STUDIES ON DROUGHT TOLERANCE POTENTIAL OF MODERN *HEVEA* CLONES IN AN EXTREME DROUGHT PRONE AREA

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BY

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CERTIFICATE

This is to certify that the dissertation entitled, "**Studies on Drought tolerance potential of modern *Hevea* clones in an extreme drought prone area**" is an authentic record of the project work done by **Ms.Anumol Joseph** at Rubber Research Institute of India, Kottayam, under the guidance of Dr.K.Annamalainathan, Scientist, Crop Physiology Division, Rubber Research Institute of India, Kottayam, in partial fulfillment of the requirement for the award of the Degree of Master of Science in Biochemistry at the School of Biosciences, Mahatma Gandhi University, Kottayam and this dissertation has not formed the basis for the award of any other degree or diploma earlier.

August 2011

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CERTIFICATE

This is to certify that the project work entitled "**studies on drought tolerance potential of modern *Hevea* clones in an extreme drought prone area**" submitted to Mahatma Gandhi University, Kottayam by **Anumol Joseph** for the award of the Degree of **Master of Science in Biochemistry** was carried out under my supervision and guidance in the Crop Physiology Division, Rubber Research Institute of India, Kottayam, Kerala. It is also certified that this work has not been presented for any other degree or diploma elsewhere.

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DECLARATION

I here by declare that the dissertation entitled "**studies on drought tolerance potential of modern *Hevea* clones in an extreme drought prone area**" submitted to Mahatma Gandhi University in the partial fulfillment of the requirements for the award of the Degree of **Master of Science in Biochemistry** at School of Biosciences is a bonafide record of dissertation work done by me, under the guidance of Dr. K. Annamalainathan, ~~Joint~~ Dy. Director, Crop Physiology Division, Rubber Research Institute of India (RRII), Kottayam and it has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or similar title to any candidate of any university.

Anumol Joseph

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CONTENTS

ABSTRACT

A study was conducted to elucidate the drought tolerant potential of modern *Hevea* clones, at Regional Research Station of Rubber Research Institute of India, Dapchari, Maharashtra. The young plants were subjected to drought stress by withholding irrigation for 10 days during peak summer season. The stress tolerance traits in these rubber plants were analyzed by various physiological and biochemical parameters. There was significant reduction in photosynthetic pigments such as Chlorophyll *a, b* and carotenoids content in drought imposed plants when compared to the irrigated plants indicated that drought stress with concomitant occurrence of high solar light and high temperature resulted in photooxidation of pigments. The effective quantum yield of PSII (Φ PSII) was drastically inhibited in drought imposed plants, however, there was existing clonal difference. A consistently over expressing 23 k Da heat shock protein in the chloroplast was observed in plants stressed with drought and high light intensity. The expression level of this stress protein was relatively higher in drought tolerant clones indicated that they have a probable role in abiotic stress tolerance mechanism. From this study it was found that clones such as RRII 430 and RRIM 600 are relatively drought tolerant among four rubber clones studied. However, exceptionally a drought susceptible clone RRII 105 also showed prominent expression of this stress protein, most probably due to extreme climatic condition prevailed at Dapchari, during summer season.

INTRODUCTION

Hevea brasiliensis generally known as Para rubber tree belongs to the family Euphorbiaceae. It is the most economically important member of the genus *Hevea*. Natural rubber is a tropical tree and native to the Amazon basin in Brazil and adjoining countries. It was introduced to Asia in 1876 by Henry Wickham and Robert Cross through Kew gardens in England. It is the most important commercial source of natural rubber a product of vital importance recovered from its latex.

Rubber tree is quick growing, tall and sturdy. It grows on many type of soils provided they are deep and well drained. A warm humid equable climate and fairly distributed annual rainfall of not less than 200 cm are necessary for the optimum growth. The temperature must be of about 20°C to 34°C with a monthly mean of 25°C to 30°C. The atmospheric humidity (RH) might be of around 70 - 80%. Bright sunshine amounts about 2000 hours per year at the rate of 6 hours per day through out the year may be required. The absences of strong winds are suitable (Rubber Grower's Companion 2010).

