility and other agronomic traits (Aarts et al., 1995; Jung et al., 2006). It has been reported that the amount of cuticular wax increases by approximately two fold in drought-treated *Arabidopsis* and tree tobacco (*Nicotianaglauca*) compared with plants grown in well-watered conditions (Cameron et al., 2006; Kosma et al., 2009). Similarly, total wax loads of cotton (*Gossypium hirsutum*) and sesame (*Sesamumindicum*) leaves increased by approximately 70 and 30 % underwater stress conditions, respectively (Bondada et al., 1996; Kim et al., 2007). More recently, it has been reported that over expression of several genes encoding transcription factors that activate cuticular wax biosynthesis increases drought tolerance in transgenic plants. For example, over expression of WIN1/SHN1 gene encoding an AP2/EREBP family transcription factor increased total wax amounts by six fold and confers drought resistance in transgenic *Arabidopsis* (Aharoni et al., 2004). Increase in cuticular wax content and resistance to drought were observed in transgenic alfalfa (*Medicagosativa*) and *Arabidopsis* over expressing *Medicago truncatula* WXP1 and WXP2 genes (Zhang et al., 2005; 2007).Studies in sorghum, camelina, garden cress, *Arabidopsis thaliana* (Bao et al., 2016; Lee et al., 2014; Mackova et al., 2013; Seo et al., 2011) have shown that the epicuticular wax increases with increase in drought intensity.Naresh Kumar et al. (2000) and Kurup et al. (1993) have reported similar increase in the ECW content as the water stress increase in coconut.

2.6.5 Membrane leakage

Cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells. The method is based on an *in vitro* stress of leaf tissues by a PEG

solution and a subsequent measurement of electrolyte leakage into an aqueous medium (Sullivan and Ross 1979). It has an enduring appeal because it requires readily available and inexpensive equipment, it is not destructive of whole plants, is easily used on plant material from a variety of cultural systems and it is suitable for analyzing large number of samples. It has been demonstrated recently that electrolyte leakage measurements may be correlated with several physiological and biochemical parameters conditioning the plant responses to environmental conditions such as spectral reflectance (Garty et al., 2000; Vainola and Repo 2000), antioxidative enzyme synthesis (Liu and Huang 2000; Sreenivasulu et al., 2000), membrane acyl lipid concentrations (Lauriano et al., 2000), water use efficiency (Franca et al., 2000; Saelim and Zwiazek, 2000), transverse relaxation time of leaf water (Maheswary et al., 1999), stomatal resistance, osmotic potential and leaf rolling index (Premachandra et al., 1989). It is therefore not surprising that electrolyte leakage has been recommended as a valuable criterion for identification of stress resistant cultivars in several crop species (Leopold et al., 1981; Stevanovic et al., 1997). In the washing treatment, PEG stressed condition, rehydrating condition of durum wheat the major part of electrolytes were removed during washing treatment. During the rehydration period PEG influenced electrolytic leakage is increased. When the PEG concentration is high, larger will be the increase of electrolytic leakage in the initial condition. After re hydration there was no further increase in the electrolytic leakage (Bajji et al., 2001a).Similar studies by Khan et al. (2017) and Bajji et al. (2001b) showed that the electrolytic leakage increased as the drought or water stress increased.

2.6.6 Lipid Peroxidation

Lipid peroxidation is the oxidative degradation of lipids and is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism which can be provided by the ROS formed due to water stress in the plant. MDA (Malondialdehyde) is a naturally occurring product of lipid peroxidation and it is carcinogenic and mutagenic substance. When the MDA is reacted with DNA, deoxyguanosin and deoxyadenosine is formed (Marnett, 1999). In seven species of Avena, plants subjected to moisture stress the level of lipid peroxidation increased in terms of malondialdehyde content. Some *Avena* species

shows high level of MDA which means that they have high lipid peroxidation and high membrane permeability. And when compared to varieties which are more susceptible to water stress, tolerant varieties produce less amount of Malonedialdehyde and so only such varieties have adaptability under stress conditions (Pandey et al., 2009). When the effect of water stress on *Triticumaestivum* L was investigated, both the MDA content and the proline contents was increased under water stress. The malondialdehyde (MDA) increased with drought stress in wheat seedlings, but uniconazole reduced MDA accumulation of stressed soybean (Tatar and Gevrek 2008; Zhang et al., 2007). In an experiment involving two mosses, one drought tolerant *Troularurali* and an drought sensitive *Cratoneuron filicinum*, in the drought tolerant moss, the lipid peroxidation declined during the slow drying and gradually increased when re hydrated. And in the case of drought sensitive moss, there was a gradual increase in the lipid peroxidation during slow drying and declined during rehydration (Dhindsa et al., 1981). Thus estimation of MDA content during water stress can help in the identification of drought tolerant cultivars.

By keeping the above knowledge in mind, in this study in addition to the whole plant water use efficiency (WUE), the morphological, physiological and biochemical traits responsible for imparting drought tolerance in dwarf and tall coconut seedlings was studied.

CHAPTER 3
MATERIALS AND METHODS

A study was conducted to determine the water use efficiency (WUE) of coconut seedlings under water deficit stress and to ascertain the morphological, physiological and biochemical traits imparting tolerance to water deficit stress in coconut.

3.1. LOCATION AND GEOGRAPHY

The experiments were conducted at the net house of ICAR- Central Plantation Crop Research Institute (ICAR-CPCRI), Kasaragod Kerala, India. CPCRI is located at 12° 18' N latitude and 75° E longitude, and at an altitude of 10.7m above mean sea level. This region receives approximately 3400 mm average annual rain fall. Average maximum temperature (in summer) is about 31.5°C and the average minimum temperature (winter) is about 21.3°C. The average relative humidity of this region is about 88% and the predominant soil type is sandy loam with the pH of 4.3- 5.5.

3.2. EXPERIMENTAL SETUP

For the present investigation four coconut varieties two dwarf *viz*., Chowghat Green Dwarf (CGD) and Malayan Yellow Dwarf (MYD) and two tall viz. Kalpatharu (KT) (Selection of Tiptur Tall) and Kalpa Pratibha (KP) were selected. These are the most popular dwarf and tall varieties grown in different parts of India. Besides, these varieties have been short-listed from the extensive screening procedures to identify coconut cultivars for better water use efficiency (WUE) characteristics.

The experiment was carried out in large plastic buckets (64×49 cm) of 100 kg dry soil capacity. Outer surface of the plastic buckets were painted black to prevent the entry of sunlight. Two holes were made on the opposite ends at the bottom of the bucket to facilitate draining of the excess water. Then small mud tiles were placed inside the buckets parallel to the drainage holes so that the holes wouldn't be choked by the soil later. Half of the bucket was filled with locally available soil which is of sandy loam in texture. After filling the buckets to half with the soil, one year old coconut seedling was

transplanted from the nurseryon 11 July, 2016. The seedlings along with their nuts were placed at the current soil level and then the bucket was filled with soil leaving two inches from the top of the bucket. Each variety was grown in 15 buckets (15 seedlings of each variety) and these 60 pots were arranged in a factorial completely randomized design (as shown in Fig 3.1). In addition to the above 15 pots, two more pots with seedlings were maintained for each variety and kept next to the rest of seedlings. In all the pots soil was continuously wetted for two days for settling the soil and extra soil were added if deemed necessary. After the soil was settled, normal watering was provided for all the 68 plants for next four months for the establishment of seedlings. These seedlings were used for the determination of water use efficiency (WUE) and for phenotyping for the morphological, physiological and biochemical traits which imparts tolerance to water deficit stress.

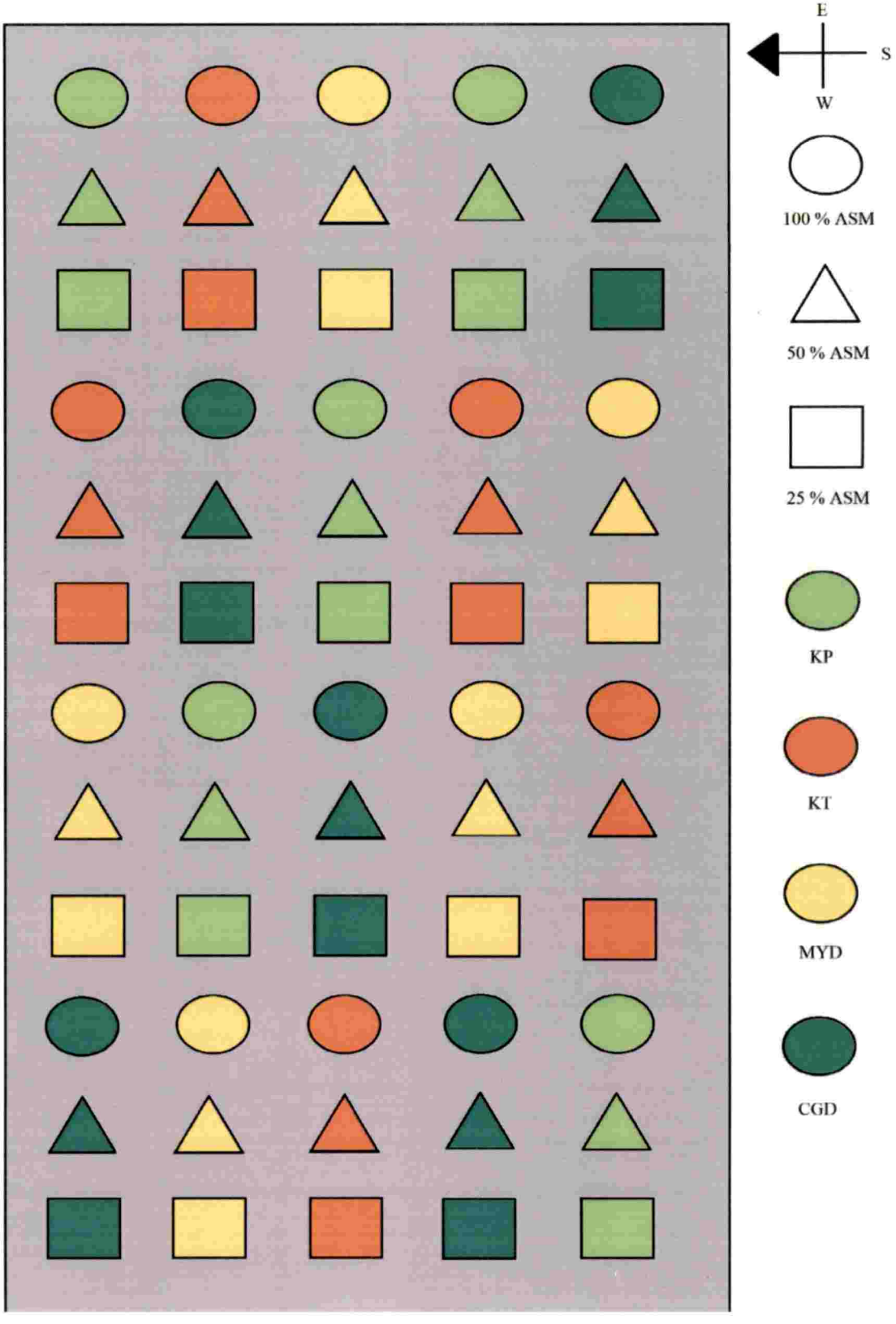

Fig 3.1 Layout of the experiment depicting the randomization of treatments

Fig 3.2 Picture showing the growth of coconut seedlings in large buckets

3.2.1. Cultural Practices

Recommended dose of fertilizers (NPK) along with vermicompost at a rate of 1000 g per plant for every one month were applied and seedlings were maintained free from pests and diseases. Various soil-moisture stress treatments were imposed from 07 November 2016 and observations were recorded for seven months and plants were uprooted on 04 May, 2017. Morphological observations were taken at an interval of 15 days. Physiological and biochemical observations were recorded from the top most fully opened leaf at periodical intervals.

3.2.2. Imposition of Water-Deficit Stress

On 7 November, 2016, fifteen potswith coconut seedlings of each variety were divided into 3 groups and distributed randomly and exposed to different moisture regimes (M1, M2 and M3).

Moisture regimes

M1 – 100% ASM

M2 – 50% ASM

M3 –25% ASM

3.2.3. Determination of 100% Available Soil Moisture (100% ASM)

The total available water (holding) capacity or the available soil moisture (ASM) is the portion of water that can be absorbed by plant roots. By definition it is the amount of water available, stored or released between field capacity and the permanent wilting point water contents. The average amount of total available water in the root zone for a sandy loam soil which we have used in our experiment is around 20% (NRCCA Cornell University website https://nrcca.cals.cornell.edu/soil/CA2/CA0212.1-3.php). Soil moisture was measured using soil moisture probe (PR2 profile probe, delta T devices, UK) supplemented with a data logger. The measurement of soil moisture was taken at 40 cm depth. Protection tubes were placed permanently at this depth and for measurement

the moisture probe was inserted into the tube and connected to data logger. Data logger connected to computer automatically starts reading soil moisture and the data was stored in computer as MS-Excel format. A day before the start of the drought treatment i.e. on 6 November 2016 all the pots were saturated with water. After overnight saturation of soil, the amount of water which must be provided daily to maintain the 100% ASM (20% moisture) was estimated by trial and error method. It was ensured that no water was lost from the bottom hole. Every day morning the soil moisture content was measured and the amount of water required to bring back the soil inside the buckets to 100% ASM was determined and recorded. Treatment M2 received 50% of the quantum of water as that of M1 whereas M3 received 25% of the amount of water that has been supplied to M1. The occurrence of precipitation (rainy days) were also taken in to consideration while supplying water to the seedlings. The amount of rainfall received during the preceding day was factored in before irrigating the seedlings at M1, M2 and M3. The water deficit treatments were maintained continuously till 10 May 2017 (194 days) during which various morphological, physiological and biochemical parameters were measured at regular intervals and water use efficiency was determined. Each treatment (moisture regime) was replicated 5 times.

3.2.4. Initial Biomass

A day before the start of the drought treatment (i.e. on 6 November 2016), two plants of each variety were uprooted. A jet of water was applied to wash off the soil, thus the plant with the intact roots were extracted from the bucket. The primary, secondary and minor roots were separated from the stem and carefully washed, without causing any loss of roots itself. Then the above ground plant parts such as shoot and leaves were separated and dried to obtain the initial biomass.

3.2.5. Final Biomass

At the end of the experimental period (i.e.) on 12 May 2017, 36 plants (9 of each variety and 3 of each moisture regime within each variety) were uprooted with roots intact (Fig 3.3). The soil in the roots was carefully washed with the help of running water. The leaf and, shoot of the uprooted plants were separated and dried in an oven at 65°C and their dry weight was recorded. Leaves that became dry during the experimental

period were collected and their dry weight was added to the final leaf dry weight. Dry weight of roots, leaves and stem was added to obtain the total biomass of the plants under different treatments. The difference in the final and initial dry weight is the biomass accumulated during the experimental period.

Fig 3.3 Extraction of intact seedlings from the soil

3.2.6. Determination of Water Loss through Evaporation

During the experimental period two identical buckets similar to the ones used in the experiment with same amount and type of soil with a hole at bottom were kept nearby the experimental site. These buckets didn't have any coconut seedlings. However, in order to mimic the shade of the original plants, two dry coconut leaves were kept inside the buckets in a vertically pointed manner as 'no-plant control' (Fig 3.4). The rational for this 'no plant control' was to determine the amount of loss of water through evaporation occurred so as to infer the actual amount of water that was being used by the plants. The amount of water provided to the 100% ASM treatments was also provided to these buckets and the water drained out throughout the day was collected and measured. This amount was considered as the amount of water consumed by the plants while the rest was equivalent the evaporative loss.

Fig 3.4 Experiment to determine the evaporative loss of water

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3.2.7. Addition of Water Through Rainfall

The amount of rainfall received was recorded in the nearby weather station during the work duration. This amount of rainfall (in cm) multiplied by the bucket area (π x (49 cm)^2) gives the amount of water added by the rainfall in cm^3 which when divided by 1000 gives us the amount of water added by rainfall in liters.

3.2.8. Amount of Water Consumed By the Seedlings

The total amount of water consumed by the seedlings was calculated based on the observations made above including from the ' no plant control'. Hence, the total amount of water consumed by plants was calculated by deducting the evaporative loss from the quantum of water supplied to the seedlings including the water received from the precipitation/rainfall.

$$\textit{Amount of water consumed by the seedlings} = \text{sum of quantum of water irrigated} + \text{rainwater} - \text{evaporative loss}$$

3.2.9. Water Use Efficiency

Water use efficiency is the amount of dry biomass produced by the seedling/ or plant per unit amount of water consumed. The initial and final biomasses of the seedlings of each variety were calculated as described above. Further, the total amount of water consumed from the quantum of water provided to the seedlings during their growth period was calculated. The whole plant water use efficiency was determined using the following equation and expressed as g biomass/liter water.

$$\text{WUE} = \frac{\Delta\ \text{Biomass (final biomass} - \text{initial biomass)}}{\text{Amount of water consumed by the seedlings over the experimental period}}$$

3.3. MORPHOLOGICAL OBSERVATIONS

The following morphological observations were recorded during the initial day, first week of the months within the experimental period and after uprooting the seedlings.

3.3.1. Plant Height

Height from the base of seedling to the longest leaf (amongst the last three leafs) was measured with a measuring scale and it was expressed in centimeters.

3.3.2. Collar Girth

The circumference at a fixed point (top of the bucket was taken as the fixed point) was measured with measuring tape and expressed in centimeters. This measurement was taken as collar girth.

3.3.3. No. Of Leaves and Leaf Area

Observations were made regarding the number of fresh and dry leaves in each seedling. For estimating leaf area of individual leaf, length and width of each fresh leaf was measured. Length was measured from origin of leaflets in a leaf to the tip of petiole of that leaf and width was measured by stretching the leaf lets on either side of petiole to maximum. Leaf area was calculated by using the linear regression equations developed by Mathes *et al.* (1989), $y = 5.9647 + 0.6314\ x$, $y = 3.9325 + 0.7044\ x$ and $y = 8.4507 + 0.6798\ x$ with the reliabilities of 94.5percent, 98.3percent and 97.8 percent respectively, where x is the product of the length and breadth at the broadest position of the leaflet and y is the area of the leaflet.

3.4. PHYSIOLOGICAL PARAMETERS

3.4.1. Photosynthesis, Transpiration and Stomatal Conductance

Leaf gas exchange parameters were measured from the fully opened leaf of the seedlings at regular intervals starting from one month of exposure to the treatments. The portable Infra-Red Gas Analyzer (IRGA, LICOR-6400XT, USA) equipped with leaf chamber was used for the measurements. Net photosynthetic rate, stomatal conductance and transpiration rate were measured using this instrument. The measurements were

taken between 9.30 AM to 11 AM on bright sunny day and gas exchange parameters were analyzed at fixed light intensity level of PAR 1000 μmol m^{-2} s^{-1}. Before starting the measurements IRGA was calibrated for zero CO_2. Both the reference and sample IRGA were matched for CO_2 and H_2O concentrations at regular interval by using equipment in match mode. The leaflet was clipped in to leaf chamber and readings were logged after the leaf internal carbon dioxide concentration became stable. All measurements were stored in a file and downloaded to the PC using retrieving software 'LI6400XTerm'and files were opened in MS-Excel for further processing.

3.4.2. Chlorophyll Fluorescence

Chlorophyll fluorescence was measured by direct method using chlorophyll fluorometer (Opti-sciences-30p, USA). Dark adapted leaflets were used for the observation of chlorophyll fluorescence. The dark adaptation clips with 4 mm aperture size with a sliding shutter (to prevent the entry of light during the insertion of sample probe) were used for the leaflet surface to undergo dark adaptation. Clips were plugged on the surface of middle leaflet of topmost fully opened leaf and leaflets were dark adapted for 30 minutes. After that the sample probe was inserted into the clip and sliding shutter was opened to start the measurement. The start and end of the measurement is indicated by an audible beep sound. Minimal fluorescence (F_0), maximum fluorescence (F_m) and F_v/Fm (ratio of variable fluorescence to maximal fluorescence) were recorded in a file. Data was retrieved using hyper-terminal software.

3.4.3. Leaf Water Potential

The leaf water potential in coconut seedlings was measured on the middle leaflets of the first fully opened leaf from top using LWP meter (Skye SKPM 1400, UK). The measurements were taken between 9.30 and 11.30 am. The instrument was taken to the field and it was connected to 2 L nitrogen cylinder through connecting hose. Instrument was switched on and 0 to 20 bar pressure mode was selected since it gives 0.01 bar resolution. Display was set to zero by turning the adjusting knob. Leaflet from top leaf was cut and a small portion of leaf lamina was removed from leaflet base to help insert midrib into the hole of chamber lid. The lid with leaflet was placed in leaf chamber and was made air tight. Pressure inside the chamber was increased using the nitrogen gas. The

pressure was recorded when the water oozed out from the tip of midrib and expressed in bars. A magnifying lens was used to visualize water oozing. The chamber pressure was then released by exhausting the gas using a knob.

3.4.4. Chlorophyll Index

Chlorophyll index in the seedlings was measured using leaf chlorophyll meter (at LEAF+, USA).The meter measures optical density at two wavelengths (660nm and 940nm) to estimate relative chlorophyll content. The leaf sensor was placed on leaf mesophyll tissue. Measurements were made at a photon flux density of 800 to 1200 µmol m^{-2} s^{-1}. From each leaf five measurements were taken and an average of these measurements provided chlorophyll index. The measurements were stored in a separate file and were retrieved using software.

3.4.5. Stomatal Resistance

Leaf stomatal resistance was measured using porometer (Porometer AP4, USA). It measures the stomatal resistance or conductance, leaf temperature and light intensity. The instrument was taken into field and the sensor clip was clipped into the middle of a leaflet. Measurements were made by pressing 'GO' button to start and an audible double beep indicating the end of the measurement. The readings were saved by pressing the GO button once again. The data was retrieved using RS232 port in computer. Before the measurement the instrument was calibrated using calibration plate and silica gel.

3.5. BIOCHEMICAL PARAMETERS

3.6.1 Leaf Tissue Preparation Scavenging Enzyme Assay

Enzyme extraction from leaf tissue

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

Reagents

1. Sodium phosphate buffer (0.1 M; pH 7.6): Disodium hydrogen phosphate heptahydrate (Na_2HPO_4. $7H_2O$; 26.805g) was dissolved in distilled water (500ml) to get solution A. Sodium dihydrogen phosphate dehydrate (NaH_2PO_4. $2H_2O$: 15.605g)

was dissolved in distilled water (500ml) to get solution B. Solution A (435ml) and solution B (65ml) were mixed and volume was made to 1000 ml with distilled water to prepare sodium phosphate buffer (0.1M: pH 7.6). The pH of solution was adjusted.

2. Insoluble poly vinyl polypyrrolidone (PVPP)

Procedure

Plant tissue sample (2 g) was homogenized in pre-chilled mortar and pestle with 0.1M sodium phosphate buffer (20 ml) and insoluble PVPP (1g). This homogenate was centrifuged at 12000 rpm at 4°C for 15 minutes. Supernatant was collected and again centrifuged at 12000 rpm for 15 minutes at 4°C. This enzyme extract was used for the assay of anti-oxidant enzymes *viz.*, superoxide dismutase (SOD), peroxidase (POD) and polyphenol oxidase (PPO).

3.6.1.1. Assay of Superoxide Dismutase (SOD)

Superoxide dismutase specific activity was assayed by following the method of Beauchamp and Fridovich, (1971).