Rubber obtained from the tree is in the form of a milky sap called latex. It is the cytoplasm of specialized tissues called laticifers which are embedded in the bark tissues of the trees. Upon tapping laticifers open and latex expelled. Natural rubber (NR) is a hydrocarbon polymer and is found in 2000 species of plants belonging to 311 genera of 79 families. Rubber molecules are made of long 1, 4 -cis isoprene units. Natural rubber and the different types of synthetic rubbers are used in many different end-products. More than 60 percent of natural rubber is used for automobile tyres, which is the major driving force behind changes in NR demand. The other category general rubber good include hoses, belting, footwear, surgical goods, and rubberized cloth.

The growth of Indian rubber plantation industry has been mainly in Kerala and parts of Tamil Nadu and Karnataka. The traditional rubber growing belt in India is between 80° and 120° N latitude between Arabian Sea coast and Western slopes of Western Ghats and their foothills. At present about 25% of arable land in Kerala is under this crop and therefore expanding rubber cultivation into newer areas in Kerala is not advisable or feasible. To meet the growing demand for natural rubber it

has become necessary to produce more rubber by extending its cultivation to newer areas outside the state of Kerala. The Konkan region of the West coast, the Coromandal coast on the East, the Andaman and Nicobar islands in the Bay of Bengal, Northern West Bengal and North Eastern states have been identified as potential areas for new rubber cultivation in the country (Hajra and Potty 1986).

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang *et al.*, 2003). Most of the field grown plants tolerate environmental stresses through many metabolic adaptations at cellular level. Plants can tolerate certain level of environmental stresses through modulating their metabolic activities and developing some defense mechanisms (Halliwell and Gutteridge 1999). An universal reaction under stress condition is the accumulation of compatible solutes many of which are osmolytes (Bohnert and Shen 1999). Almost all stresses induce the production of a group of proteins called heat-shock proteins (Hsps) or stress-induced proteins. The induction of transcripts of these proteins is a common phenomenon in all living things (Vierling 1991).

The agro climatic and pedological factors prevailing in some of the non traditional areas can be stressful to *Hevea*. In India drought and high temperature in the North Konkan and chilling winter in the North East are the two major limiting factors that restrict the growth and productivity of *Hevea*. Summer in the North Konkan can last for more than 6 months from mid December onwards with practically no rain during this period. Summer in this region is characterized by fast depletion of soil moisture, high temperature and very low relative humidity. The fairly warm air and low atmospheric relative humidity (RH) lead to high evaporative demand causing atmospheric drought in North Konkan. Both in the North Konkan and North East, the environmental stress is associated with light intensities of sunlight, much more than what is required to saturate photosynthesis of leaves. Excess light can aggravate the harmful effects of environmental stresses like drought

and chilling (Alam and Jacob, 2002). Increasing spell of drought and uncertain weather conditions are reality in this changing climatic regimes.

For situations where rubber cultivation is extended to non traditional areas, planting materials of suitable clones for withstanding stress conditions like drought, cold and high elevation need to be developed. Ideal clones of *Hevea* with high production potential and desirable secondary characters can be developed through breeding programs (Varghese *et al., 2000).*Many new clones with high growth vigour and yielding potentials are being released by Rubber Research Institute of India. However, their drought tolerance potential was yet to be ascertained. In the present study a few modern *Hevea* clones were tested for their drought tolerance potential in a severe drought prone region namely Dapchari, Maharastra in the North Konkan region.

OBJECTIVE

1. To study the drought tolerant capacity of modern *Hevea* clones like RRII 400 series in a severe drought prone area in North Konkan Region of India.

2. To study the level of chloroplast stress protein and its role in water deficit stress tolerance of young plants of *Hevea.*

REVIEW OF LITERATURE

Establishment of young rubber plants

Polybag plants of 2-3 whorls (8-10 months old) raised from budded stumps are used as planting material in the field. Planting may be carried out during favorable climate with sufficient soil moisture. It should be either during pre monsoon period or immediately after the intensive rainy season. If polybag plants are used the top storey of the leaves should be mature at the time of planting. When the polybag plants are taken out of the trench dressing of the roots is necessary. While planting the scion of the polybag plants should be directed towards the North East to minimize the adverse effect of direct sunlight on the bud patch (Rubber Growers companion 2010).

Recent studies showed that there are indications of climate change in the NR growing regions in India and increasing temperature is the most prominent change indicated (Shammi *et al.,* 2010). Under the changing climate, survival/establishing young plants during summer is a major problem to be addressed seriously.