Reagents

1. Potassium phosphate buffer 0.1M (pH 7.8): Potassium hydroxide (1.122g) was dissolved in distilled water (100ml) to get solution A and potassium dihydrogen phosphate (2.722g) was dissolved in distilled water (100ml) to get solution B. Solution A (90ml) and Solution B (100ml) were mixed and made to 200ml using distilled water to prepare potassium phosphate buffer (0.1M; pH 7.8).
2. Sodium carbonate solution 1.5M (pH 10.2).
3. Nitro blue tetrazolium (NBT) solution (0.00067M): NBT (5.5mg) was dissolved in distilled water (10 ml).
4. Methionine solution (0.13M): Methionine (194mg) was dissolved in distilled water (10 ml).
5. EDTA disodium salt solution (0.0127M): EDTA (37.2mg) was dissolved in distilled water (10 ml).
6. Riboflavin solution (0.00129 M): Riboflavin (4.89mg) was dissolved in distilled water (10 ml) and 1ml from the solution was diluted to 100 ml using distilled water.

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Procedure

Reaction mixture

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

Light control

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

Dark control

Reaction mixture with enzyme extract was kept in dark condition.

Sample

Reaction mixture with enzyme extract was kept in light condition.

Reagent blank

Potassium phosphate buffer served as reagent blank.

Enzyme assay

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

Calculation of specific activity

$$(100\ (OD_S/ODL_C)\ 100)/50 = X$$

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

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

$$OD\ S = OD_T - OD_C$$

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OD_T = Absorbance of sample

OD_C = Absorbance of dark control

OD_{LS} = Absorbance of light control

3.6.1.2 Assay of Peroxidase (POD)

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

Principle

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

Reagents

1. Sodium phosphate buffer (0.1 M; pH 7.6): Disodium hydrogen phosphate heptahydrate (Na_2HPO_4. $7H_2O$; 26.805g) was dissolved in distilled water (500ml) to get solution A. Sodium dihydrogen phosphate dehydrate (NaH_2PO_4. $2H_2O$: 15.605g) was dissolved in distilled water (500ml) to get solution B.

   Solution A (435ml) and solution B (65ml) were mixed and volume was made to 1000ml with distilled water.
2. Hydrogen peroxide (H_2O_2): Hydrogen peroxide (0.5%) was diluted up to 10ml using sodium phosphate buffer.
3. 0.2% O-dianisidine: O-dianisidine (20mg) was dissolved in methanol (10ml)

Procedure

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

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

Reagent blank

Reaction mixture except enzyme extract served as reagent blank.1 unit is defined as a change in OD to 1 per minute per gram tissue

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3.6.1.3 Assay of Polyphenol Oxidase

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

Principle

Polyphenol oxidase is one of the enzymes involved in the oxidation of phenolic compounds into brown pigments. In plants PPO is involved in conferring resistance to the infection. The enzyme activity is measured as rate of increase in absorbance calorimetrically at 480nm.

Reagents

1. Potassium phosphate buffer 50 mM (pH 6.8)
2. Pyrogallol
3. Sulphuric acid (2N)

Procedure

Control

Potassium phosphate buffer (2 ml) and pyrogallol (1 ml) were mixed together and then sulphuric acid (0.5ml) was added and kept at room temperature for 3 minutes for incubation. Enzyme extract (0.1ml) was added after 3 minutes of incubation and immediately OD was taken at 480 nm using UV visible spectrophotometer (Shimadzu UV 160A, Japan).

Sample

Potassium phosphate buffer (2ml) and pyrogallol (1ml) were mixed together. To this enzyme extract (0.1ml) was added and kept at room temperature for 3 minutes for incubation. Reaction stopped by adding sulphuric acid (0.5ml). The OD was taken at 480 nm using UV visible spectrophotometer (Shimadzu UV 160A, Japan).

Reagent blank

Reaction mixture without enzyme extract served as a reagent blank.

Specific activity calculation

$(A \times 3/0.1)/3$ = Units/min/mg protein

Where, A = Difference in absorbance of sample and the control (OD S – OD C).

3.6.2. Estimation of Total Protein

The Lowry's reaction for protein estimation is an extension of the Biuret method provided by Lowry *et.al.* (1951).

Principle

Protein reacts with the Folin-Ciocalteu Reagent (FCR) to give a blue colored complex. The color so formed is due to the reaction of alkaline copper with the protein as in the biuret test and the reduction of Phospho molybdic-phospho tungstic components in FCR by the amino acids tyrosine and tryptophan present in the protein. The intensity of the blue color is measured calorimetrically at 660nm. The intensity of the color depends on the amount of these aromatic amino acids present and will thus vary for different protein.

Reagents

1. Solution A: 0.2% sodium carbonate (anhydrous) in 0.1N NaOH.
2. Solution B: 0.5% copper sulphate in 1% sodium potassium tartrate (prepare fresh).
3. Solution C: Alkaline copper sulphate
   100 ml of Solution A was mixed with 2 ml of Solution B just prior to use.
4. FCR: The commercial reagent (2N) was diluted with an equal of volume water on the day of use (1:1).
5. Stock standard protein solution: 50mg of Bovine Serum Albumin (BSA) in 50ml of water.
6. Working standard solution: 1ml of the stock solution was diluted in 10ml of water.

Procedure

Aliquots of 0.1 or 0.2 ml 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution were pipetted out in to a series of test tubes and the volume was made upto to 1 ml with distilled water. In a separate tube, 1 ml of water was taken that served as a blank. In all the tubes 5ml of Solution C was added, mixed well and incubated at room temperature for 10 min. After incubation, 0.5ml of FCR was added, immediately mixed well and incubated at room temperature in dark for 30 min. Absorbance of blue colour at 660 nm was read using UV- spectrophotometer (Shimadzu UV 160A, Japan). From the standard graph prepared, the amount of soluble protein present in the sample was calculated.

3.6.3. Estimation of Epicuticular Wax (ECW)

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

Reagents

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

Procedure

Twenty leaflets segment of 3x2 cm^2 area from the third fully opened leaf from the top were immersed in chloroform (15 ml) and then vigorously shaken for 20 seconds and chloroform was completely collected in test tubes. After complete evaporation of chloroform, 5 ml potassium dichromate reagent was added and kept it in a boiling water bath for 30 minutes. After that the volume was made up to 17 ml using distilled water and OD readings was taken at 590 nm using UV- Visible spectrophotometer (Shimadzu UV 160A Japan)

3.6.4 Estimation of Chlorophyll Content

The chlorophylls are the essential components for photosynthesis and occur in chloroplasts as green pigments in all photosynthetic plant tissues. They are bound loosely to proteins but are readily extracted in organic solvents such as acetone or ether.

Principle

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

Reagents

Analytical grade acetone was diluted to 80 percent (pre-chilled).

Procedure

Chlorophyll of 500mg finely chopped leaf tissue was extracted with 10 ml 80 percent acetone. After keeping it overnight, the supernatant was decanted and the residue was extracted with 10 ml 80 percent acetone. The supernatant was decanted and pooled to make up the volume to 25ml. The OD was measured at 663 and 645 nm against 80 percent acetone.

Calculation

$$\text{Total Chlorphyll } \left(\frac{\text{mg}}{\text{g}}\right) = \frac{[20.2(A645) + 8.02(A663)] \text{ x V}]}{1000 \text{ x W}}$$

Where,

A = Absorbance at specific wavelengths.

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

W = Fresh weight of the tissue extracted.

3.6.5. Leaf Tissue Extraction for the Starch and Total Soluble Sugar

Leaf tissue (0.250g) was homogenized in a pestle and mortar using 80 percent ethanol (10 ml) and kept in preheated water bath for 30 minutes at 70 °C. The homogenate was filtered through Whatman No.1 filter paper. The step of extraction and filtration was repeated. Thereafter ethanol was evaporated completely by placing the filtrate at preheated water bath (70 °C), and the remaining portion was dissolved in 10 ml distilled water and vortexed for 2 minutes. This extract was used for the estimation of the total soluble sugar. The residue is treated with 75 % perchloric acid and kept for 30 min. Then the sample is kept in 4^0C to stop the hydrolysis process and vortexed for 2 minutes at 4^0C. The extract is then used for starch estimation.

3.6.5.1. Estimation of Total Soluble Sugars

Concentration of total soluble sugars was estimated by following the phenol sulphuric acid method (Dubois *et al.,* 1956).

Principle

In a hot acidic medium glucose was dehydrated to hydroxyl methyl furfural. This furfural forms an orange-yellow colored product with phenol and has absorption maximum at 490 nm. The basis of this reaction is the differential production of furfural, when pentose are heated with dilute acids and of hydroxyl methyl furfural, when hexoses are heated with dilute acids when either product condenses with phenol an orange to yellow product is formed.

Reagents

1. Phenol reagent (5%): Redistilled phenol (50g) was dissolved in distilled water and then diluted to 1000 ml using distilled water to prepare phenol reagent (5%).
2. Sulphuric acid (96%).
3. Glucose standard: The standard stock solution was prepared by dissolving 100g of glucose in distilled water and the volume was made up to 100 ml. Working standard was prepared by diluting 10 ml of stock standard solution to 100 ml distilled water. Series of standard solutions were prepared by taking 0.1 to 1 ml of working standard solution and made up volume to 1ml in each tube using distilled water.

Procedure

The extract (0.1 ml) and the working standard solution (0.1 ml to 1 ml) were pipetted in a separate series of test tubes and the volume was made up to 1 ml with distilled water. To this, phenol reagent (1 ml) 96 percent sulphuric acid (5 ml) was added and incubated for 30 minutes at 30°C. The OD values were taken at 490 nm in UV-spectrophotometer (Shimadzu UV 160A, Japan).

Reagent blank

Reaction mixture without enzyme extract served as reagent blank. From the standard graph drawn, the amount of total soluble sugar was calculated by using glucose standard (100-500 µg/ml) and the results are expressed in mg/g fresh weight.

3.6.5.2. Estimation of Starch

Concentration of starch was estimated by following the phenol sulphuric acid method (Dubois *et al.,* 1956).

Principle

Starch is a non-soluble polysaccharide carbohydrate. However, under acidic condition, starch hydrolyses and give mono and disaccharides which are soluble. Then phenol sulphuric acid method is done in order to obtain the total amount of starch

Reagents

1. Perchloric Acid (75 %)
2. Phenol reagent (5%): Redistilled phenol (50g) was dissolved in distilled water and then diluted to 1000 ml using distilled water to prepare phenol reagent (5%).
3. Sulphuric acid (96%).
4. Glucose standard: The standard stock solution was prepared by dissolving 100g of glucose in distilled water and the volume was made up to 100 ml. Working standard was prepared by diluting 10 ml of stock standard solution to 100 ml distilled water. Series of standard solution were prepared by taking 0.1 to 1 ml of working standard solution and made up volume to 1ml in each tube using distilled water.

Procedure

The extract (0.1 ml) and the working standard solution (0.1 ml to 1 ml) were pipetted in a separate series of test tubes and the volume was made up to 1 ml with distilled water. To this, phenol reagent (1 ml) 96 percent sulphuric acid (5 ml) was added and incubated for 30 minutes at 30°C. The OD values were measured at 490 nm in UV-spectrophotometer (Shimadzu UV 160A, Japan).

Reagent blank

Distilled water (1 ml) + FCR (0.5 ml) + sodium carbonate (5 ml, 20%). Blank is also run simultaneously

OD was read at 650 nm by using UV- VIS spectrophotometer (Shimadzu UV 160A, Japan) and from the graph drawn using standards, the amount of total phenol was

calculated by using catechol standard and the results were expressed as mg catechol equ. g FW^{-1}.

3.6.6 Estimation of Lipid Peroxidation

The extent of membrane damage and lipid peroxidation were studied following the protocol suggested by Heath and Packer(1968)

Reagents

- 0.1% w/v Tri Chloro Acetic acid (TCA)
- 0.5% Thiobarbituric Acid(TBA) diluted in 20 % (w/v) Tri Chloro Acetic acid

Procedure

A. 0.1g of leaf tissue was homogenized by adding 0.5 ml of 0.1 % (w/v) TCA
B. The homogenate was centrifuged for 10 min at 15000 x g, at 4,0°C
C. The supernatant was collected and 0.5 ml of supernatant was mixed with 1.5 ml 0.5% TBA
D. 0.5 ml of supernatant was taken in a test tube and 3ml of TBA+TCA mixture was added and incubated in water bath at 95°C for 25 min.
E. The reaction was stopped by incubating it on ice. Centrifugation was carried for further 5 min (15000 x g, 4,0°C) whenever the solution was found to be turbid to obtain clear solution
F. The absorbance was measured at 532 and 600 nm.
G. OD600 values are subtracted from the MDA-TBA complex values at 532 nm and MDA
H. The concentration was calculated using the Lambert-Beer law with an extinction coefficient εM= 155

3.6.7 Estimation of Membrane Stability

Coconut leaf membrane stability was determined following the method of Lu et al. 2003. Conductivity measured ($\mu S\ cm^{-1}$) were recorded with a conductivity meter. Eight leaflet segments of 3 cm^2 area each were immersed overnight at 25°C in 25ml of distilled water in a beaker and the initial conductivity was measured (C_1). Then the leaf with water was placed in boiling water bath for 30 minutes, and cooled to room temperature.

Conductivity was again measured (C_2). Conductivity of double distilled water was also measured and was considered as blank (C_0The membrane stability index was calculated using the following formula.

Membrane stability index (MSI) $= 1 - \left[\frac{(C1-C0)}{(C2-C0)}\right] 100$

C_0 – conductivity of distilled water

C_1 – initial conductivity

C_2 – conductivity after boiling

3.7 STATISTICAL ANALYSIS

The data was analyzed in two way factorial analysis using SAS 9.3 software. Mean values were compared for significance using the F-protected LSD test.

Chapter 4

RESULTS AND DISCUSSION

Coconut (*Cocos nucifera* L.) is one of the important plantation crops grown in the southern parts of India. The areas wherein the coconut is grown are usually along coastal belts and hilly areas which are highly vulnerable to the climate change. As per the IPCC AR5, along with an increase in the global temperature, sea level rise and greenhouse gas content, the chances of extreme climate events like drought (medium confidence) also increases despite a prediction in increase of global precipitation. Since coconut plant requires a defined quantum of water for optimum growth and yield (at least 40 L of water for coconut grown under drip irrigation, the most efficient method) (POP 2017) a drought period can adversely affect the plants. Also, the effects of water stress on coconut can persist years after the water stress has been disappeared, affecting livelihood of millions of people worldwide. Thus it is essential to develop coconut genotypes which are drought tolerant. Water use efficiency (WUE) is an important character in determining the drought tolerant capability of a plant as an increase in the WUE with the water stress increases the drought tolerant potential of the crop.

This study had been designed to investigate and estimate the whole plant WUE of four coconut cultivars (Tall and Dwarf cultivars 2 each) under three different moisture regimes such as 100 % ASM, 50% ASM and 25% ASM. Two dwarf cultivars (Malayan Yellow Dwarf (MYD) and Chowghat Green Dwarf (CGD)) and two tall cultivars (Kalpa Pratibha (KP) and Kalpatharu (KT)) were chosen for a comparative assessment of WUE. One year old coconut seedlings which were grown in large plastic pots (capacity 100 kg dry soil) were subjected to water deficit stress treatments for a period from November 2016 to May 2017. Each treatment was replicated three times. The initial and final biomass and the total water consumed by the crop was recorded to determine the water use efficiency under each treatment. Time course measurements of morphological, physiological and biochemical parameters were recorded to phenotype for water deficit tolerance.

4.1 CLIMATIC CONDITIONS

The experiment was conducted from 7 November 2016 to 10 May 2017. During the period daily maximum and minimum temperature, rainfall, solar radiation and evaporation were recorded. The average values of the observed weather parameters on a weekly basis are presented here.

4.1.1 Temperature (°C)

The average maximum temperature (T_{max}) during the period of the study was 31.12°C while the average minimum temperature (T_{min}) was 23°C. Low T_{max} was recorded during the month of December (28.4°C) and it increased during the months of April and May (39°C) (Fig 4.1). Similarly, T_{min} ranged from19.2°C (January) to 28.4°C (May) and showed an increasing trend from December to May (Fig 4.1).

4.1.2 Rainfall

A total of 2504.4 mm of rainfall was received during 2016-17. However, during the experimental period, only 70.6 mm total rainfall was recorded, in which, the first ten weeks received 26.8 mm rainfall followed by 11.2 mm of total rainfall during next ten weeks. Another32.6 mm of rainfall was recorded from 20thto 27th week of the experiment. (Fig 4.2).

4.1.3 Sunshine hours

On an average, 7.98 hours of sunshine was recorded during the period of this study. The maximum sunshine hour was 10.6 hrs, which was recorded during 1st of May 2017 (Fig 4.3) with the lowest sunshine hour, being zero hours, observed during 14th and 15th of December.

4.1.4 Evaporation

The maximum evaporation of 6.3 mm was recorded during the 26th week and the minimum evaporation of 0.6 mm was recorded during the 7thweek of the experimental period. Throughout the period, about 3.88 mm of average evaporation was recorded and the rate of evaporation increased during a period extending from 15th to 30th weeks (Fig 4.4).

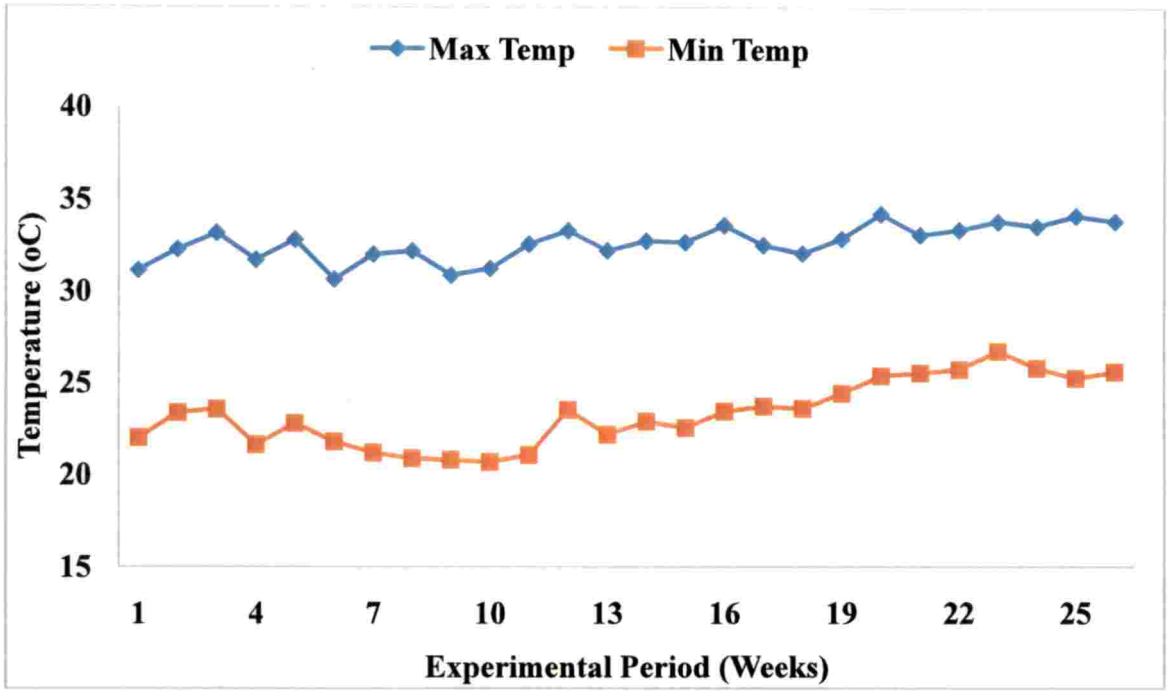

Fig 4.1. Weekly maximum (T_{max}) and minimum temperature (T_{min}) during the experimental period November 2016 - May 2017.

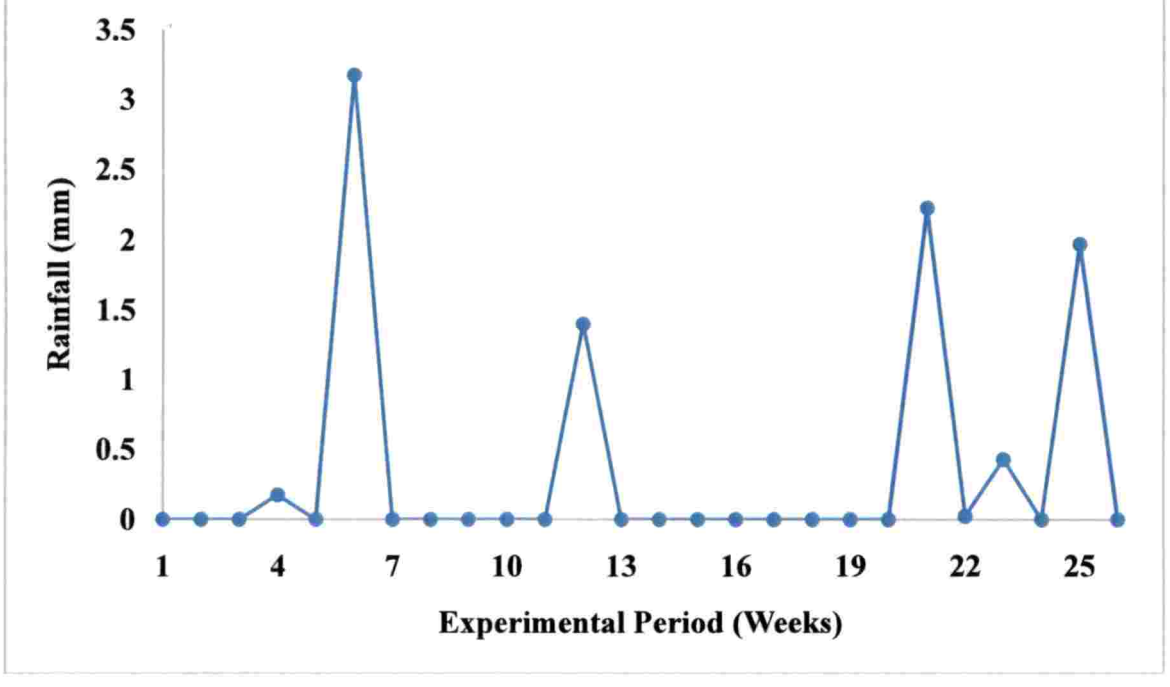

Fig 4.2. Weekly Rainfall during the experimental period November 2016 - May 2017.

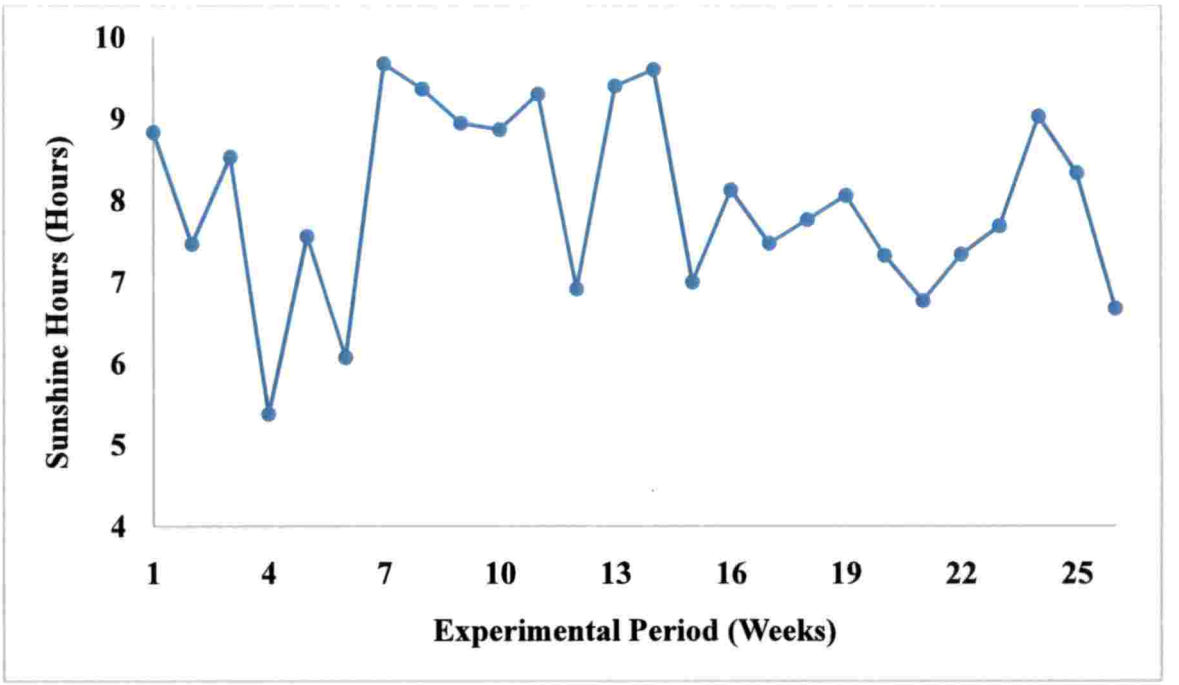

Fig 4.3. Weekly sunshine hours during the experimental period November 2016 - May 2017.

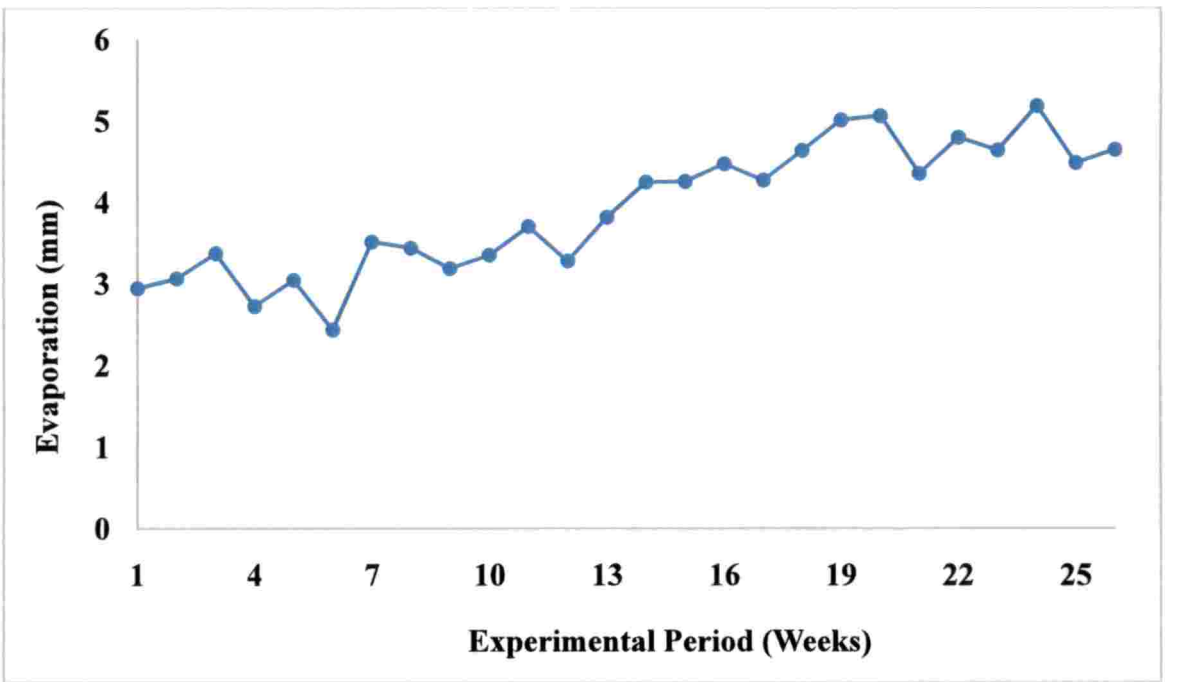

Fig 4.4. Weekly evaporation during the experimental period November 2016 - May 2017.

4.2 MORPHOLOGICAL RESPONSE

Morphological parameters of a plant are resultant of both physiological and biochemical parameters for a long term and thus can be regarded as long term effects, unlike physiological or biochemical parameters. Morphological measurements such as plant height, number of green leaves, leaf area and collar girth are important indicators to know the effect of stress on plants. Significant differences were observed in coconut seedlings subjected to moisture regimes. The results of the experiments are presented below.

4.2.1 Plant Height

Mean plant height of all genotypes were significantly high in seedlings grown at 100% ASM (Table 4.1). At 50 % ASM it was significantly reduced to180.53cm short by 30.8cm (14.58%) from the 100% ASM plants. At 25 % ASM it was further reduced by 29.85 to 150.68 cm. Among the varieties, the tall cultivar (KP) grown under 100% ASM exhibited a total height of 244.22 cm while the shortest seedling belongs to dwarf cultivar (MYD) (126.31 cm) grown under moisture-deficit stress (25 % ASM). Height was very sensitive to moisture and within 25 days after stress imposition significant difference was observed amongst the moisture regimes. At 50% ASM and 25% ASM, height was reduced by 6.37 cm and 35.37 cm with respect to 100% ASM (Fig 4.5). In control, height continued to increase throughout the experimental period while at 50% ASM and 25% ASM very little increase in height was observed. The trend in height increase across the varieties remained the same (Fig 4.6).

Table 4.1 Plant height of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	**100% ASM (cm)**	**50% ASM (cm)**	**25% ASM (cm)**	**Mean (cm)**
MYD	180.78	152.33	126.31	153.13^{D}
CGD	216.94	164.36	143.50	174.93^{C}
KP	244.22	223.75	163.64	210.54^{A}
KT	203.42	181.69	169.28	184.80^{B}
Mean	211.34^{A}	180.53^{B}	150.68^{C}	
CD at 5%				
Variety	5.28			
Moisture Regime	4.58			
V*M	9.15			

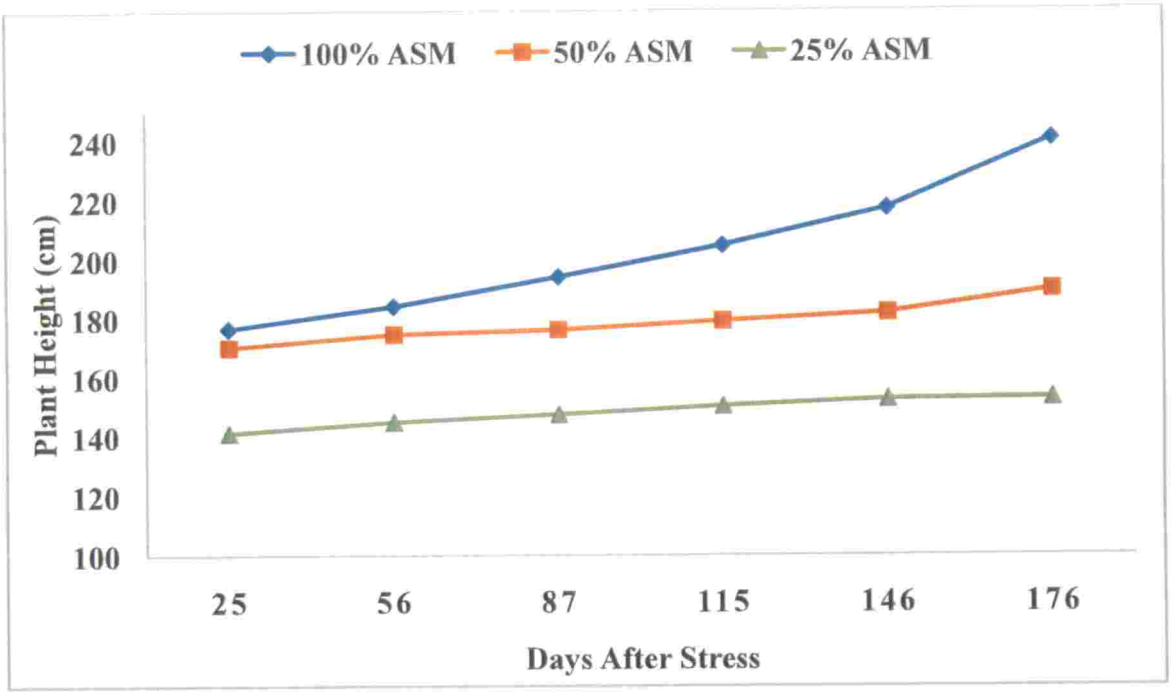

Fig 4.5. Change in the mean total height of the seedlings under different moisture regimes

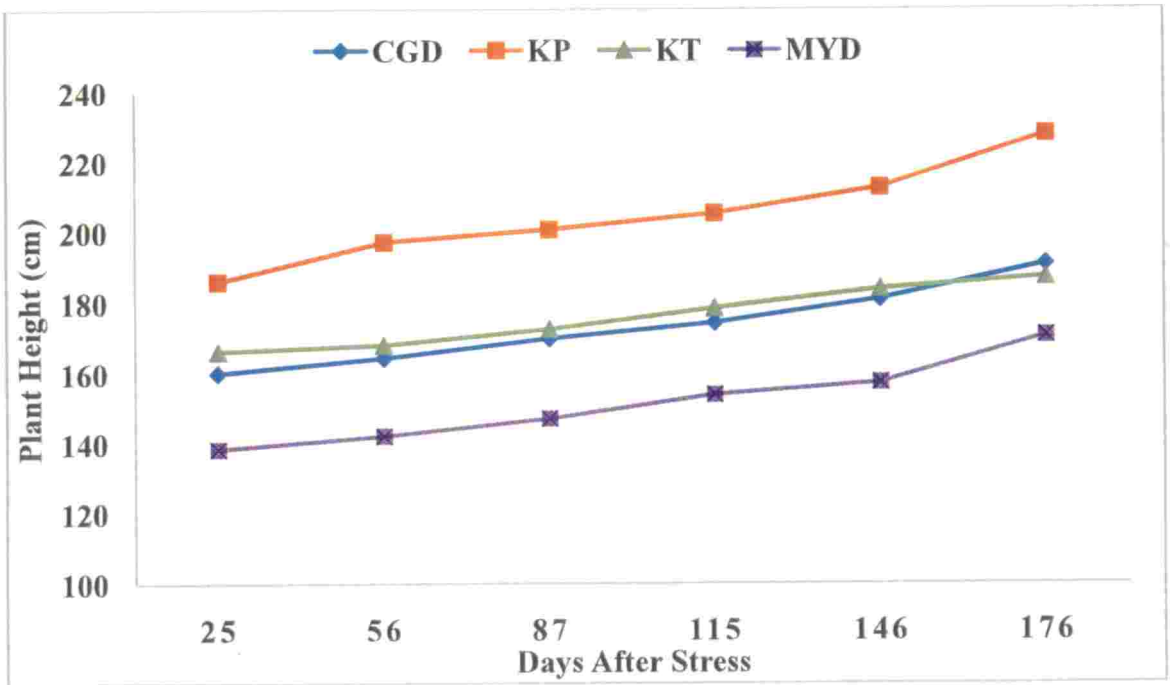

Fig 4.6. Variety-wise change in the plant height of the seedlings subjected to moisture-deficit stress

4.2.2 Number of fresh leaves

Seedlings grown under 100% ASM produced on an average of nine leaves and it significantly reduced to 8 and 6 leaves for plants grown at 50% ASM and 25% ASM respectively. Under 100% ASM leaf number was high in CGD (11) and low in KP but at 25% ASM, the leaf number was high in KP (7) while low in KT (5) (Table 4.2). It was also noticed that the cultivars CGD and KT had shown the largest decline in the number of fresh leaves with an almost 5 leaf difference between the 100% ASM and 25% ASM. Leaf number showed a steady increase up to 87 days. After this point, the leaf number in the control plant remained constant while at 50% ASM, the seedlings showed a gradual decline. On the other hand, at 25% ASM, there was a steady decline from 25th day itself (Fig 4.7). Leaf number in all the genotypes showed increasing trend upto 87th day except KP which showed the increasing trend only till 57th day, beyond which the number of leaves decreased in all the genotypes (Fig 4.8).

Table 4.2 The number of fresh leaves of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Varieties	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**
MYD	8.33	6.67	5.67	6.88^{C}
CGD	11.00	8.67	6.00	$8,55^{A}$
KP	8.00	7.00	6.67	7.22^{BC}
KT	10.00	8.33	5.00	7.77^{B}
Mean	9.33^{C}	7.66^{B}	5.83^{C}	
CD at 5%				
Variety	0.61			
Moisture Regime	0.53			
V*M	1.05			

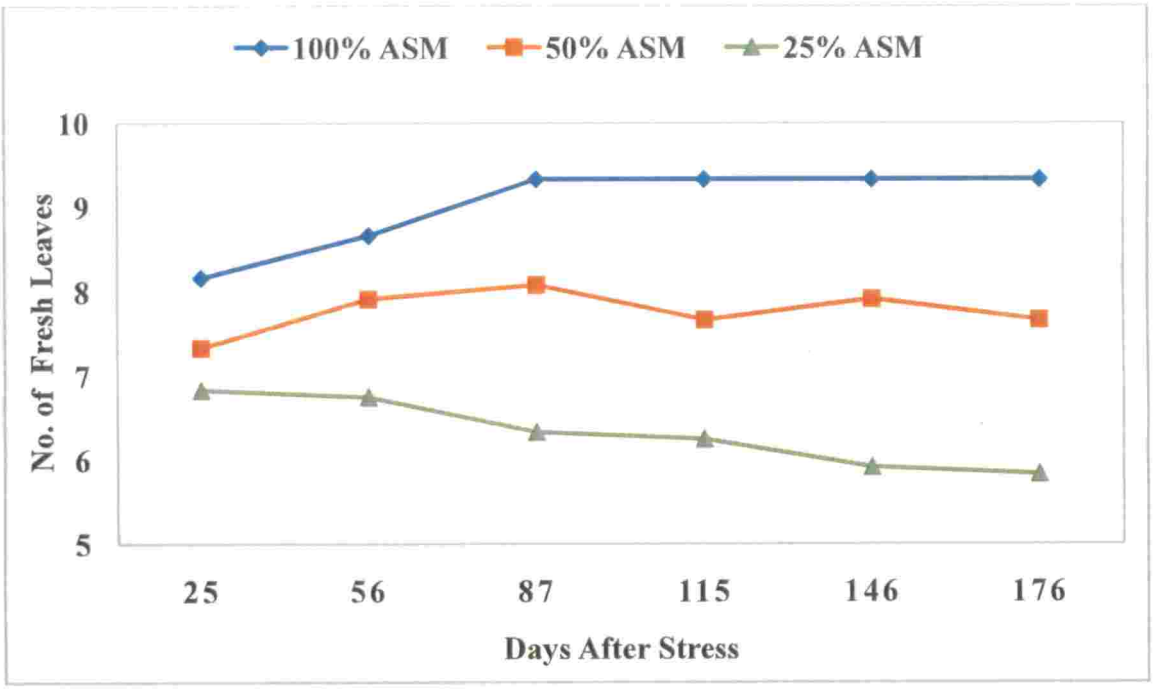

Fig 4.7. Change in mean number of fresh leaves of the seedlings under different moisture regimes

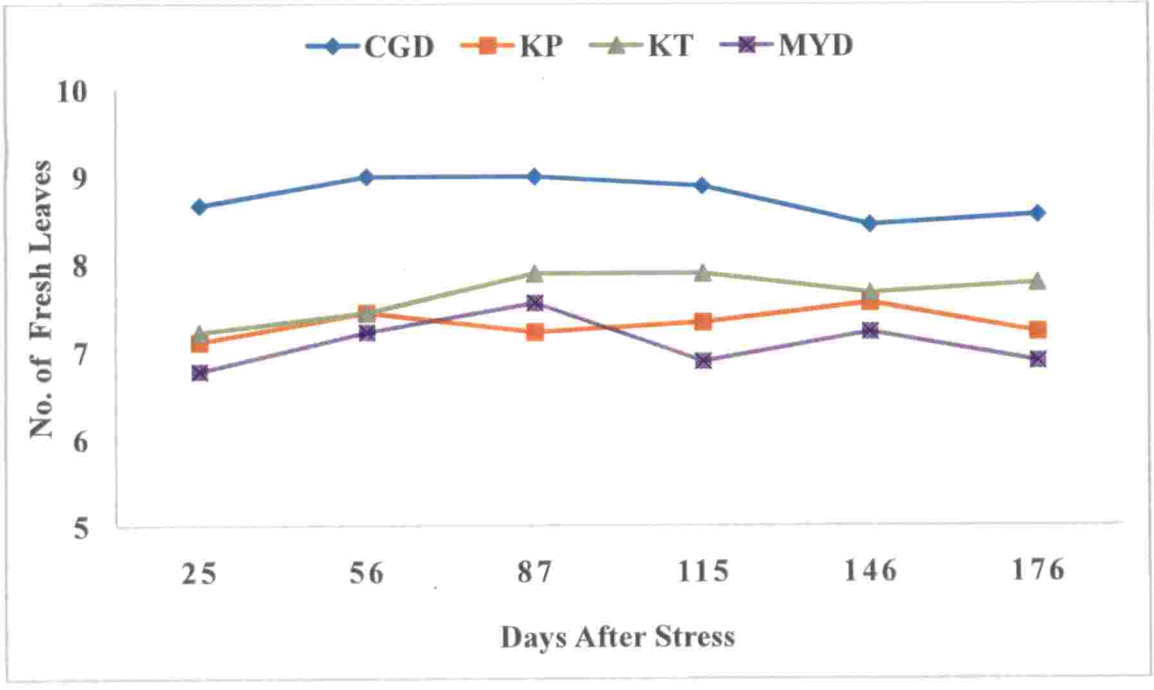

Fig 4.8. Variety wise change in the number of fresh leaves of the seedlings

4.2.3 Leaf area

Mean leaf area of the seedlings grown under various moisture regimes showed significant difference (Table 4.3). 100% ASM seedlings had the highest leaf area (67069 cm^2) followed by seedlings subjected to 50% ASM at 50488 cm^2which was 24.72% lower than the 100% ASM plants (Table 4.3). Seedlings grown at 25% ASM had 28936cm^2 (17.5%) and 16581 cm^2 (13.2%) lesser leaf area than 100% ASM plants and 50% ASM plants respectively. At 100% ASM condition, the highest mean leaf area was observed in the variety KP (84523 cm^2) while the least was observed in KT (51712 cm^2) and MYD (51242 cm^2). At 25% ASM, the leaf area was significantly high in KP when compared to rest of the genotypes while it was least in KT (27483 cm^2). Across the genotypes, the initial difference in leaf area with moisture regime was less (Fig 4.9). However, the leaf area showed significant difference as the growth advanced and at the end, it was 67069 cm^2, 50488 cm^2 and 38133 cm^2 for 100% ASM, 50% ASM and 25% ASM respectively. Leaf unfolding requires longer duration at initial stages in KT when compared to rest of the genotypes and thus showed a large increase in leaf area at later stages (Fig 4.10). Even though leaf numbers reduced by 87^{th} day (Fig 4.10), the leaf area continued to increase suggesting that there were more expansion of leaves.

Table 4.3. Leaf area of coconut cultivars grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM)

Varieties	**100% ASM (cm^2)**	**50% ASM (cm^2)**	**25% ASM (cm^2)**	**Mean (cm^2)**
MYD	51242	41409	31063	41238^{B}
CGD	80801	57968	40730	59833^{A}
KP	84523	57689	53258	65156^{A}
KT	51712	44888	27483	41361^{B}
Mean	67069^{A}	50488^{B}	38133^{C}	
CD at 5%				
Variety	5082.23			
Moisture Regime	4401.34			
V*M	8802.68			

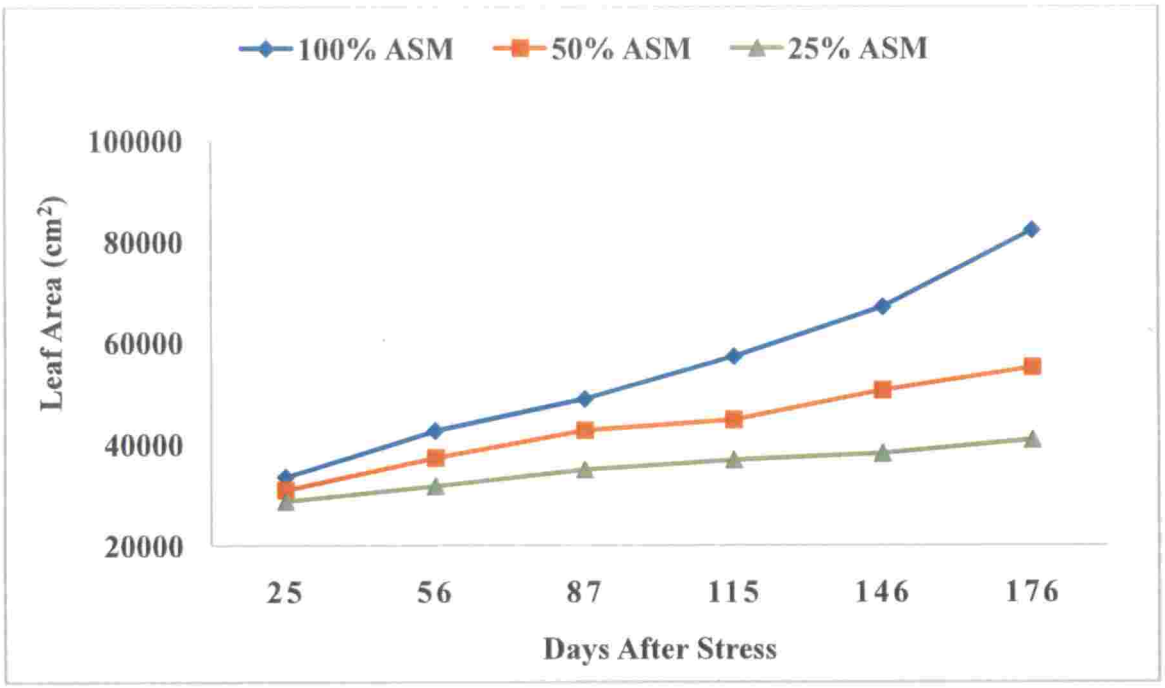

Fig 4.9. Change in leaf area of the seedling grown under different moisture regimes

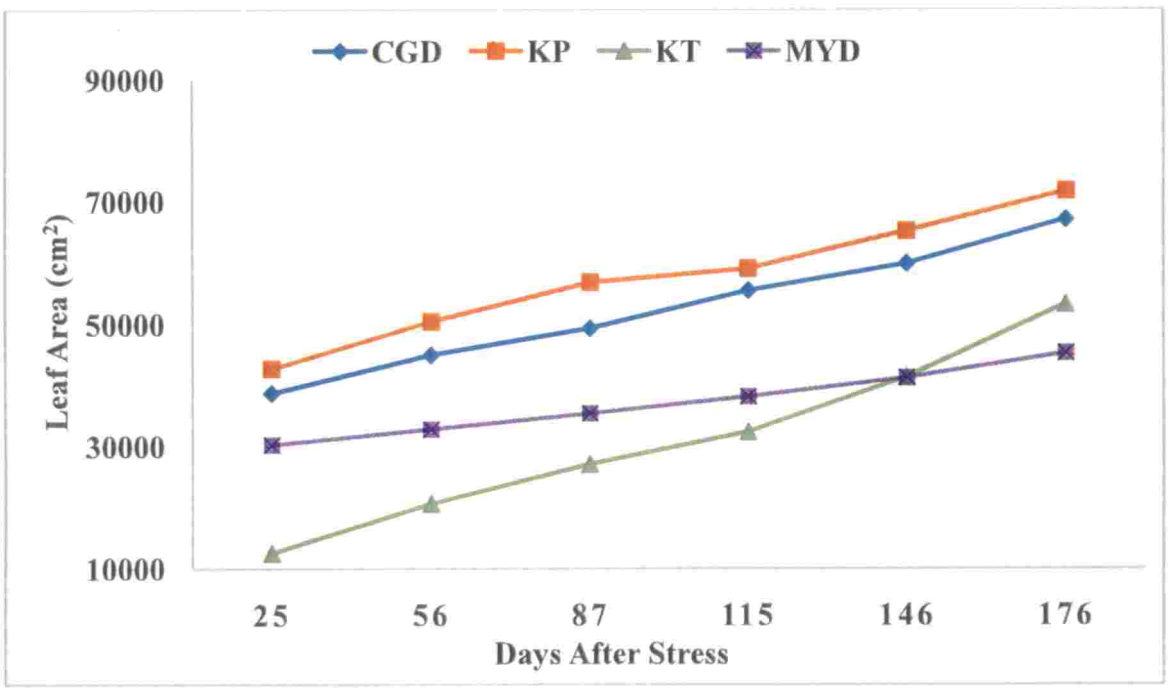

Fig 4.10. Variety wise change in the leaf area of the seedlings.