In the non-traditional rubber growing region like the Konkan region in India, long dry spell and high temperature are the limiting factors for establishment of rubber plants. Providing irrigation, either basin or drip irrigation, at the rate of 0.5 ETc is found beneficial for the successful establishment of rubber (Mohan Krishna *et al.*, 1991). In a few trials life saving irrigation was provided for the first three years for establishing young plants. Adequate irrigation resulted in good growth and thus reduced the immature period to six years as compared to 9 years in the rainfed control in non-traditional Central India (Vijayakumar *et al.,* 1994.). Irrigation led to higher leaf area index resulting in greater solar radiation interception and these plants showed greater and uniform growth in North Konkan region.Clonal variation existed in the degree of drought tolerance in non-traditional areas. RRIM 600 and RRII 208 are the potential clones that seem to have better growth and yield in North Konkan region than other clones like RRII 105.

The following management practices which have been tested in various experiments at Rubber Research Institutes of India can be practiced for the

protection of immature and mature plants in the field. Partial shade (30 % shade) is advisable for the establishment of young nursery plants (Nair *et al.*, 2004). During the year of planting in main field the plants may be provided with shade before the onset of summer. Plaited coconut leaf or used gunny bags can be used as shade providing materials. Mulching the base with cut grasses, dried plant materials or plastic with punched holes can be practiced in young immature plants (Rubber Growers Companion 2010). From the second year onwards till the canopy development the young brown stems are generally white washed with lime during summer in almost all rubber growing regions of India.

Experiments conducted with tilling the plant base in young plants resulted significant level of soil moisture retention and the growth was significantly superior to that of untilled plants. Both tillage and life saving irrigation were found effective overcoming the transient drought condition. Under short dry spell, application of potassium and silicon were found effective in reducing the adverse effect of water stress in young plants. Thus to enhance the ability of plants to tide over drought stress in addition to irrigation proper nutritional management is also required .Adopting both these management practices together may give adequate protection to plants to withstand drought stress. Life saving irrigation (150 litre water/tree/week during summer) in young immature plantation is a recommended practice in non-traditional areas of rubber cultivation. In mature plantations partial irrigation (0.5 or 0.25 ETc) may be practiced in water deficit areas like North Konkan region etc.

Irrigation requirement in non-traditional areas

Attempts were made to quantify the optimum irrigation requirement for immature and mature phase of two rubber clones for summer period. Irrigation during summer months significantly improved the growth of immature rubber leading to early tappability from 9 years to 6 yrs. Results indicated that irrigation at 50% of the crop evapotranspiration (0.5 ETc) was sufficient for improving the growth in immature phase (Mohan Krishna *et al.*, 1991.). Significant increase in yield was observed with higher level of irrigation in comparison to lower level of irrigation in the first few years of tapping, thereafter yield was stabilized in all levels

of irrigation. Results revealed that when water availability is limited, irrigation can be provided at 50% and 25% of saturated level for immature and mature phase of rubber plants respectively, without affecting growth, tappability and yield. In Konkan region with limited water resource, deficit irrigation can be practiced as a strategy to manage water more efficiently while maintaining good productivity under the prevailing sub humid climatic condition (Meenasingh *et al.*, 2010).

Abiotic Stress

Any unfavorable condition or substance that affects a plant's metabolism, growth or development is regarded as stress (Lichtenthaler 1998). The factors responsible for stress may be either natural or anthropogenic. Many environmental stresses and weather induced losses affect yield. Important stresses include damage caused by temperature, drought, cold, high wind, nutrient imbalance etc (Bora *et al,* 2001). Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. Abiotic stress factors affect every aspect of plant growth. Strong stresses can cause considerable damage which may leads to cell and plant death. Light is one of the major environmental factor which determines the whole structural and functional development of plants. The plants have a capacity to adapt themselves to such conditions. Changes in light conditions drastically affect the structure and composition of photosynthetic apparatus (Anderson and Barber1996). It is effective for the utilization of available light and also to withstand short and long term changes in light quantity and quality. Drought combined with high solar light intensity has been reported as major environmental constraint for establishing rubber cultivation in areas such as North Konkan (Jacob *et al.*, 1999, Alam *et al.*, 2005). Drought and high light drastically inhibit light reactions and damage the thylakoid membrane proteins in young plants of *Hevea* (Annamalainathan *et al.*, 2006). It has been reported that a partial shade provides a photo protective role against photo inhibition in young rubber plants during summer (Nair *et al.*, 2004).