4.2.4 Collar Girth

Mean collar girth of seedlings raised under different moisture regimes showed a significant difference (Table 4.4). Plants grown under 100% ASM have the highest collar girth (33.83 cm) followed by seedlings grown under 50% ASM(24.75 cm) that showed 9.08 cm (26.85%) lesser collar girth than the 100% ASM plants. Seedlings grown at 25% ASM showed collar girth of 13.125 cm that is 38.79% lesser than 100% ASM plants and 4.0417cm (16.33%) lesser than 50% ASM plants. In normal grown plants, the largest mean collar girth was observed with the variety CGD (39.67 cm) and the least was observed in KT (27.5 cm) (Table 4.4). On the other hand, at 25% ASM it was the least in MYD (15.83 cm) and the highest in CGD (26 cm). Similar to plant height and leaf number, distinct difference were observed in collar girth among different moisture regime treatments. Normal grown plants showed a continual growth throughout the experimental period. At 50% ASM and 25% ASM, there was significant decline in collar girth and at the end of experiment; it was only 26.85% and 53.03% respectively of normal plants (Fig 4.12). Amongst the varieties, CGD and KP had higher collar girth than KT and MYD and all the four cultivars showed an increasing trend from the starting till the conclusion of the experiment (Fig 4.13).

Table 4.4. Collar girth of various coconut varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	100% ASM (cm)	50% ASM (cm)	25% ASM (cm)	Mean (cm)
MYD	31.83	21.33	15.83	23.00^{C}
CGD	39.67	31.50	26.00	32.38^{A}
KP	36.33	24.17	21.33	27.27^{B}
KT	27.50	22.00	19.67	23.05^{C}
Mean	33.83^{A}	24.75^{B}	20.70^{C}	
CD at 5%				
Variety	1.82			
Moisture Regime	1.57			
V*M	3.15			

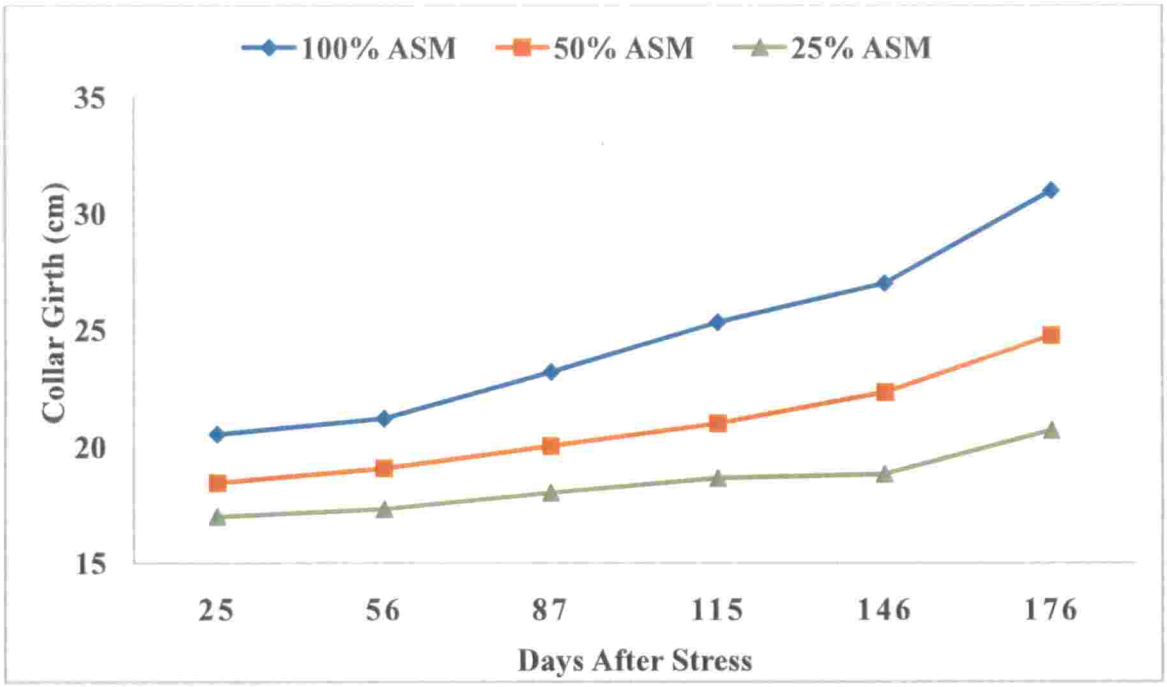

Fig 4.11. Change in collar girth of the seedlings under different moisture regimes

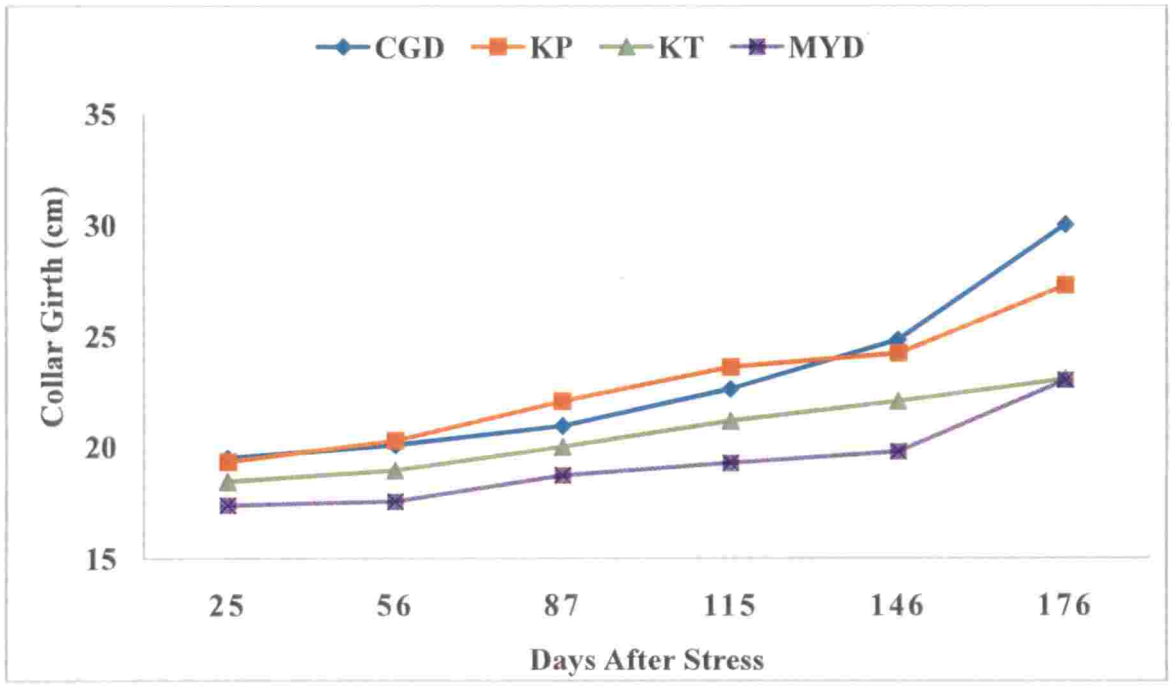

Fig 4.12. Variety wise change in the collar girth of the seedlings

Coconut genotypes selected for this study showed distinct difference in their morphological traits when grown under normal (100% ASM) condition. Plant height, leaf area and collar girth were significantly high in CGD, and KP while number of leaves were significantly low in KP. Coconut seedlings are highly sensitive to water deficit stress and response could be seen as early as 25 days after stress. Rajagopal et al. (1989) reported that exposure of palms to moisture stress for 16 or 24 days led to reduction in the vegetative dry matter by 15% and 18%, respectively. Number of fresh leaf production at 50% ASM and 25% ASM is highly sensitive and declined beyond 87 days after stress while leaf area of these treatments continue to increase suggesting new leaf production is more sensitive than area expansion. When exposed to such severe moisture stress coconut palms exhibit adverse effects such as bending and breaking of older leaves and drying of leaves (Rao 1985). It is a known fact that in some of the crops new leaf emergence is more sensitive than leaf expansion. Both for leaf area and leaf number of MYD is more sensitive compared to the rest of the genotypes.

4.3 PHYSIOLOGICAL RESPONSE

Physiological parameters such as photosynthetic rate, stomatal conductance, transpiration, leaf water potential, stomatal resistance, chlorophyll index and chlorophyll fluorescence were measured in coconut seedlings under normal and water deficit conditions to estimate the changes in physiological parameters. The results are presented as below.

4.3.1 Net Photosynthesis (Pn)

Photosynthetic rate of CGD and KP was high (9.59 and 8.26 μ moles $m^{-2}s^{-1}$ respectively) while it was low in KT and MYD (5.9 and 5.71 μ moles $m^{-2}s^{-1}$respectively) under 100% ASM. It significantly reduced to 3.75 μ moles $m^{-2}s^{-1}$ and 0.88 μ moles $m^{-2}s^{-1}$ at 50 and 25% ASM respectively. At 50% and 25% ASM Pn was reduced by 19.6% and 83.6% from 100% ASM respectively. Net photosynthetic rate was significantly high (7.36 μ moles $m^{-2}s^{-1}$) for seedlings grown under 100% ASM conditions. Among the varieties, CGD seedlings under 100% ASM conditions, exhibited the highest net photosynthetic rate (9.59 μ moles $m^{-2}s^{-1}$) while KT grown under moisture-deficit stress (25% ASM)

showed the lowest Pn (0.28 μ moles $m^{-2}s^{-1}$). It was also observed that the net photosynthetic rate in dwarf varieties was higher than the tall varieties under the moisture regimes of 50% ASM and 25% ASM (Table 4.5). Earlier Kasturi-bai et al. (1998) showed a decline of photosynthetic rate from 7.97 μ moles $m^{-2}s^{-1}$ to 4.66 μ moles $m^{-2}s^{-1}$ under stress condition. In comparison to these values Pn at 25% was very low indicating plants were under severe stress Table 4.5 Net Photosynthesis rate of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Table 4.5 Net Photosynthetic rates of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

VARIETY	100% ASM	50% ASM	25% ASM	Mean
MYD	5.71	5.73	1.29	4.24^{AB}
CGD	9.59	3.94	1.42	4.98^{A}
KP	8.26	2.41	0.54	3.73^{BC}
KT	5.90	2.94	0.28	3.04^{C}
Mean	7.36^{A}	3.75^{B}	0.88^{C}	
CD at 5%				
VARIETY	0.94			
MOISTURE REGIME	0.81			
V*M	1.62			

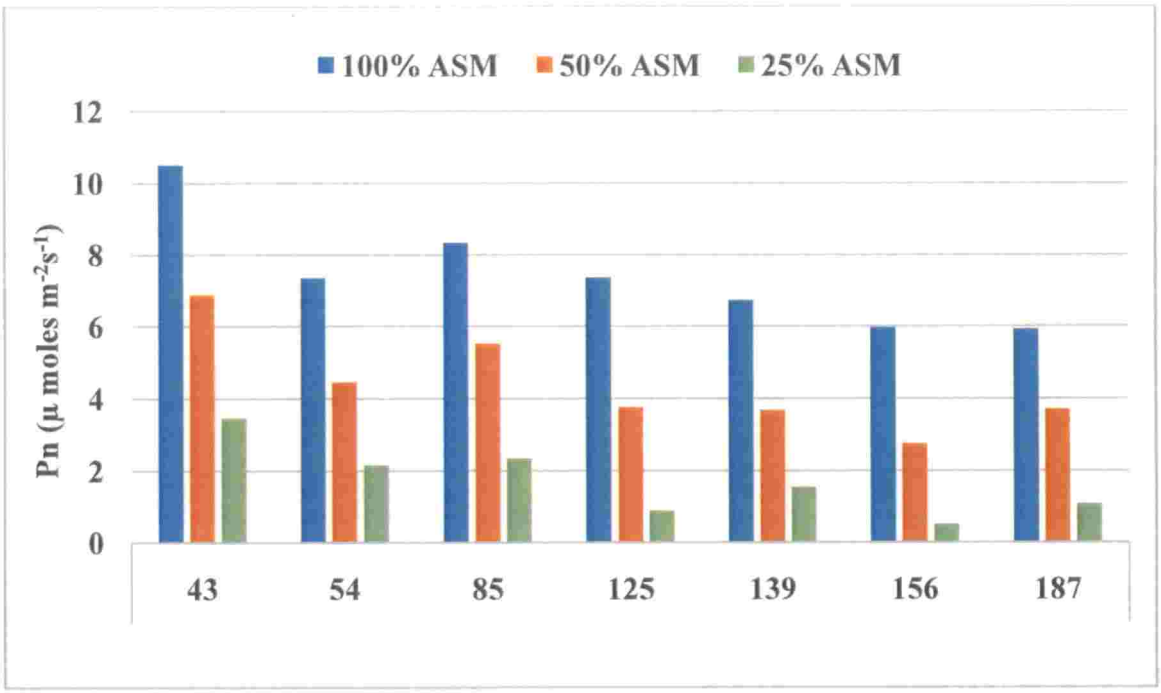

Fig 4.13. Net Photosynthesis rate of genotypes under 100% ASM, 50% ASM and 25% ASM

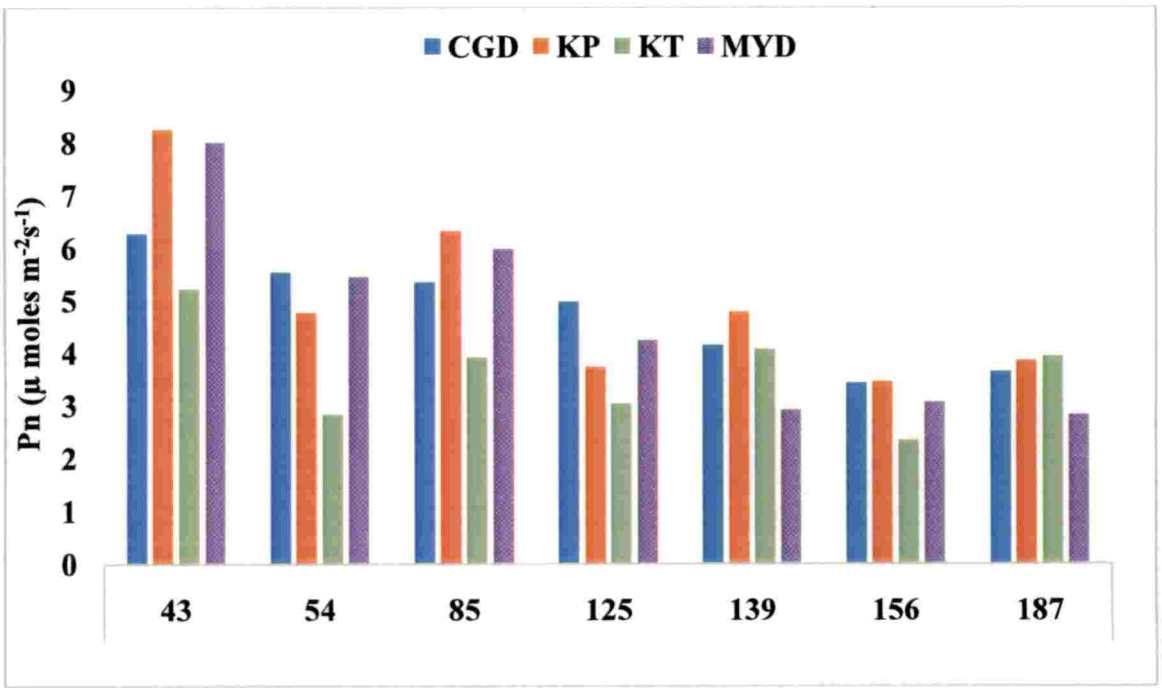

Fig 4.14. Time course measurement of net Photosynthesis rate of various coconut varieties

4.3.2 Stomatal conductance (gs)

Similar to Pn, highest stomatal conductance was recorded in100% ASM plants (0.109 moles $m^{-2}s^{-1}$) which significantly decreased with water deficit. It decreased by 19.8% and 40.3% at 50 and 25% ASM. At 100% and 50% ASM, CGD had highest gs (0.15 and 0.11mole $m^{-2}s^{-1}$) while it was least for MYD (0.05 and 0.02). At 25% ASM gs was on par across the genotypes (Table4.6). Stomatal closure is one of the first-line of defense for plants under any stress condition especially under water stress. Closure of stomata ensures that the lesser amount of water is lost from the plant through evapotranspiration process. Thus the stomatal closure causes decreased gaseous exchange with the outside atmosphere along with decreased water loss through evapotranspiration which decreased the stomatal conductance of the plant, as the plant is subjected to water stress. The decrease in the stomatal conductivity due to water deficit was also found in research works done by Anyia and Herzog (2004); Arndt et al. (2001); Lu and Zhang (1999). Similar results on coconut conductance was observed by Kasturi-bai (1997). However, unlike the results observed in the previous studies which observed significant difference between hybrid and tall varieties, a significant difference was only found when comparing KT to the dwarf varieties while the KP showed similar values as that of dwarf varieties.

Table 4.6. Stomatal conductance of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

VARIETY	100% ASM	50% ASM	25% ASM	Mean
MYD	0.05	0.02	0.02	0.031^{C}
CGD	0.15	0.11	0.03	0.097^{A}
KP	0.13	0.04	0.02	0.060^{B}
KT	0.11	0.04	0.05	0.066^{B}
Mean	0.109^{A}	0.055^{B}	0.027^{C}	
CD at 5%				
VARIETY	0.02			
MOISTURE REGIME	0.02			
V*M	0.04			

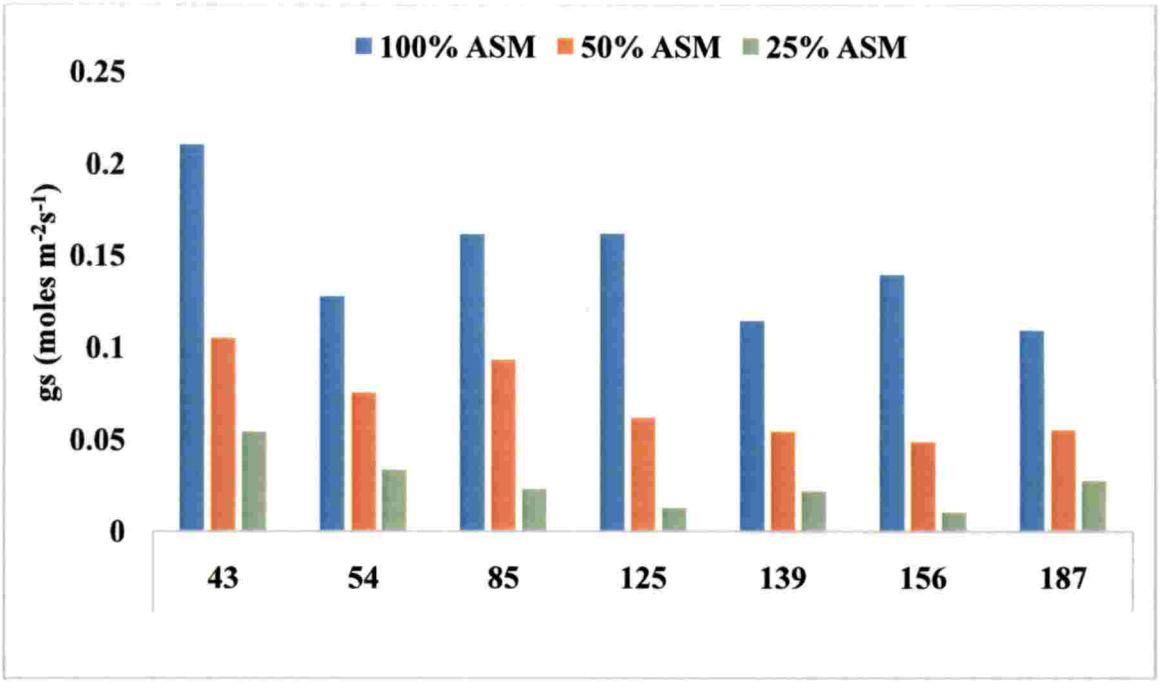

Fig 4.15. Stomatal conductance of genotypes under 100% ASM, 50% ASM and 25% ASM.

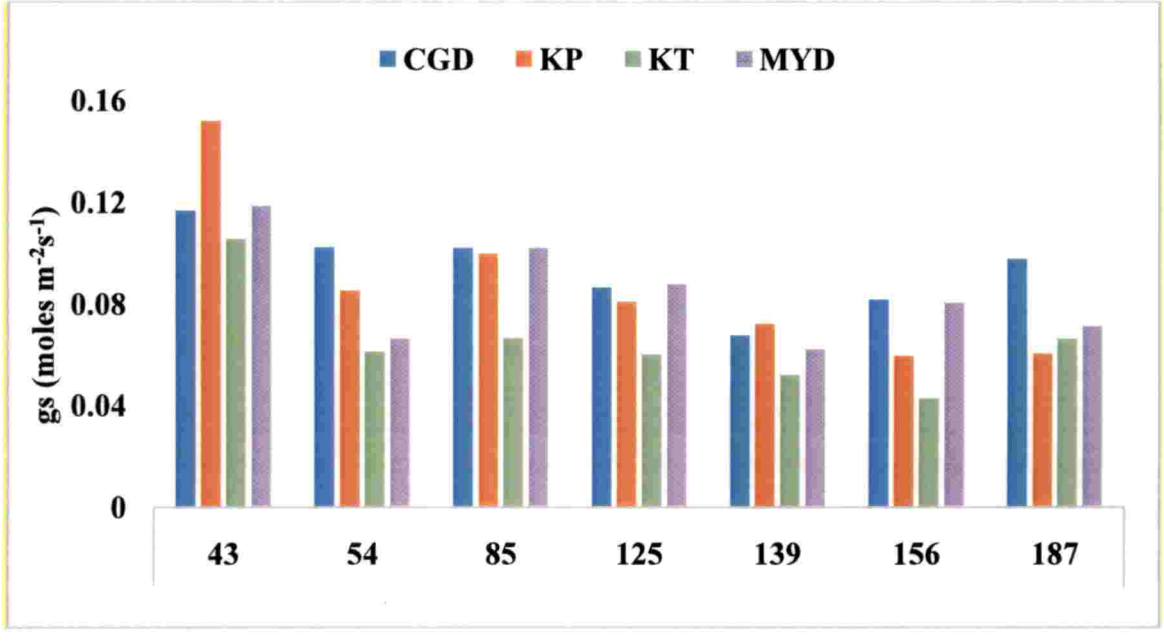

Fig 4.16. Stomatal conductance of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Similar decrease in the net photosynthesis rate of coconut palms due to water deficit which results in lower stomatal conductivity and lower water potential has been extensively reported in previous studies (Nareshkumar et al., 2006; Naresh Kumar et al.,2002; Repellin et al., 1994; 1997; Passos et al., 1999; Rajagopal and Kasturi-bai, 2002).

4.3.3 Stomatal resistance (rs)

Stomatal resistance was 1.41 s cm^{-1} for 100% ASM plants and it increased to 3.27 at 50% ASM however, it was not significant. At 25% it was very high (15.44 s cm^{-1}). Time course measurement showed almost similar trend throughout the experimental period with and without water deficit stress (Fig. 4.17). The above trend was seen as early as 16 days after stress imposition which was the first observation available. At 25% dwarfs showed high rs compared to talls. It was highest in CGD (21.73 s cm^{-1}) followed by MYD (15.57) and was low in KT (12.4) and KP (12.07). Under water stressed conditions, ion-transport and water-transport systems across membranes reduce the turgor pressure in guard cells. This will result in the closure of stomata thus increasing the stomatal resistance in plants. This mechanism ensures that the water loss through the stomatal pores is minimized (Anyia and Herzog, 2004; Arndt et al., 2001; Lu and Zhang, 1999). Similar difference in stomatal resistance was observed by Shivashankar et al. (1991) and Kasturi-bai et al. (1988). Shivashankar et al. (1991) also observed an increase in stomatal resistance from December till May.

Table 4.7. Stomatal resistance of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM)

Variety	100% ASM	50% ASM	25% ASM	Mean
MYD	1.09	2.65	15.57	6.43^{B}
CGD	1.93	3.23	21.73	8.96^{A}
KP	1.07	2.89	12.07	5.34^{B}
KT	1.58	4.33	12.40	6.10^{B}
Mean	1.41^{B}	3.27^{B}	15.44^{A}	
CD at 5%				
Variety	2.16			
Moisture Regime	1.87			
V*M	3.73			

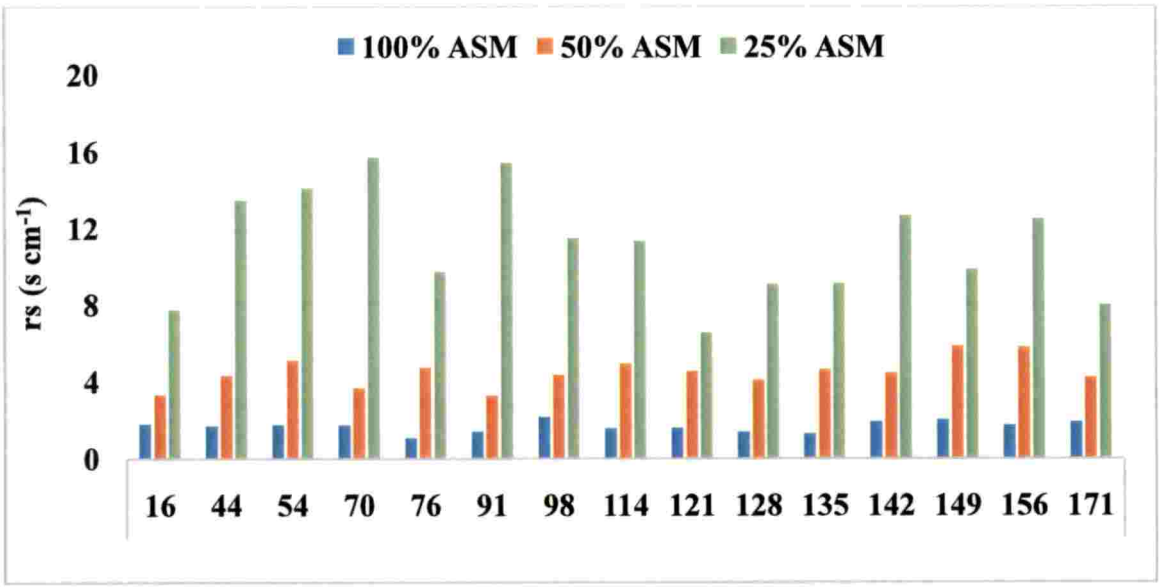

Fig 4.17. Stomatal resistance of genotypes under 100% ASM, 50% ASM and 25% ASM

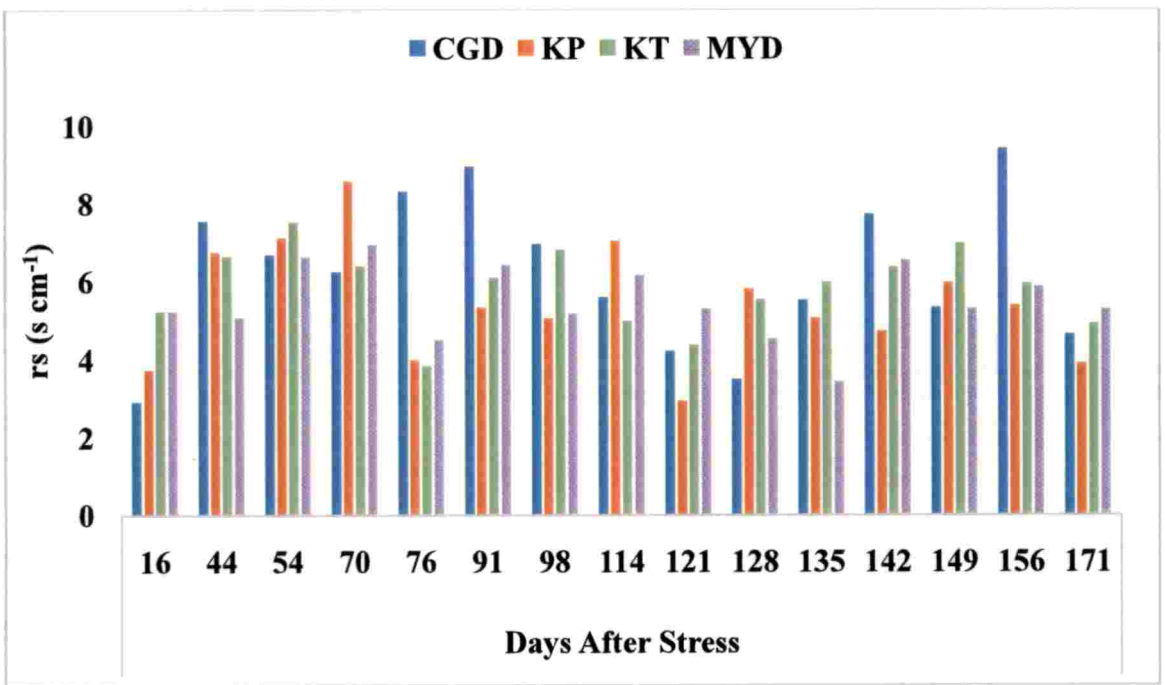

Fig 4.18. Time course measurement of stomatal resistance of coconut varieties

4.3.4 Transpiration (Tr)

The highest mean transpiration rate was recorded in coconut seedlings grown under controlled conditions (3.64 mmole $m^{-2}s^{-1}$). Significant decrease in transpiration rate was observed with the increase in water-deficit stress. At 50 and 25% ASM it declined to 1.47 m mole $m^{-2}s^{-1}$ and 0.95 m mole $m^{-2}s^{-1}$ respectively. In control plants Tr ranged between 3.37(CGD) and 3.92 (KP) and it showed a steep decline at 50% ASM which ranged from 0.84 (MYD) to 2.17 (CGD), At 25% it was too low and ranged between 0.59 (KP) and 1.59 (KT) (Table 4.8). Time course measurement showed that Tr declined beyond 85 days after stress imposition in 50 and 25% ASM (Fig 4.19). Transpiration was high in KP and low in KT (Fig 4.20). The decrease in the transpiration rate observed with the increase in water stress could be attributed to the phenomenon of closure of stomata in the water stressed plants which is aimed to reduce the transpirational loss of water. Hence, imposition of water deficit stress enhances stomatal resistance and thus low transpiration rate. Similar decrease in the transpiration loss as the water stress increased in coconut palms was observed in the works conducted by Kasturi-bai et al. (1997) and Kasturi-bai and V Rajagopal (1997)

Table 4.8 Transpirational Rate of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	100% ASM	50% ASM	25% ASM	Mean
MYD	3.46	0.84	0.67	1.65^{C}
CGD	3.37	2.17	0.98	2.17^{AB}
KP	3.92	1.32	0.59	1.94^{C}
KT	3.85	1.55	1.59	2.32^{A}
Mean	3.64^{A}	1.47^{B}	0.95^{C}	
CD at 5%				
Variety	0.32			
Moisture Regime	0.28			
V*M	0.55			

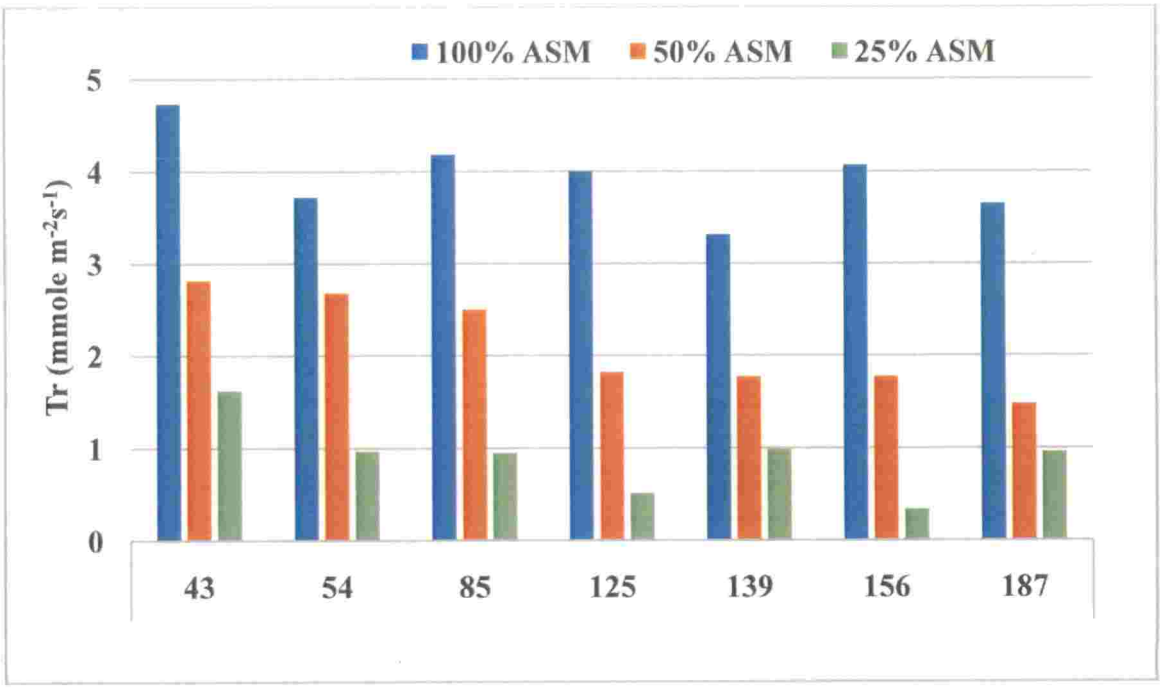

Fig 4.19. Transpiration rate of the leaves of the genotypes grown under100% ASM, 50% ASM and 25% ASM

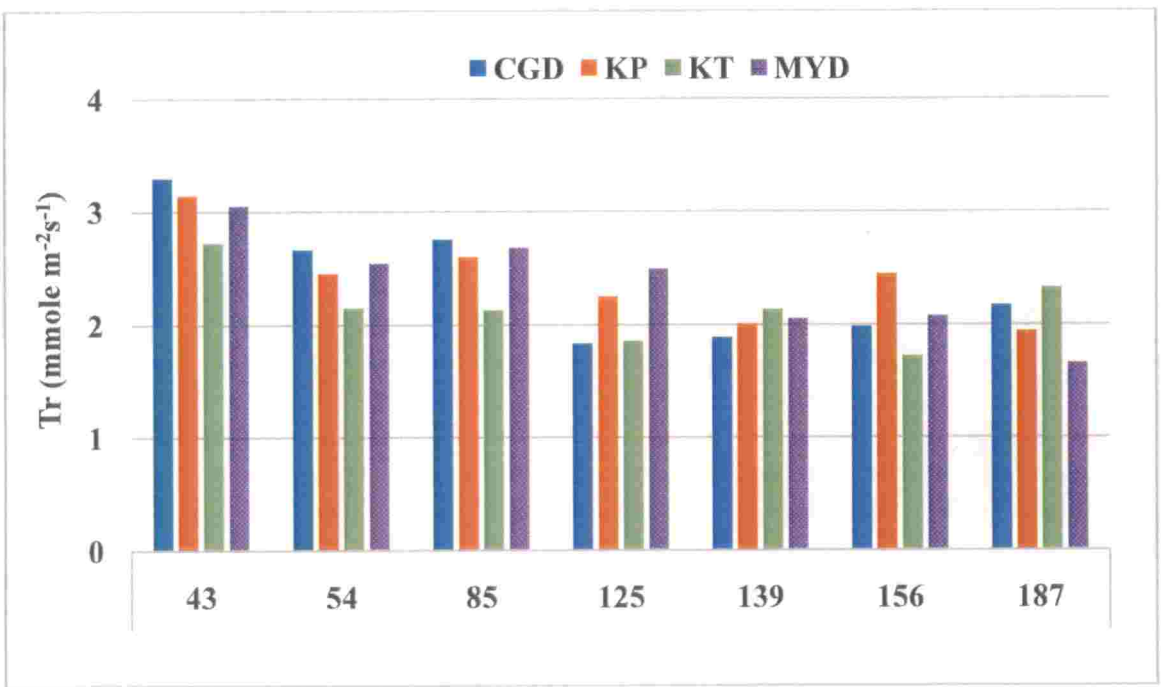

Fig 4.20. Transpiration of various coconut varieties

4.3.5 Leaf water potential (LWP)

Leaf water potential of 100% ASM seedlings was low 9.62 bars. Plants that were subjected to stress (25% ASM) showed average LWP of 19.13 bars as against -14. 58 bars exhibited by the seedlings grown under 50% ASM conditions. Thus, imposition of moisture stress significantly decreased the LWP across the varieties studied. Difference in LWP between the genotypes was low at 100% ASM (8.01(KP) to 10.9 (CGD) bars) compared to 25% ASM 16.9 (KT) to 20.83 (CGD) bars) (Table 4.9). The entire water balance of the plant is lost during water stress. As the amount of available water within the soil pores decreases, plants require more energy to extract them and this causes lowering of leaf water potential. Thus, lesser the available soil moisture greater the water deficit and accordingly larger is the negative potential required by the plants to absorb the water, which, decreases the leaf water potential. Decrease in the leaf water potential due to water stress in coconut palms has been observed in works conducted by Shivashankar et al. (1991) and in works conducted by Dee-Roo (1969)

Table 4.9 Leaf water potential of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**
MYD	9.83	15.28	18.10	14.40^{AB}
CGD	10.9	14.73	20.83	15.50^{A}
KP	8.01	14.48	20.70	14.39^{AB}
KT	9.73	13.87	16.90	13.50^{B}
Mean	9.62^{C}	14.58^{B}	19.13^{A}	
CD at 5%				
Variety	1.24			
Moisture Regime	1.08			
V*M	2.15			

4.3.6 Chlorophyll Index

Chlorophyll index (CI) value was high in 100% ASM (57.60) and declined significantly with water deficit stress treatment. It decreased by 11.4 % and 12.6 % at 50 and 25% ASM respectively (Table 4.10). At 100% ASM KT exhibited the highest chlorophyll index (63.29) while it was the least in MYD (52.8). Since MYD has yellow colored leaves it has low CI (Hebbar et al., 2016). Under 25% ASMCI reduced to 36.29 in MYD and it was 58.11 in KT. Foliar chlorophyll content is good indicator of plant stress and potential for plant carbon dioxide uptake which ultimately shows growth (Datt, 1999; Hebbar et al., 2016). Earlier work showed that the relationship between CI and chlorophyll across the coconut genotypes was not good but the relation between CI and a genotype with different management options was strong (Hebbar et al., 2016). Compared to Pn, gs and Tr, the CI was more stable upto 36 days after stress (Fig 4.21). Beyond this, it started declining at 25% ASM, while at 50% ASM it increased upto 56 DAS and started declining. It was high in100% ASM throughout the experimental period.CI was high in KT followed by CGD and KP and was the least in MYD (Fig 4.22).

Table 4.10 Chlorophyll index of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Varieties	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**
MYD	52.8	38.04	36.29	42.37^{C}
CGD	59.72	51.27	46.29	52.42^{B}
KP	54.59	60.21	41.81	52.20^{B}
KT	63.29	52.28	58.11	57.89^{A}
Mean	57.60^{A}	50.45^{B}	45.62^{C}	
CD at 5%				
Variety	2.53			
Moisture Regime	2.19			
V×M	4.38			

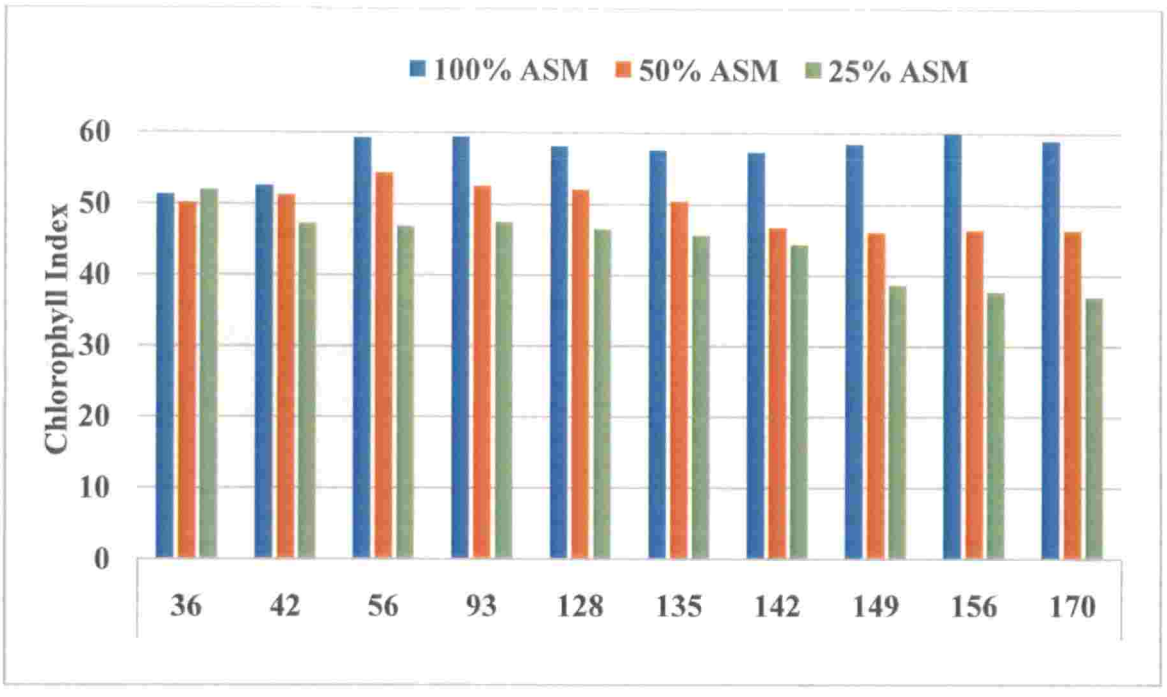

Fig 4.21. Chlorophyll index of genotypes under 100% ASM, 50% ASM and 25% ASM

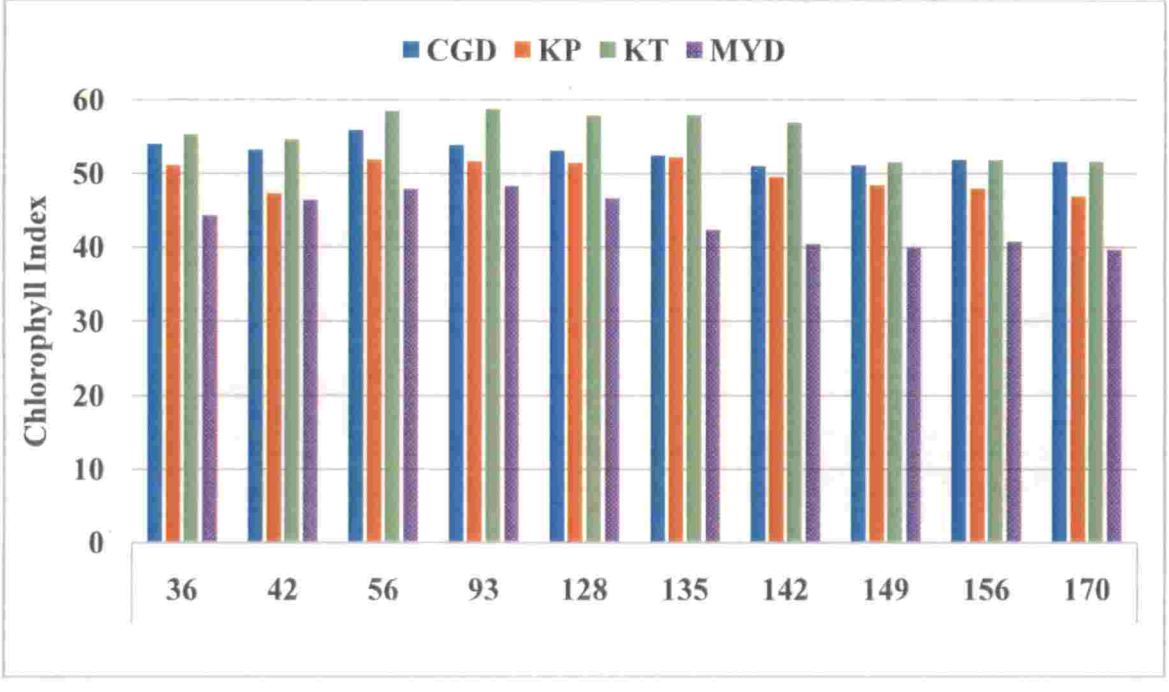

Fig 4.22. Chlorophyll index of coconut cultivars

4.3.7 Chlorophyll fluorescence (Fv/Fm ratio)

PS II quantum yield (Fv/Fm) was high in seedlings grown under 100% ASM conditions (0.7916) and the chlorophyll fluorescence decreased progressively with the water stress. Among the genotypes studied, MYD showed highest Fv/Fm ratio (0.8) while CGD under moisture-stress showed (25% ASM) had the least Fv/Fm ratio (0.73) (Table 4.11).Genotype and moisture deficit interaction was not significant.

The decrease in the chlorophyll fluorescence (Fv/Fm ratio) is an indication that the photosynthetic apparatus have been damaged and the plant is under stress (Daymond and Hadley, 2004). Severe water stress led to chronic photo inhibition in coconut varieties, which was indicated by low *Fv*/*F*m. It has been demonstrated that the light harvest reductions due to the reduction in pigments content and the similar *Fv*/*F*m values may suggest that photochemistry down-regulation rather than PSII damage is occurring (Pastenes et al., 2004). Coconut PS II system is indeed sensitive to soil water deficit and other micrometerological variables and proper maintenance of soil and leaf water status is essential for seedling survival during summer months (Kasturi-bai et al., 2006).