Drought is one of the most important manifestations of abiotic stress in plants. It is the most yield limiting factor of crop plants and it determines the distribution of plant species (Helena, 2008). Stressers like cold or drought induce various primary effects on the cellular level termed strains (Levitt 1980) which in turn leads to uncontrolled (damage) or controlled (adaptation) effects on that level. In plant tissues some strains produced by drought includes shrinkage of protoplast by water loss, negative turgor which forces the cell wall to bend inwards and increasing the water potential of the cell. Besides changes also occurred in the concentration of cellular solutes and membrane potentials. This further lead to loss of membrane integrity and change in metabolic activity (Beck *et al.*, 2007). It affects the leaf expansion, rate of leaf production, leaf senescence etc (Sharp and Davies 1979, Richardson and Mcree 1985). Water deficit also causes yield reduction.

The climatic condition of North Konkan region during summer is associated with high solar radiation, high temperature and low relative humidity. These environmental conditions are known to inhibit the growth and productivity of *Hevea.* Water deficit for the dry season in this region can be as high as 1070 mm compared to 350 mm in traditional rubber growing regions. Several studies have shown that with adequate irrigation during summer months, *Hevea* can be successfully cultivated in this region (Sethuraj *et al.*, 1989, Chandrasekhar *et al.*, 1990).

Drought and Photosynthesis

Photosynthesis is the process where conversion of solar light energy into usable chemical energy takes place. It is associated with action of green pigment chlorophyll. This process splits water, liberates oxygen and fixes CO_2 into sugar. The pigment-protein complexes that gather light for photosynthesis are embedded within cell membrane. This membrane may be tightly folded into cylindrical sheet called thylakoids. In plants and algae photosynthesis takes place in organelle called chloroplast. In addition to chlorophyll, carotenoids and xanthophyll are also present.

These are embedded in a special antenna protein called light harvesting complex. (Anderson and Barber 1996). The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy to a specific chlorophyll pair in the reaction center of the photosystems. Carotenoids are essential components in the photosynthetic apparatus in plants where they protect membrane from photo oxidative damage and contribute to the light harvesting in photosynthesis (Goodwin, 1980). Caroteniods including xanthophylls are also important for avoiding photo inhibitory damage during water deficit. It is by the dissipation of excess energy through the xanthophyll cycle. A decline of pigment ratio is found in drought susceptible clones of wheat during water stress (Loggini *et al.*, 1999). A drought induced reduction in pigment contents was previously reported in several plant species, including pea (Moran *et al.*, 1994) and *Nerium oleander* (Demmig-Adams *et al.*, 1988).

Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sink for PSII activity during mild drought (Cornic and Fresneau 2002). Many studies investigated the impact of drought on the PSII activity. Any environmental perturbation is quickly reflected in the activity of PSII as it is more sensitive to environmental changes (Adams *et al.*, 2001). The quantum yield of PSII as related to Calvin cycle metabolism is reduced only under drastic water deficit in some species (Flagella *et al.*, 1998). Long term drought mediated reduction in water content of tissue led to considerable depletion of Pea PSII core. The decline in Φ PSII efficiency is regulatory probably serving a photo protective role. Increased levels of energy dissipation which decrease Φ PS2 may help to protect PS2 from over excitation and photo damage (Schindler and Lichtenthaler 1994).

Drought stress usually leads to oxidative stress due to stomatal closure (Ozkur *et al.*, 2009), which causes the over-reduction of photosynthetic electron chain (Bacelar *et al.*, 2007; Ben Ahmed *et al*, 2009) and high formation of reactive oxygen species (ROS) in chloroplasts and mitochondria (Asada, 1999; Fu and Huang, 2001). ROS including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-) and singlet oxygen could disrupt normal metabolisms of plants through oxidative damages to lipids, proteins, nucleic acids, and

photosynthetic pigments and enzymes (Fu and Huang, 2001; Ozkur *et al.*, 2009). In order to overcome oxidative stress, plants have developed enzymatic and non-enzymatic antioxidant defense mechanisms to scavenge ROS (Smirnoff, 1993). The most important antioxidant enzymes are superoxide dismutase (SOD) ,catalase (CAT) and peroxidase (POD). SOD converts O_2 - into H_2O_2 and O_2 and CAT and POD scavenge H_2O_2 into H_2O (Yang *et al.*, 2008; Wang *et al.*, 2009). Besides, non-enzymatic antioxidative carotenoids (Car) such as carotene and xanthophylls can also quench ROS and stabilize photosynthetic complexes (Adams *et al.*, 1999; Bassi and Caffarri, 2000; Munne-Bosch and Penuelas, 2003).

The effect of drought on various clones of natural rubber plants has been studied and reported. Apparently the growth light conditions influence the photosynthetic pigments content. It was reported that chlorophyll contents are significantly different among open light and shade grown plants (Nair *et al.*, 2004). Chlorophyll content of leaves were found increased considerably in shaded plants. The light use efficiency of shade grown plants were better under low measurement light than high light (Schiefthaler *et al.*, 1999). High intensity solar radiation concomitant with soil moisture deficit leads to an imbalance between light and dark reaction of photosynthesis and causes an increased diversion of electrons for the production of active oxygen species in the leaves of rubber plants (Jacob *et al.*, 1999).

Drought stress leads to a substantial reduction in the rate of photosynthetic CO_2 assimilation. Under mild to moderate drought stress photosynthesis is mainly limited by reduced intercellular CO_2 concentrations, due to stomatal closure (Quick *et al.*, 1992). ATP synthesis and thus ribulose 1, 5 bisphosphate regeneration impair photosynthesis at mild water deficits (Tezara *et al,* 1999). Stomatal closure is the major cause for the decline of CO_2 up taking during mild drought stress. During onset of drought stomatal conductance normally declines before photosynthesis and the inhibition of photosynthesis under mild stress can be mostly explained by a restriction of CO_2 diffusion to the mesophylls (Chaves 1991, Cornic 2000). The decline in the intracellular CO_2 after stomatal closure under prolonged water deficits may induce an adjustment of photosynthetic machinery to match the available

carbon substrate and decreased growth. This is also consistent with the decrease in the activity of enzyme of the C_3 cycle (Medrano *et al.*, 1997, Maroco *et al.*, 2002). High CO_2 to the leaf may compensate for the increased resistance of the mesophyll under stress leading to similar rates of photosynthesis in well watered and water stressed plants (Quick *et al.*, 1992). It is reported that total leaf area and leaf number was decreased in *Hevea* plants under drought (Dey and Vijay Kumar 2005). Tezara *et al.*, (1999) suggested that decreased coupling factor and photophosphorylation was the cause for decreased photosynthesis under water stress but later they showed that decrease in net photosynthesis with water deficiency was related to lower Rubisco activity rather than to ATP and RuBP contents (Tezara *et al.*, 2002).

Loss of chlorophyll contents under water stress is considered to be a main cause of inactivation of photosynthesis. Furthermore, water deficit induced reduction in chlorophyll content has been ascribed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation, and the appearance of lipid droplets (Kaiser *et al.*, 1981). Low concentrations of photosynthetic pigments can directly limit photosynthetic potential and hence primary production. From a physiological perspective, leaf chlorophyll content is a parameter of significant interest in its own right. Studies by majority of chlorophyll loss in plants in response to water deficit occurs in the mesophyll cells with a lesser amount being lost from the bundle sheath cells. A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006). When leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate with a concomitant increase in leaf temperature (Siddique *et al.*, 2001).

The percentage inhibition in photosynthetic O_2 evolution in drought stressed *Hevea* plants as compared to the irrigated controls was as low as 33% in shade (30%light) grown plants and as high as 51% in open light grown plants (Annamalainathan *et.al.*,2006). Several studies have shown that various components of photosynthetic metabolism are very sensitive to drought stress in *Hevea* (Jacob *et al.*, 1999, Devakumar *et al.*, 2002).

Along with proteins, lipids are the most abundant component of membranes and they play a role in resistance of plant cell to environmental stresses (Kuiper 1980, Suss and Yordanov 1986). Strong water deficit leads to a disturbance of the association between membrane lipids and proteins as well as to decrease in the enzyme activity and transport capacity of the bilayer (Caldwell and Whitman 1987). When *Vigna unguiculata* plants were submitted to drought the enzymatic degradation of galacto and phospholipids increased. The stimulation of lipolytic activities was greater in the drought sensitive than in drought tolerant cultivars (Sahsah *et al.*, 1998).