Table 4.11 Chlorophyll fluorescence of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	100% ASM	50% ASM	25% ASM	Mean
MYD	0.80	0.75	0.75	0.76^{A}
CGD	0.79	0.76	0.73	0.76^{A}
KP	0.79	0.77	0.75	0.77^{B}
KT	0.79	0.77	0.75	0.77^{B}
Mean	0.79^{A}	0.75^{B}	0.74^{B}	
CD at 5%				
Variety	NS			
Moisture Regime	0.01			
V*M	NS			

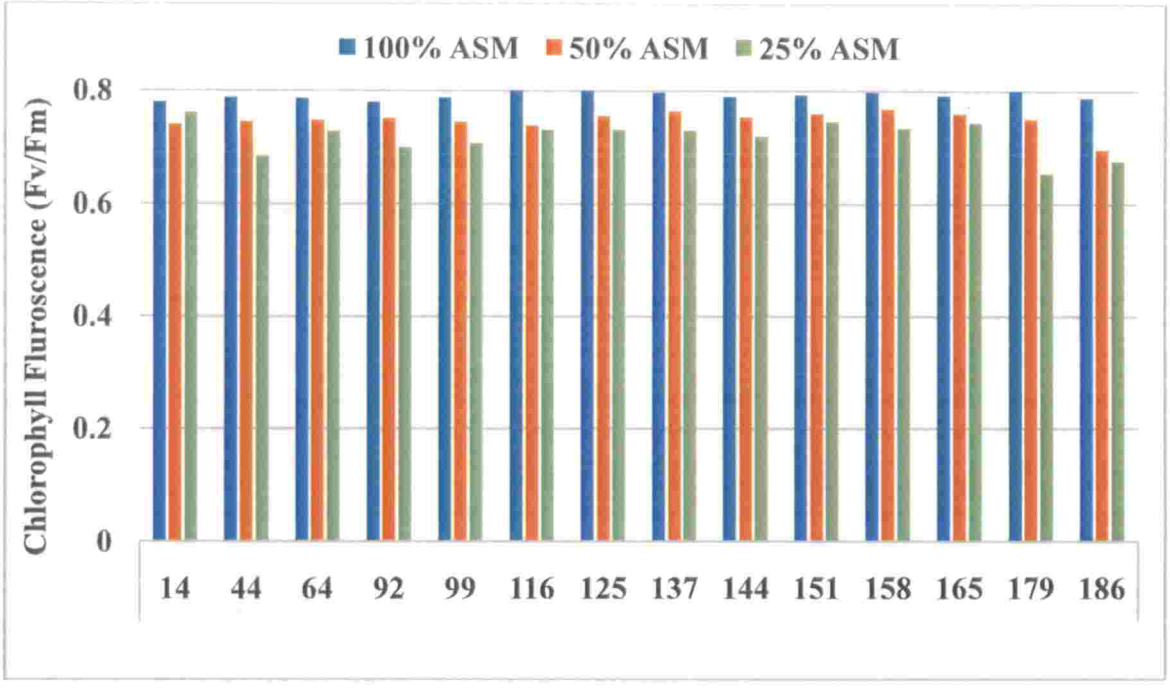

Fig 4.23. Change in chlorophyll fluorescence of genotypes under 100% ASM, 50% ASM and 25% ASM

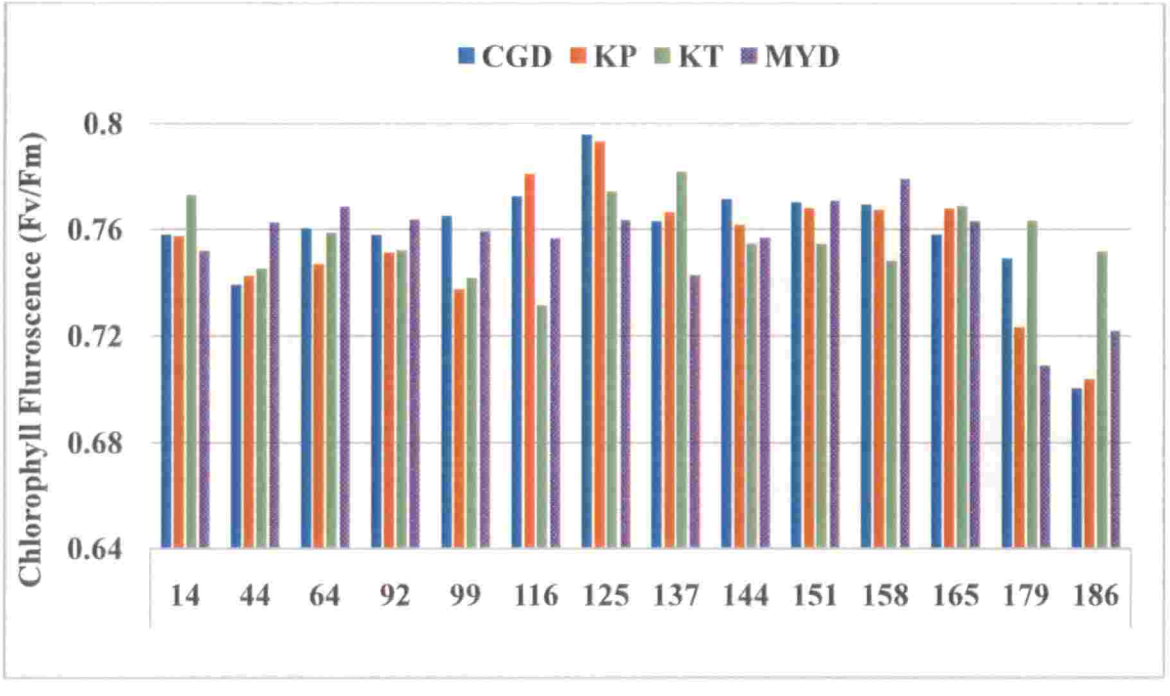

Fig 4.24. Chlorophyll fluorescence of coconut varieties

4.4 BIOCHEMICAL RESPONSE

Most of the physiological responses are an immediate response to the water stress while the biochemical response takes time. However, how a plant responds to the water stress biochemically is one of the important aspects by which plants survive long term water stresses like annual droughts. Biochemical parameters like chlorophyll content, total sugar, reducing sugar, soluble protein, and total phenol content were measured in coconut seedlings in response to normal and water deficit conditions. The resultant values are presented below.

4.4.1 Total soluble protein

The plants grown under 25% ASM showed the highest soluble protein value (26.4101mg g^{-1} fresh leaf tissue) and the amount of soluble protein decreased as the water stress decreased. Variety wise, CGD at 25% ASM had the highest (30.75 mg g^{-1} fresh leaf tissue) protein value while KT 100% ASM had the lowest soluble protein (15.32 mg g^{-1} fresh leaf tissue) value. Variety wise, moisture regime wise and the interaction between variety and moisture regime were all statistically significant (Table 4.12). During water stress, plants produce more proteins due to two reasons. The first reason is that the plants are producing stress proteins to cope with the water deficit and the other reason is that the plants are producing water soluble proteins like proline which can yield in lower water potential in plants allowing plants to maintain the soil plant air continuum. Similar observations has been also seen in studies done by Singh and Rai (1982); Vartania et al. (1987).

4.4.2 Starch

Mean value of showed that in 100% ASM, the plants showed the highest starch value (115.67 mg g^{-1} fresh leaf tissue) and the amount of starch decreased as the water stress increased. Variety wise, CGD 100% ASM had the highest (135.94 mg g^{-1} fresh leaf tissue) starch value while KT 25% ASM had the lowest starch (77.01 mg g^{-1} fresh leaf tissue) value. Variety wise and moisture regime wise, the total plant starch content showed statistical significance (Table 4.12). However the interaction between variety and moisture regime was not statistically significant.

4.4.3 Total soluble sugar

The plants grown under 25%ASM showed the highest soluble sugar value (63.01 mg g^{-1} fresh leaf tissue) and the amount of soluble sugar decreased as the water stress decreased. Variety wise, CGD at25% ASM has the highest (71.63 mg g^{-1} fresh leaf tissue) sugar value while KP 100% ASM had the lowest sugar (41.83 mg g^{-1} fresh leaf tissue) value. Variety wise and moisture regime wise, the total soluble sugar was showing statistical significance. However the interaction between variety and moisture regime was not statistically significant. (Table 4.12)

Under water stress, higher potential is required to maintain the soil plant air continuum. This is achieved by the plant cells by dissolving soluble carbohydrate (like glucose and other monosaccharaides) which are converted from insoluble carbohydrate (like starch and other polysaccharides). Thus with the help of enzymes like acid invertase, neutral invertase, phosphate synthase and sucrose synthase, a reduced amount of starch and increased amount of soluble sugars is obtained which will yield an increased osmotic potential, thus helping the plant combat water stress. Similar observations were made by Zaher-Ara et al., (2016); Turner et al., (1978) and Ghate et al., (2017).

Table 4.12 Total soluble protein, Starch and Total Sugar content of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM)

Variety	Total Soluble Protein (mg g^{-1} f.wt leaf tissue)				Starch (mg g^{-1} f.wt leaf tissue)				Total Soluble Sugar (mg g^{-1} f.wt leaf tissue)			
	100% ASM	**50% ASM**	**25% ASM**	**Mean**	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**
MYD	25.21	24.39	24.73	24.77^{A}	118.56	93.02	88.99	100.18^{AB}	47.52	55.69	66.57	56.59^{B}
CGD	18.69	21.00	30.75	23.48^{AB}	135.94	115.81	106.25	119.33^{A}	50.99	65.43	71.63	62.68^{A}
KP	16.23	20.59	24.12	20.31^{C}	112.51	90.33	90.28	97.70^{AB}	41.83	45.38	55.81	47.67^{D}
KT	15.32	24.9	26.05	22.09^{BC}	95.68	84.71	77.01	85.80^{B}	46.01	51.86	58.00	51.95^{C}
Mean	18.86^{C}	22.72^{B}	26.41^{A}		115.67^{A}	95.96^{AB}	90.63^{B}		46.58^{C}	54.58^{B}	63.00^{A}	
					CD at 5%							
Variety			2.46			22.77				3.48		
Moisture Regimes			2.13			19.72				3.01		
V*M			4.27			NS				NS		

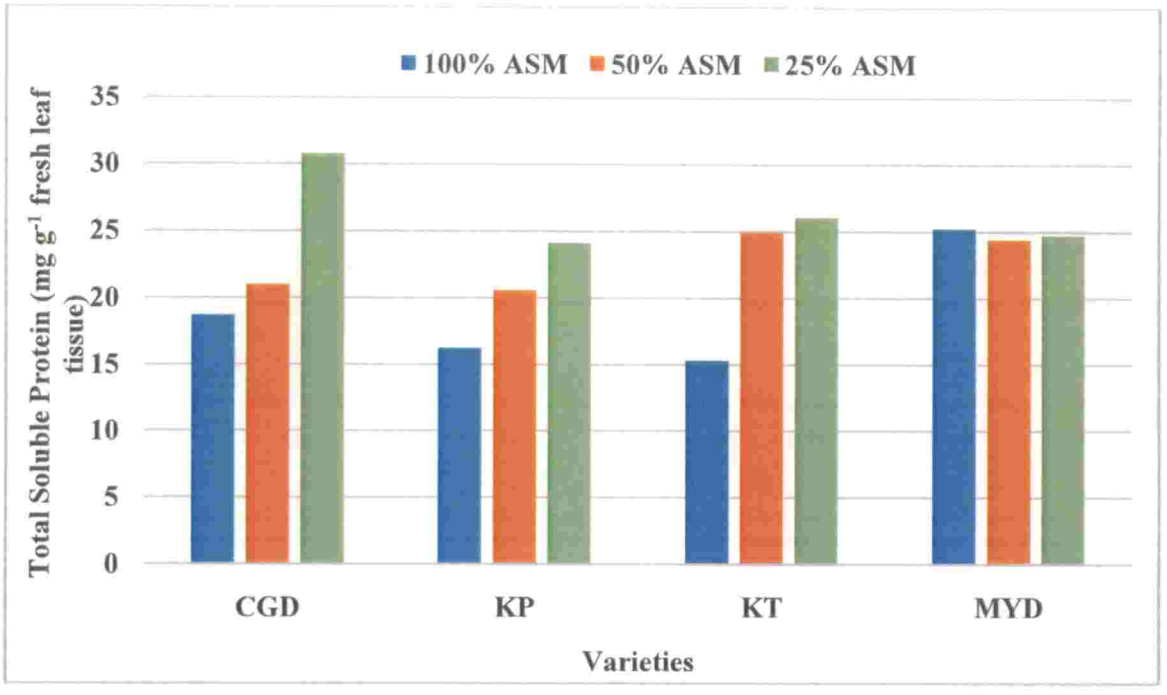

Fig 4.25. Soluble protein of genotypes under 100% ASM, 50% ASM and 25% ASM

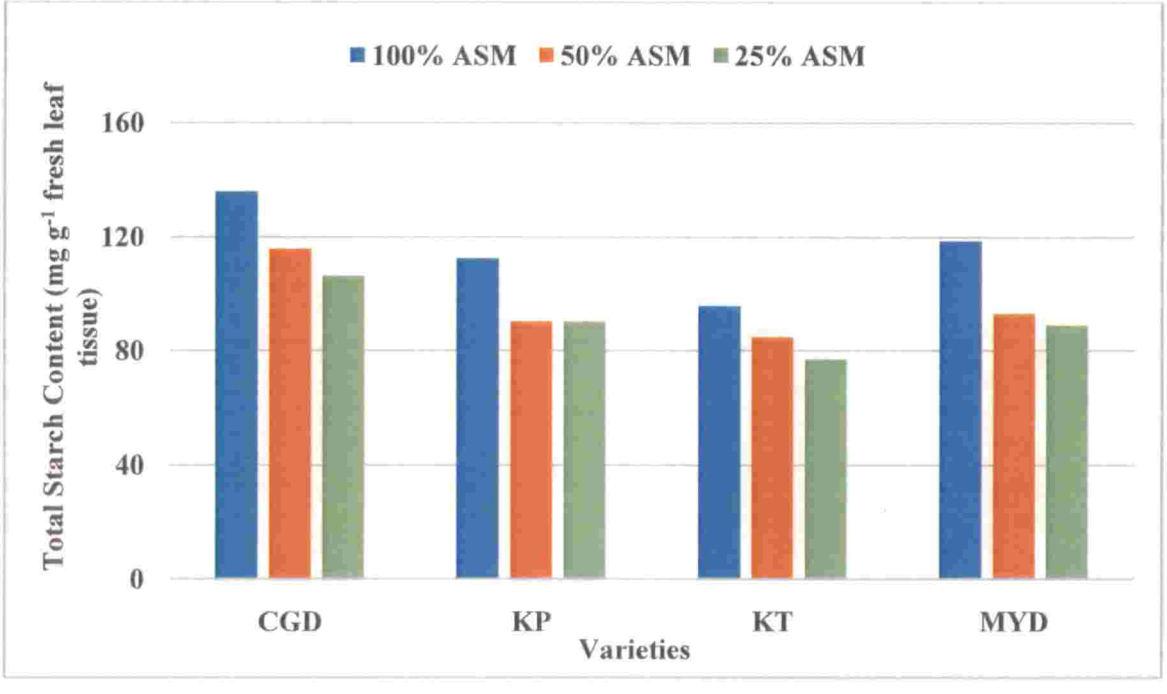

Fig 4.26. Starch content of genotypes under 100% ASM, 50% ASM and 25% ASM.

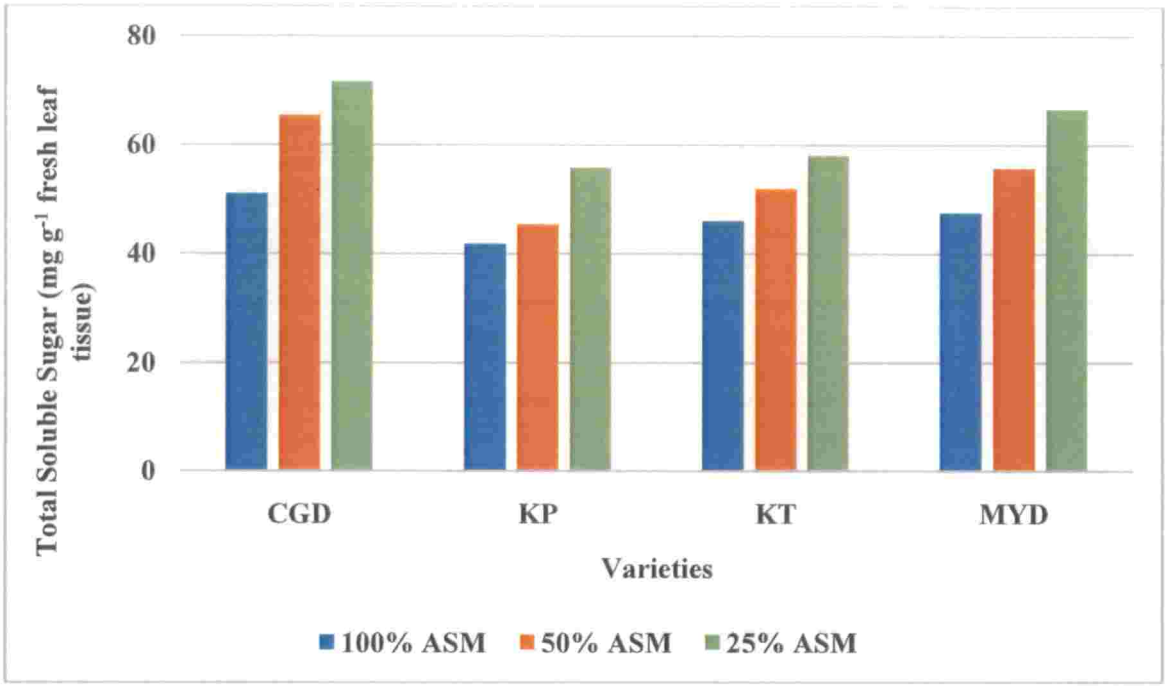

Fig 4.27. Total soluble sugar content of genotypes under 100% ASM, 50% ASM and 25% ASM

4.4.4 Chlorophyll content

Mean values of chlorophyll content in the leaves of coconut seedlings showed highest values (1.91 mg g^{-1} fresh leaf tissue) in the samples derived from 100% ASM seedlings and the chlorophyll content decreased as the water stress increased. Variety wise, 100% ASM grown KP seedlings had the highest chlorophyll content (1.98 mg g^{-1} fresh leaf tissue) while CGD under stress (25% ASM) had the lowest chlorophyll content (1.02 mg g^{-1} fresh leaf tissue) (Table 4.13). Similar to the observations obtained in the physiological parameter, the chlorophyll content of the plant decreased as the water stress is increased as a part of plant to prevent the formation of ROSs.

Table 4.13 Chlorophyll content of various coconut varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	100% ASM	50% ASM	25% ASM	Mean
MYD	1.86	1.54	1.20	1.54
CGD	1.89	1.34	1.02	1.42
KP	1.98	1.62	1.14	1.58
KT	1.9	1.45	1.11	1.48
Mean	1.91^{A}	1.49^{B}	1.12^{C}	1.49
CD at 5%				
Variety	NS			
Moisture Regime	0.15			
V*M	NS			

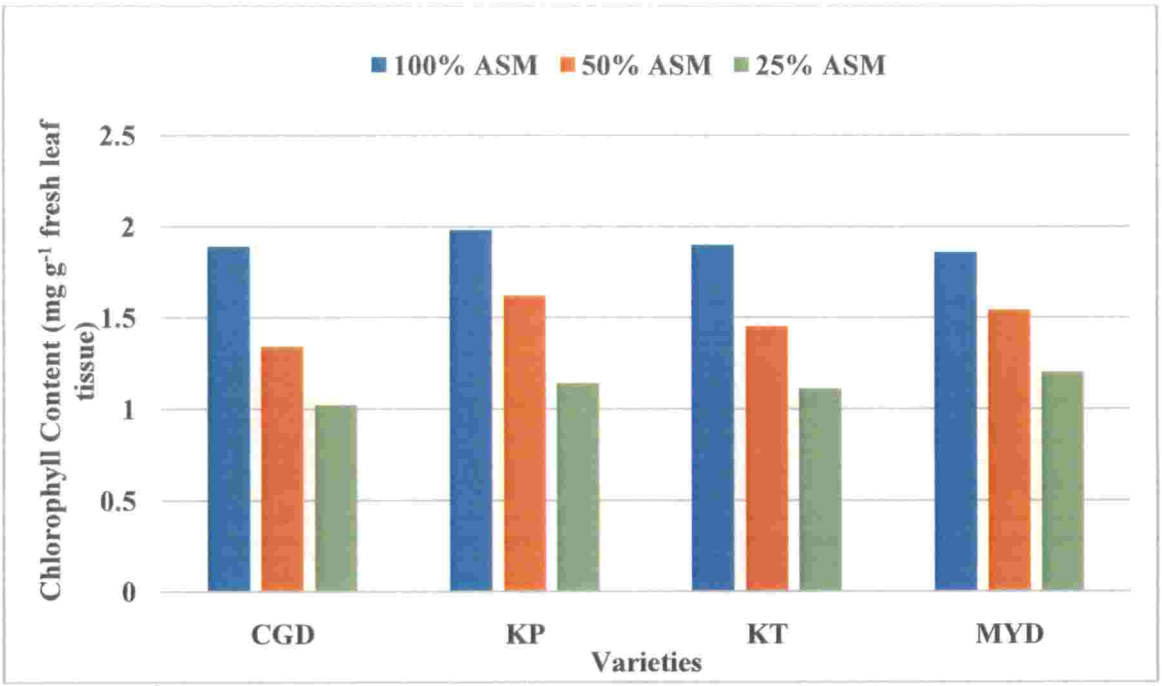

Fig 4.28. Chlorophyll content of cultivars under 100, 50 and 25% available soil moisture

4.4.5 Epicuticular Wax

Studies regarding epicuticular wax content revealed that in seedlings grown under 25% ASM, showed relatively high amount of epicuticular wax (67.95 0 μg cm^2) and the epicuticular wax content decreased with the improved water status of the seedlings. Among the varieties, KT grown under moisture stress (25% ASM) exhibited the highest epicuticular wax content (88.59 μg cm^2) while the leaves of the 100% ASM MYD seedlings had the lowest epicuticular wax content (45.62 μg cm^2) (Table 4.14). Individually, variety wise and moisture regime wise the differences in the epicuticular wax content was statistically significant; however the interaction between variety and moisture regime was not statistically significant. Epicuticular wax is known to act as a barrier for the loss of water through transpiration from the plants. Under water stress condition wherein the water is scarce, plants will produce more epicuticular wax so that the loss of water from plants is minimized. Prior to this, Palmer 1992; Hwang et al. (2002); Riederer (2006); Raffaele et al. (2008); Schreiber (2010) have shown that the wax production increased in the plants under water deficit conditions as observed here. Studies in coconut by Naresh Kumar et al. (2000) and Kurup et al. (1993) have yielded similar increase in the ECW content as the water stress increased.

4.4.6 Lipid Peroxidation

Mean value of MDA (Malondialdehyde), which is an indicator of lipid peroxidation, showed higher MDA content (31.8854 mmol g^{-1} fr. wt) in the stressed plants (grown under 25% ASM), and the MDA content decreased as the amount of water provided increased. Variety wise, KP 25% ASM had the highest MDA content (46.98 mmol g^{-1} fr. wt) while CGD grown under 100% ASM had the lowest MDA content (10.44 mmol g^{-1} fr. wt) (Table 4.14). The differences observed in the MDA content of various varieties, different moisture regimes and their interaction effect were all found to be statistically significant. The oxidative degradation of lipids by the free radicals in the cell can result in the end product MDA. Thus under stress condition when the free radical content is high, lipid peroxidation is enhanced leading to higher amount of the end product of lipid peroxidation, MDA . However, there is no trend regarding to the MDA content. This may

be due to the decrease in the free radicals due to sudden increase in the enzymes like POD, PPO and SOD after 50% ASM100% ASM which can reduce the amount of free radicals which causes the lipid peroxidation (and MDA content) in the first place. Studies by Pandey et al. (2010); Dhindsa et al. (1981) have shown that MDA content will increase as the stress induced by the water is increased

4.4.7 Electrolytic leakage

Mean value of electrolytic leakage in the leaves of the coconut seedlings showed highest values (19.6408 %) in the drought imposed plants (25% ASM), and the electrolytic leakage was low with the improved water status of the seedlings. Among the varieties, KT (under 25% ASM) recorded the highest electrolytic leakage (22.45%) while under 100% ASMCGD exhibited the lowest electrolytic leakage (13.83%) Further, statistical analysis also showed that statistically significant values were obtained between the varieties studied, moisture regime imposed and their interaction effects (Table 4.14). Due to the presence of free radicals in water stressed plants, the cell membrane integrity is lost as the phospho-lipid bilayer is readily disrupted by the free radicals formed due to the water stress. Thus when the cell membrane is compromised, the contents within the cell is released outside the cell, increasing the electrolytic leakage of the plant as observed in these experiments. Similar observations have been made in studies conducted by Garty et al., (2000) and Vainola and Repo (2000).

The study of MDA (Malondialdehyde) show that dwarf cultivar CGD and tall cultivar KT under controlled condition exhibit low MDA values implying their drought tolerant nature. Furthermore, MDA content of the cultivars grown under moisture-deficit stress showed a progressive increase implying the influence of stress on plant mechanism. To corroborate the findings membrane leakage studies show that membrane damage was observed irrespective of the cultivars implying moisture deficit stress severely damages the membranes. Thus it is certain that extent of stress imposition and genotypic differences were observed with respect to MDA content. However, drought condition showed increase in MDA content with the corresponding membrane damages.

Table 4.14 Epicuticular Wax, MDA and Electrolytic Leakage of various varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	Epicuticular Wax (µg cm^{-2})				MDA (mg g^{-1} f.wt leaf tissue)				Electrolytic Leakage (%)			
	100% ASM	50% ASM	25% ASM	Mean	100% ASM	50% ASM	25% ASM	Mean	100% ASM	50% ASM	25% ASM	Mean
MYD	45.62	54.26	56.79	52.22^{C}	19.54	26.39	28.62	24.84^{B}	15.13	16.17	18.62	16.64^{B}
CGD	50.08	54.01	54.63	52.90^{C}	10.44	12.4	17.32	13.38^{C}	13.83	15.86	20.01	16.56^{B}
KP	46.79	68.46	71.79	62.34^{B}	34.61	34.87	46.98	38.82^{A}	14.53	18.63	17.49	16.88^{B}
KT	74.7	73.77	88.59	79.01^{A}	17.14	27.91	34.62	26.55^{B}	16.49	19.57	22.45	19.50^{A}
Mean	54.29^{B}	62.62^{A}	67.95^{A}		20.43^{C}	25.39^{B}	31.88^{A}		14.99^{C}	17.55^{B}	19.64^{A}	
CD at 5%												
Variety	7.02				1.92				0.45			
Moisture Regimes	6.08				1.66				0.39			
V*M	NS				3.32				0.77			

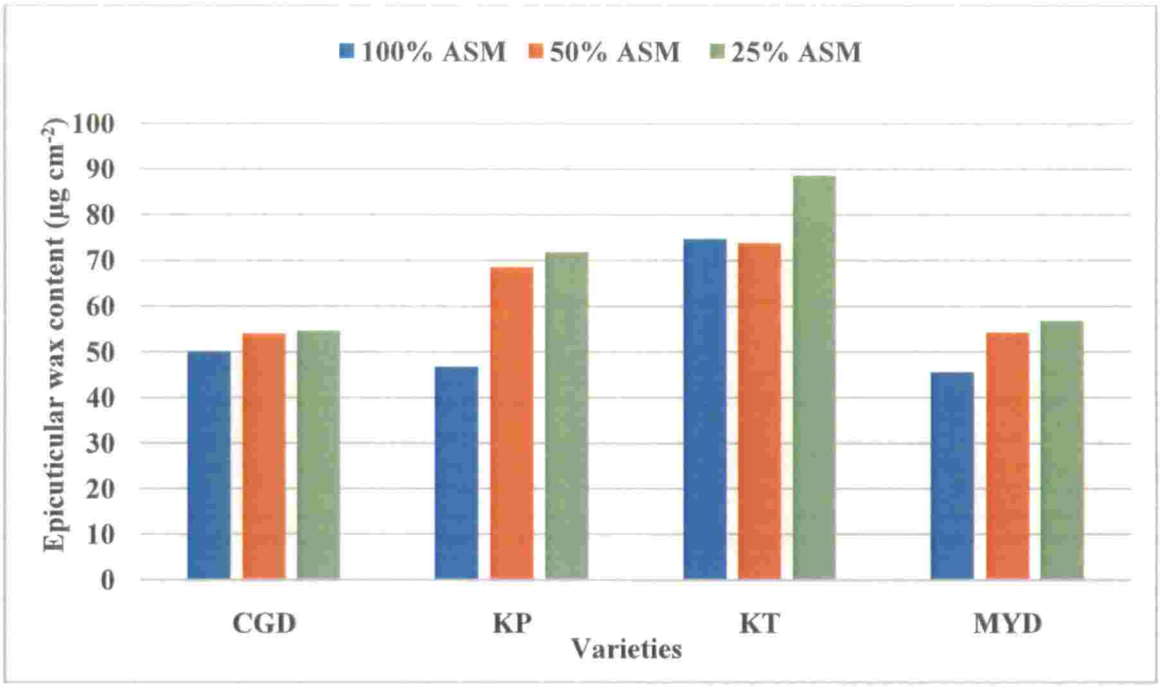

Fig 4.29. Epicuticular wax content of cultivars under 100%, 50% and 25% available soil moisture

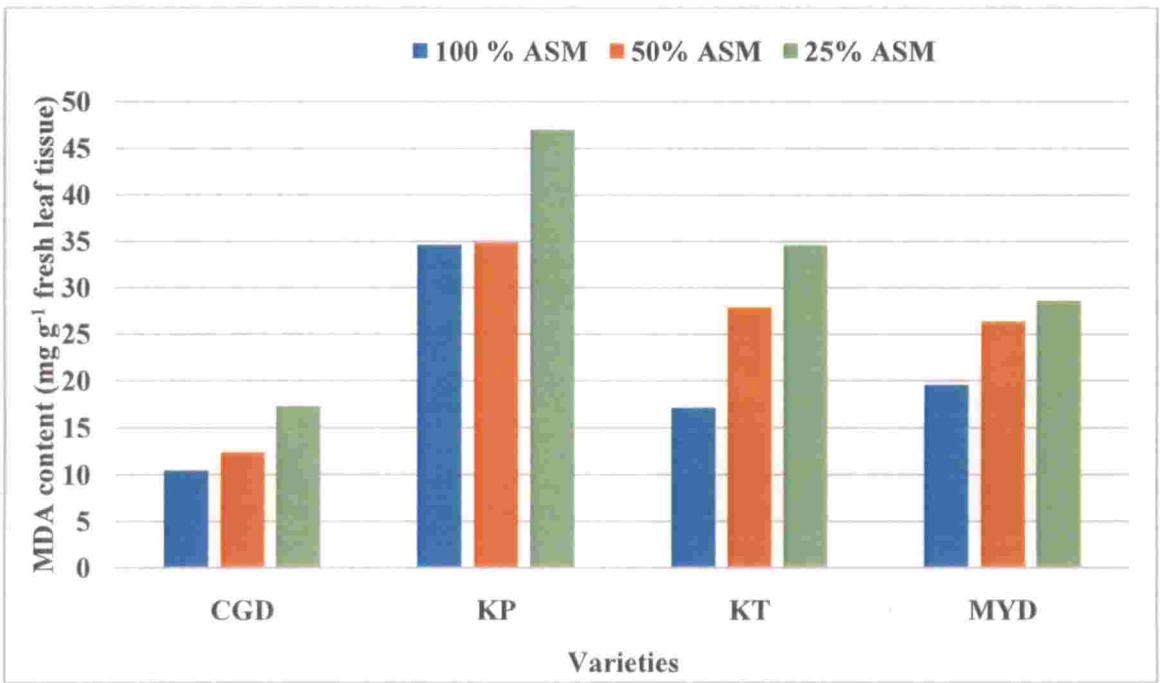

Fig 4.30.MDA content of cultivars under 100%, 50% and 25% available soil moisture

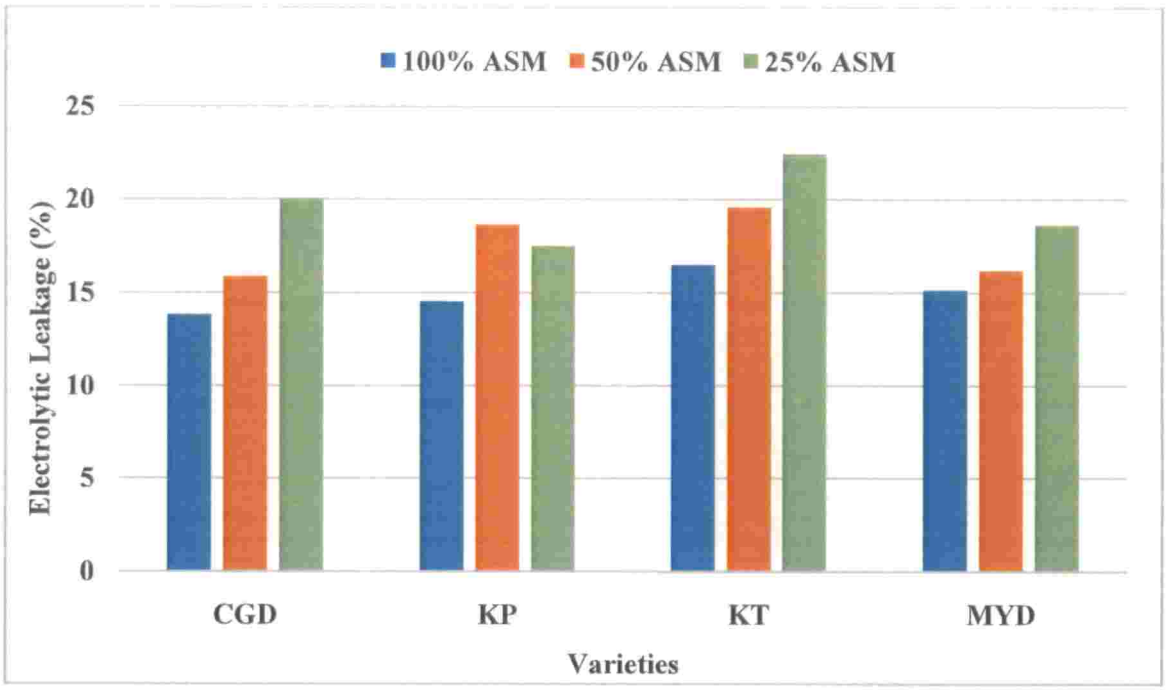

Fig 4.31. Electrolytic leakage of cultivars under 100%, 50% and 25% available soil moisture

4.4.8 Super-oxide dismutase (SOD)

Super-oxide dismutase (SOD) enzyme is a major anti-oxidant enzyme that plays greater role in scavenging reactive oxygen species (ROS) generated during stress. The results reveal that upon induction of moisture-deficit stress, both tall and dwarf cultivars show a significant increase in water stress (Table 4.15). However, the increase in the SOD activity was observed only when the seedlings were subjected to moisture deficit stress of 50% ASM, hence, the specific activity at 25% ASM showed lower levels compared to seedlings grown under 100% ASM. Thus the greater imposition of water-deficit stress did not necessarily convert into greater SOD activity. Mean values of enzyme activity showed that in 50% ASM, the plants showed the highest SOD value (3.98 μg g^{-1}protein) and this value of SOD decreased in the control plants. Variety wise, KT 25% ASM1 had the highest (5.98 μg g^{-1} protein) SOD value while MYD 25% ASM had the lowest SOD (1.86 μg g^{-1} protein) value.

4.4.9 Peroxidase

Peroxidases are haem-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyze the number of oxidative reactions. The results reveal that water-deficit stress upto 50% ASM did not show significant change in the peroxidase activity compared to the control seedlings. This trend was observed across all the tall cultivars and dwarfs studied. Interestingly, the activityof the enzyme decreased significantly when the plants were subjected to severe moisture stress of 25% ASM of water supply. Thus, mean values of peroxidase activity showed that, the plants under 50% ASM showed the highest peroxidase activity (0.84575 μg g^{-1}pro) and the peroxidase activity drastically reduced with the water stress. Variety wise, CGD 50% ASM had the highest activity (1.4 μg g^{-1} protein) while KT 25% ASM had the lowest (0.09 μg g^{-1} protein) peroxidase amount. (Table 4.15)

4.4.10 Polyphenol oxidase

In plants, polyphenol oxidase (PPO) is involved in the oxidation of monophenols and/or o-diphenols to o-quinones with the concomitant reduction of oxygen to water causing protein complex and brown melanin pigments. Polyphenol oxidase (PPO) activity in coconut seedlings showed that imposition of water stress increased the activity of

enzymes to great extent. Mean value of PPO activity showed highest activity (1.24 μg g^{-1} protein) in plants subjected to moisture stress (25% ASM).Variety wise, KT 25% ASM had the highest (1.37 μg g^{-1} protein) polyphenol oxidase value while CGD under 100% ASM had the lowest polyphenol oxidase (0.14 μg g^{-1} protein) value. (Table 4.15).

SOD, POD and PPO are all enzymes which are produced to prevent the ROS, which were formed in the photosynthetic powerhouse of plants. These ROS are detrimental to the plant health as they cause the lipid peroxidation and membrane leakage.. Thus the activities of select anti-oxidant enzymes showed variable trend upon moisture-stress induction. Activities of SOD showed an increase till 50% ASM, however, Peroxidase did not show any significant increase in activity until the stress of 50% ASM but showed decrease in activity when the moisture stress was increased further to 25% ASM. On the other hand polyphenol oxidase activity showed increased activities with moisture stress. These variability in the activities of the enzymes were observed with other abiotic stressors such as elevated CO_2 or elevated temperature or under imposed moisture stress condition in the seedlings of coconut grown under open top chamber (OTC), field conditions or chamber control set up (Hebbar et al., 2013; Sunoj et al., 2014; Gomes et al., 2007).

Table 4.15 SOD, POD and PPO activities of various coconut varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

Variety	SOD (µg g^{-1} f.wt leaf tissue)				Peroxidase (µg g^{-1} f.wt leaf tissue)				Polyphenol Oxidase (µg g^{-1} f.wt leaf tissue)			
	100% ASM	50% ASM	25% ASM	Mean	100% ASM	50% ASM	25% ASM	Mean	100% ASM	50% ASM	25% ASM	Mean
MYD	2.77	3.08	1.86	2.57^{D}	0.37	0.36	0.26	0.32^{D}	0.18	1.15	1.23	0.85^{B}
CGD	2.35	3.66	2.51	2.84^{C}	1.15	1.4	0.48	1.01^{A}	0.14	1.28	1.18	0.86^{B}
KP	2.76	3.76	2.76	3.09^{B}	0.61	0.58	0.27	0.48^{C}	0.33	1.09	1.19	0.87^{B}
KT	3.49	5.43	5.98	4.96^{A}	0.82	1.05	0.09	0.65^{B}	0.16	1.24	1.37	0.92^{A}
Mean	2.84^{C}	3.98^{A}	3.28^{B}		0.73^{B}	0.84^{A}	0.27^{C}		0.20^{C}	1.19^{B}	1.24^{A}	
CD at 5%												
Variety	0.22				0.03				0.05			
Moisture Regimes	0.19				0.02				0.04			
V*M	0.37				0.04				0.08			

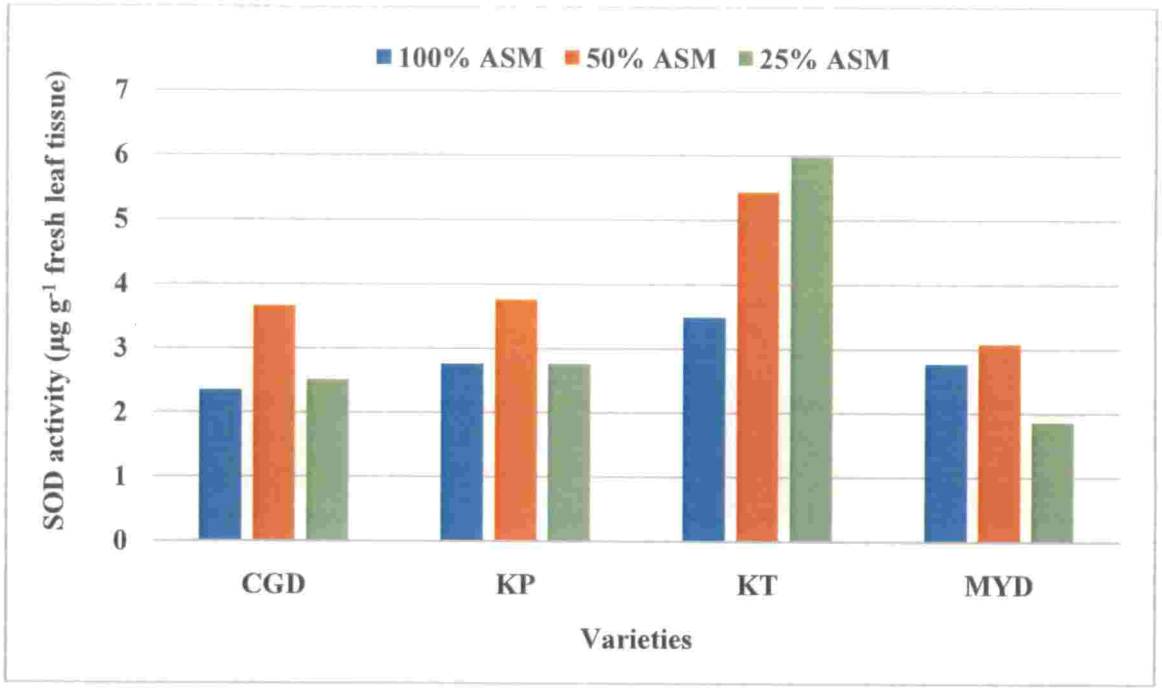

Fig 4.32. SOD content of cultivars under 100%, 50% and 25% available soil moisture.

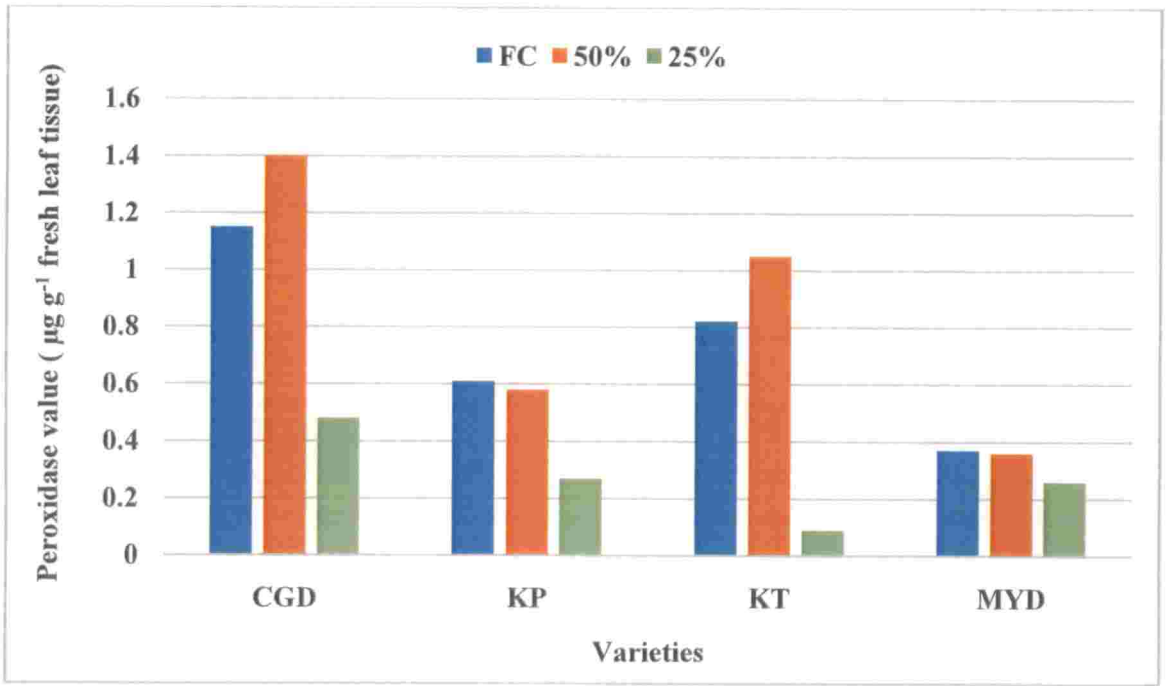

Fig 4.33. Peroxidase activity of cultivars under 100, 50 and 25% available soil moisture

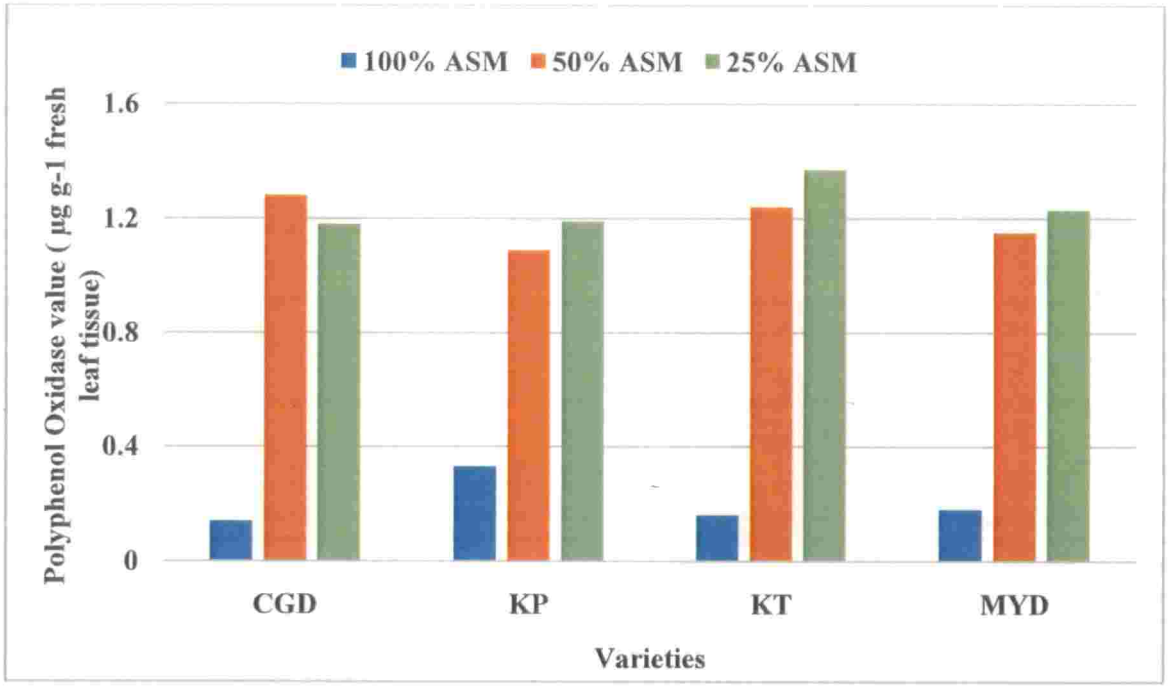

Fig 4.34 Polyphenol oxidase activity of cultivars under 100, 50 and 25% available soil moisture

4.5 WATER USE EFFICIENCY

Whole plant water use efficiency (WUE) of coconut cultivars grown in large buckets and grown under different moisture regimes was calculated. WUE is nothing but the dry matter produced per unit amount of water consumed. The difference between the final biomass and initial biomass is the dry matter produced during the experimental period. Total water consumption by the plant during the experimental period is the summation of (daily water added – evaporation loss + rain water).

4.5.1 Initial Dry Biomass

Initial biomass has been taken by uprooting two plants from each variety. The samples were dried and their dry biomass is presented in Table 4.17.Initial biomass was high in MYD followed by KP and was the least in KT. Initial leaf and root production is faster in MYD compared to rest of the varieties and thus it showed high dry biomass. Interestingly, irrespective of the total biomass the proportion of biomass partitioned to each genotype ranged from 32-34% (Fig 4.35).

Table 4.16 Total water added to pot, rain water addition, evaporative loss and the total water consumption of coconut cultivars under 100, 50 and 25% ASM

Varieties	Total water added (L)	Rain water (L)	Evaporative loss (%)	Total water consumed (L)
100% ASM				
CGD	664	13.32	45	304.79
MYD	508.5	13.32	45	234.81
KP	664	13.32	45	304.79
KT	508.5	13.32	45	234.81
50% ASM				
CGD	332	13.32		152.39
MYD	254.25	13.32		117.4
KP	332	13.32		152.39
KT	254.25	13.32		117.4
25% ASM				
CGD	166	13.32		76.19
MYD	127.12	13.32		58.7
KP	166	13.32		76.19
KT	127.12	13.32		58.7

Table 4.17 Leaf, shoot, root and total biomass of the coconut cultivars before the imposition of water deficit stress.

Variety	Leaf wt	Root wt	Shoot wt	Total Biomass
MYD	0.240a	0.164a	0.191a	0.595
CGD	0.231a	0.153a	0.185	0.569
KP	0.218a	0.164a	0.189	0.571
KT	0.150b	0.087b	0.125	0.362
CD at 5%	0.023	0.028	0.027	0.078

4.5.2Final Dry Biomass

As the plant grows older the pattern of biomass accumulation changed in the genotypes selected. MYD which had high initial biomass could accumulate only 1.23 kg biomass at the end of the experiment as against 2.06 Kg of CGD and 1.77 kg of KP under 100% ASM. It was the least in KT (1.11 kg) (Tables4.18). High biomass accumulation was seen in leaf followed by shoot and root of all the genotypes (Fig. 4.36). However, the proportion of biomass in roots ranged from 23 % (MYD) to 26% (KP and KT) which had declined from initial root proportion range of 32 to 34%.

Table 4.18 Root and Shoot Dry Biomass of various coconut varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).

	Root Dry Biomass				Shoot Dry Biomass			
VARIETY	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**
MYD	0.26	0.21	0.19	0.2187^{B}	0.34	0.2	0.21	0.2518^{C}
CGD	0.52	0.23	0.22	0.3222^{A}	0.64	0.26	0.22	0.3727^{A}
KP	0.5	0.26	0.22	0.3263^{A}	0.46	0.32	0.18	0.3224^{B}
KT	0.29	0.21	0.17	0.2236^{B}	0.34	0.27	0.18	0.2616^{C}
Mean	0.3924^{A}	0.2275^{B}	0.1982^{B}		0.4467^{A}	0.2628^{B}	0.1968^{C}	
CD at 5%								
VARIETY	0.05				0.04			
MOISTURE REGIME	0.05				0.04			
V*M	0.09				0.07			

Table 4.18 Plant Final Leaf and Total Dry Biomass of various coconut varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM)

	Leaf Dry Biomass				Total Dry Biomass			
VARIETY	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**	**100% ASM**	**50% ASM**	**25% ASM**	**Mean**
MYD	0.63	0.41	0.21	0.4168^{B}	1.23	0.83	0.61	0.8872^{B}
CGD	0.9	0.51	0.3	0.5730^{A}	2.06	1	0.74	1.2679^{A}
KP	0.81	0.58	0.35	0.5791^{A}	1.77	1.16	0.76	1.2279^{A}
KT	0.48	0.37	0.24	0.3637^{B}	1.11	0.85	0.59	0.8488^{B}
Mean	0.7033^{A}	0.4686^{B}	0.2776^{C}		1.5423^{A}	0.9589^{B}	0.6726^{C}	
CD at 5%								
VARIETY	0.07				0.12			
MOISTURE REGIME	0.06				0.1			
V*M	0.12				0.2			

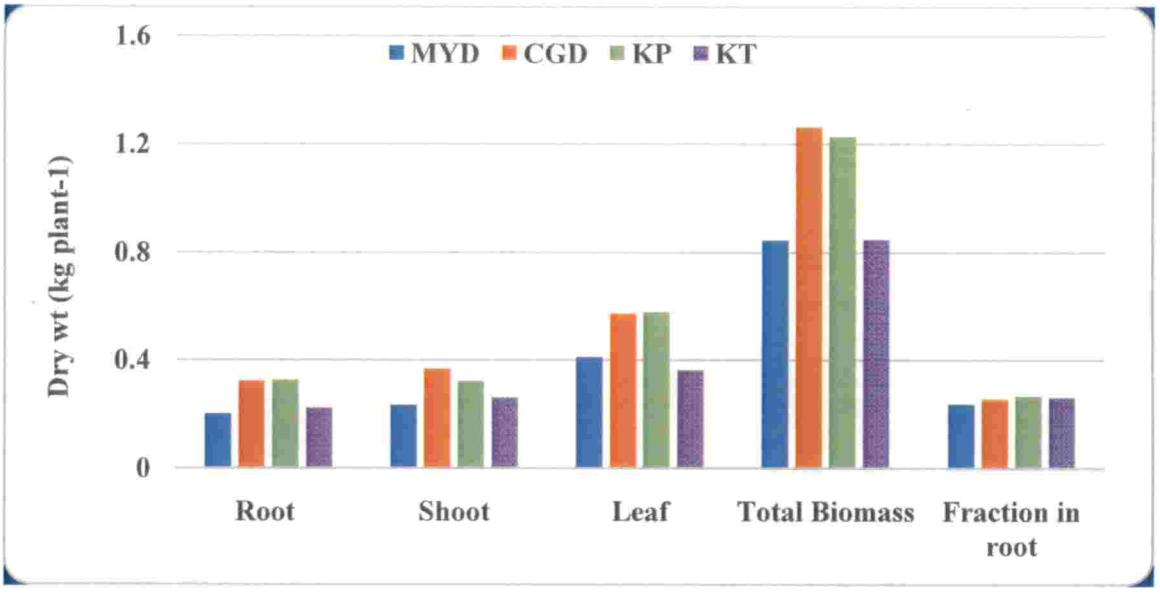

Fig.4.36 Root, shoot, leaf, total biomass and fraction of biomass in roots of MYD, CGD, KP and KT

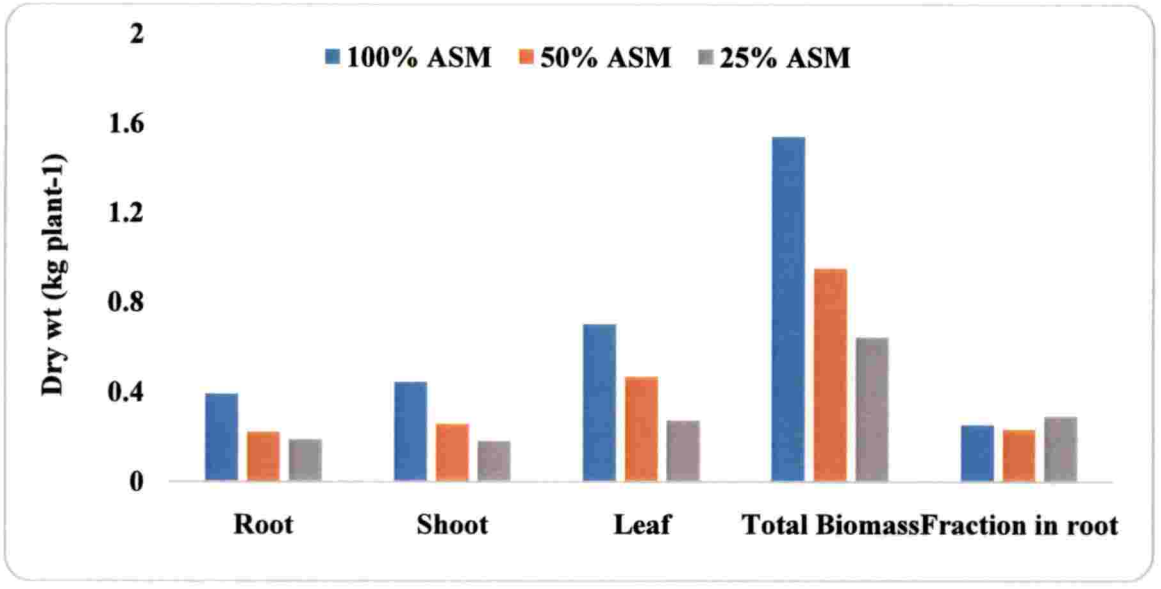

Fig. 4.37 Root, shoot, leaf, total biomass and fraction of biomass in roots at 100, 50 and 25% ASM regimes.

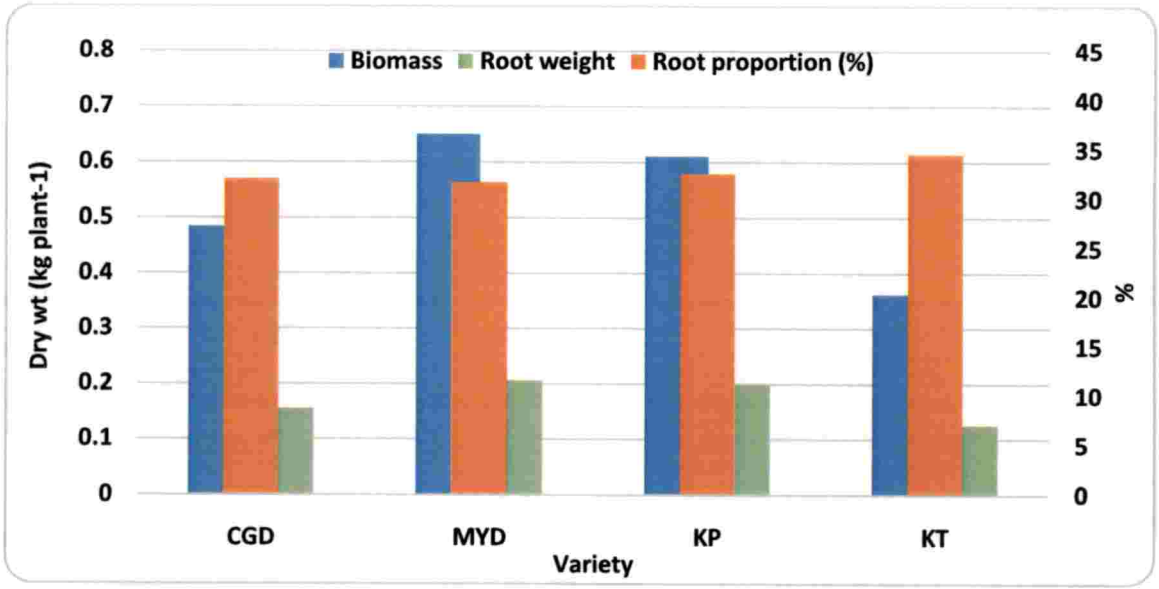

Fig 4.35. Total biomass, root biomass and proportion (%) of root to total biomass of different varieties.

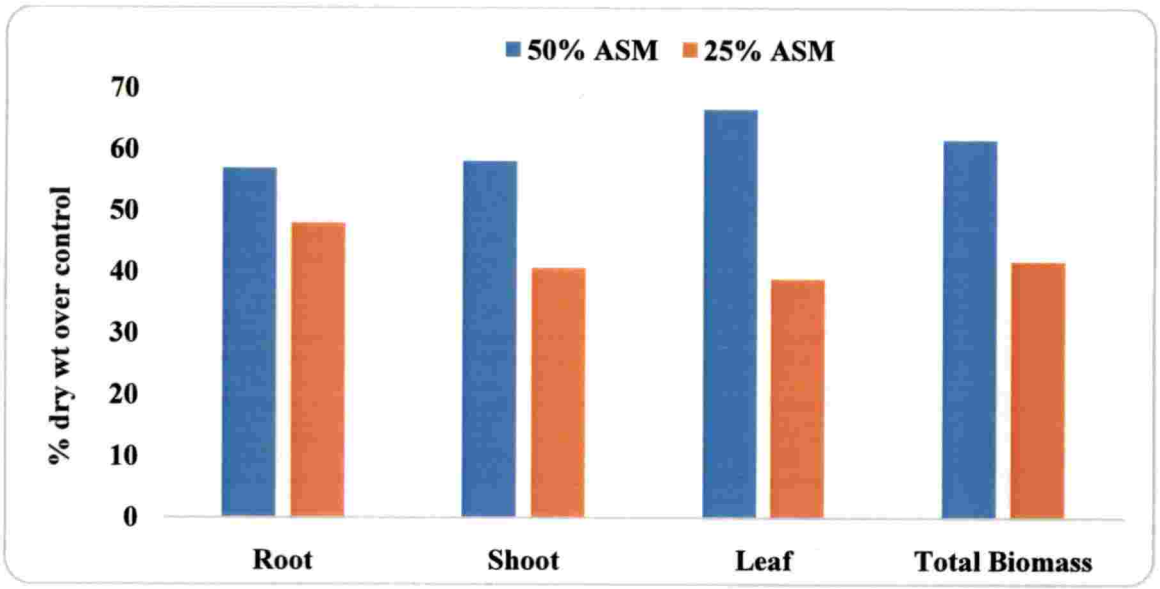

Fig. 4.38 Percentage biomass accumulation in different plant parts and total biomass of 50% and 25% ASM over 100% ASM.

Biomass declined significantly at 50% and 25% ASM by 16 % and 22 % respectively from the 100% ASM. At 25% biomass production of KP (0.76) and CGD (0.74) was on par while it was low and on par between MYD (0.61) and KT (0.59). Biomass accumulation was high in leaf (0.70) followed by shoot (0.45) and low in root (0.39) in plants grown at 100% ASM. With water deficit stress this difference was reduced amongst the plant parts. At 25% ASM biomass accumulation in leaf, shoot and root was 0.27, 0.19 and 0.19 respectively which was 39, 41 and 48% of 100% ASM. Thus, under severe stress the fraction of biomass partitioned to root is slightly more than rest of the parts (Fig. 4.36).

From the above Fig. 4.37 it is clear that as the stress increases leaf and shoot biomass declines and more biomass is partitioned towards the root. Leaf is the most sensitive followed by shoot and is the last part to get affected. Biomass is the product of leaf area and photosynthesis per unit leaf area. (Weraduwage et al., 2015) Biomass production of CGD and KP was significantly high under well watered condition compared to other genotypes. Leaf area production as well as Pnwas significantly high both in CGD and KP and thus both have contributed for biomass accumulation. However, under stress Pn was found to be more sensitive compared to leaf area and thus reduced Pn is responsible for the declined biomass(Kasturi-bai et al., 1997). Kasturi-bai et al. (1996). Rajagopal et al. (1989) also observed that there will be a higher vegetative dry mass production at the expense of reproductive dry matter production during water deficit condition in coconut palms.

4.5.3 Water Use Efficiency

The whole plant water use efficiency significantly differed amongst genotypes but not with moisture levels. However cultivar x moisture level was significant. At 100% ASM it was significantly low in MYD (3.34) followed by KT (4.06) and was significantly high in KP (4.44) and CGD (4.74) (Table 4.19). Both biomass accumulation and water consumption was low in KT and MYD as compared to that of CGD and KP.

Table 4.19 WUE of various coconut varieties grown under controlled condition (100% ASM), and moisture-deficit stresses (50% ASM and 25% ASM).The data was subjected to sin transformation.

Cultivars	100% ASM	50% ASM	25% ASM	Mean
MYD	3.34	3.54	2.74	3.2077^{C}
CGD	4.74	3.68	3.84	4.0873^{B}
KP	4.44	5.42	4.55	4.8008^{A}
KT	4.06	5.48	5.68	5.0720^{A}
Mean	4.1455	4.5299	4.2004	
CD at 5%				
Cultivar	0.49			
MOISTURE REGIME	NS			
V*M	0.84			

At 50% ASM, WUE increased in both tall genotypes while it was on par in MYD and significantly declined in CGD from control plants. In general, it was observed that WUE of crops increased under mild stress (Hebbar et al., 1994; Rajagopal et al., 1989). However, for the same water consumption KT had higher WUE than MYD suggesting higher biomass production per unit water consumption. As the soil water availability decreased WUE increased. However, under severe stress as in 25% ASM WUE significantly declined in MYD and KP from 50% ASM but it was on par in KT and CGD. The tall cultivar KT grown under 25% ASM had the highest WUE (5.68) as against dwarf variety MYD (2.74). Interestingly, the WUE values for dwarf varieties i.e., CGD and MYD under stress was lower than tall varieties. This suggested that under water limited condition talls are more efficient in utilizing the water as compared to dwarfs. Studies by Esmaeilpour et al. (2016); Hai-dong et al. (2016); Tolk et al. (2015); Choury et al. (2016); Hassan et al. (2017); Schoo. et al. (2017) have demonstrated WUE increases in the drought tolerant varieties of a plant species. Thus from the results, it could be inferred that tall coconut cultivars are more adaptive towards water stress than dwarf coconut cultivars. Kasturi-bai et al. (1996) also reported higher WUE in adult palms which are subjected to water deficit. However, Kasturi-bai et al. (1997) also reported that the instantaneous WUE do not show a significant difference when compared between irrigated and unirrigated palms. WUE has been shown to vary among various agro-climatic conditions for same coconut variety and also amongst different coconut varieties (Passos et al., 1999; Prado et al., 2001; Gomes et al., 2002).

Thus from this study it can be inferred that tall variety under moisture stress had higher water use efficiency compared to the dwarfs. KT had the most efficient use of water followed by KP and it was the least in MYD. Though distinct contribution of Pn and leaf area was not evident in imparting drought tolerance in KT and KP, it was clear that under stress they exhibited drought avoidance mechanism like more root production and tolerance mechanisms like epicuticular wax deposition, antioxidant scavenging like SOD activation.

Chapter 5

Summary

An experiment was conducted at ICAR-CPCRI, Kasaragod to study the whole plant WUE of two dwarf Varieties – Chowghat Green Dwarf (CGD) and Malayan Yellow Dwarf (MYD) and two tall varieties – Kalpatharu (KT) and Kalpa Pratibha (KP). Plants were grown in large plastic buckets (64×49 cm) of 100 kg dry soil capacity. Three month after establishment in the bucket the available soil moisture of set of buckets were maintained at 100% ASM (available soil moisture), 50% ASM and 25% ASM for a period of six months. During the period daily known amount of water was supplied to each set of pots and at the end of the experiment it was cumulated. The water lost through evaporation and water added through rainfall was adjusted to get the cumulative water consumed by the plant. The initial biomass at the start of the experiment and final biomass at the end of the experiment was used to calculate the biomass accumulation during the period. Thus WUE g biomass/liter water consumed was calculated for each of the genotypes under stress and non-stress conditions. In order to phenotype the genotypes for their tolerance various morphological, physiological and biochemical parameters were quantified during the experimental period. The salient findings of the study are summarized as follows:

The cultivars selected for the study exhibited distinct differences in their morphological, physiological and biochemical traits to water deficit stress. Coconut seedlings at 100% ASM had normal growth while it significantly declined at 50% ASM. At 25% ASM there was no new leaf emergence and did not show any biomass accumulation from the initial biomass especially in MYD. MYD and KT had the ability to use less water while it was high for KP and CGD. Accordingly biomass production was less in MYD and KT.

By the time of the first observation (25 days after stress imposition) there was distinct difference in plant height, leaf emergence and collar girth however, leaf area was almost on par suggesting it was leaf initiation rather than expansion which was most affected under water stress. At 25% ASM virtually no growth was seen in any of these parameters with further period.

Similar to morphological parameters physiological parameters like Pn, gs, and Tr significantly declined under stress. Stomatal resistance (rs) measured 16 days after stress imposition was significantly high in 50 and 25%ASM. Pn reduced to 50% at 50%ASM and less than 20% at 25%ASM from the 100% ASM. At early stages KP and MYD had high Pn but at 7 later stages high Pn was seen in KP and KT. This could have contributed for high biomass accumulation under stress condition.

Biochemically, the total soluble sugars, total soluble proteins, POD, SOD, PPO, epicuticular wax, MDA and electrolytic leakage were lowest for 100% ASM plants. These values increased as the water stress increased and was highest for plants grown under 25% ASM conditions. However, the starch content was highest in 100% ASM plants and decreased as the water stress increased and eventually became lowest in 25% ASM plants.Talls KT and KP accumulated significantly higher epicuticular wax and free radical scavengers like SOD and polyphenol oxidase compared to dwarfs CGD and MYD.

Thus, in this study we have seen differential accumulation in biomass of cultivars across different moisture levels from the initial almost similar biomass. It was high for all the genotypes at 100% ASM followed by a significant decline at 50% ASM and negligible accumulation at 25% ASM. At 25% biomass accumulation was high in KT and KP and was least in CGD and MYD. Interestingly, talls partitioned higher biomass under stress towards roots, may be to facilitate better extraction of water. This could have enabled them to have low stomatal resistance compared to dwarfs. In terms of WUE, both talls showed higher WUE compared to the dwarfs. When both KT and MYD were grown with same water input KT could accumulate higher biomass compared to MYD. Both drought avoidance mechanism like high root biomass and drought tolerant mechanism like epicuticular wax deposition, increased SOD and polyphenol oxidase activity could have imparted tolerance. Similar difference was seen in other two high water users KP and CGD where KP had better root system and accumulation of epicuticular wax and antioxidant systems.

Thus this study revealed the differences in whole plant water use efficiency of coconut cultivars under water deficit stress and the possible mechanisms imparting

tolerance to these stresses. Further, studies at molecular and transcriptome levels are required to understand the differences at genetic level.

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COMPARATIVE ASSESSMENT OF WHOLE PLANT WATER USE EFFICIENCY (WUE) OF COCONUT SEEDLINGS (*COCOS NUCIFERA*) TO DROUGHT TOLERANCE.

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ABSTRACT OF THE THESIS

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Abstract

A study was conducted at ICAR-CPCRI, Kasaragod to determine the whole plant WUE of two dwarf (Malayan Yellow Dwarf (MYD) and Chowghat Green Dwarf (CGD)) and two tall varieties of coconut seedlings (Kalpatharu (KT) and Kalpa Pratibha (KP)) of coconuts. The seedlings were grown in large buckets and exposed to three moisture regimes- 100% ASM (available soil moisture), 50% ASM and 25% ASM. Coconut seedlings were found to be highly sensitive to moisture and observed that with the first observation made i.e., within 25 days after stress imposition there was significant decline in morphological and physiological traits. Plant height, collar girth, leaf number showed an increasing trend up to 57 days after 50% ASM imposition while at 25% ASM there was no further increment in growth of above parameters. Both Pn and leaf area which contribute to the biomass significantly declined under stress condition. However biomass accumulation over the stress period was high in KT and KP compared to CGD and MYD. Thus KT and KP had high WUE both under 50% and 25% ASM 5.48 g/L and 5.68 g/L and 5.42 g/L and 4.55 g/L respectively. CGD on the other hand had lower WUE at 50% ASM (3.68 g/L) and increased slightly at 25% ASM (3.84 g/L). WUE was the least in MYD both at 50% (3.54 g/L) and 25% ASM (2.74 g/L). The higher WUE in KT and KP was found to be imparted by high biomass partitioning to roots and drought tolerance mechanisms like epicuticular wax deposition and higher activity of antioxidant scavenging systems. Thus, tall varieties KP and KT are found to be better adapted to water deficit stress compared to the dwarf varieties CGD and MYD.