Drought tolerance Mechanisms

Plants immobility limits the range of their behavioural responses to environmental cues and places a strong emphasis on cellular and physiological mechanisms of adaptation and protection. The initial stress signals would trigger downstream signaling processes and transcriptional control, which activate stress responsive mechanism to re establish homeostasis and protect and repair damaged proteins and membranes. Fast and slow desiccation stress occurs in plants due to water deficit can have totally different results in terms of physiological response or adaptation (Mc Donald and Davies 1996). Plant resistance to drought has been divided into escape, avoidance and tolerance strategies (Levitt 1972, Turner 1986). Some major tolerance mechanisms including ion transporters, osmoprotectants, free radical scavengers, late embryogenesis abundant proteins and factors involved in signaling cascades and transcriptional control are essentially significant to counteract the stress effects (Wang *et al.*, 2004). Plants that escape drought exhibit a high degree of developmental plasticity being able to complete their life cycle before physiological water deficits occur. Tolerance to low tissue water potential may involve osmotic adjustment (Morgan 1984), more rigid cell walls, or smaller cells (Wilson *et al.*, 1980). Many of the evergreen shrubs and trees in arid or semi arid regions combine the high solute concentration in living cells with sclerophylly and low photosynthetic capacity and stomatal conductance (Faria *et al.*, 1998).

Plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential thereby maintaining cell turgor (Rhodes and Samaras, 1994). Under drought, the maintenance of leaf turgor may also be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycine betaine, and other solutes in cytoplasm improving water uptake from drying soil. The process of accumulation of such solutes under drought stress is known as osmotic adjustment which strongly depends on the rate of plant water stress Osmotic adjustment has been considered one of the crucial processes in plant adaptation to drought because it sustains tissue metabolic activity and enables the regrowth upon rewetting but varies greatly among genotypes (Morgan 1984). Plants such as wheat are marked by low level of these compatible solutes and the accumulation and mobilization of proline was observed to enhance tolerance to water stress (Nayyar and Walia, 2003).

Accumulation of low molecular compounds, such as glycine betaine, sugars, sugar alcohols and proline, is a mechanism aimed at balancing water potential following drought (Pilon-Smits *et al.*, 1995). In addition to synthesis of these osmolytic compounds, specific proteins and translatable mRNA are induced and increased by drought stress (Reviron *et al.*, 1992). Hydrophilic proteins such as lea proteins, carbohydrates such as fructans and sucrose (Vijn and Smeekens 1999) and cyclitols such as D-pinitol, mannitol etc are also over synthesized in response to drought stress (Anderson and Kohorn 2001). Among solutes, proline is the most widely studied because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. Progressive drought stress induced a considerable accumulation of proline in water stressed maize plants. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2009). Accumulation of proline under stress has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. It influences protein solvation and preserves the quarternary structure of complex proteins, maintains membrane integrity under dehydration stress and reduces oxidation of lipid membranes or photo inhibition (Demiral and Turkan, 2004).

Furthermore, it also contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). Abscisic acid (ABA) is central in the response to drought stress because it stimulates stomatal closure, thus reducing water loss, which limits CO_2 fixation and reduces NADP+ regeneration by the Calvin Cycle.

Drought tolerance is a complex trait where several characteristics influence plant success during vegetative period (Ingram and Bartels, 1996). It is achieved by modulation of gene expression and accumulation of specific protective proteins and metabolites (Reddy *et al.*, 2004). Water stress tolerance has been documented in almost all plants but its extent varies from species to species.

Maintaining a higher level of antioxidative enzyme activities may contribute to drought induction by increasing the capacity against oxidative damage (Sharma and Dubey, 2005). The capability of antioxidant enzymes to scavenge ROS and reduce the damaging effects may correlate with the drought resistance of plants. The production of ROS in plants, known as the oxidative burst, is an early event of plant defense response to water-stress and acts as a secondary massager to trigger subsequent defense reaction in plants. ROS levels increase dramatically resulting in oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). Being highly reactive, ROS can seriously damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death. Scavenging of reactive oxygen species by enzymatic and non enzymatic systems, cell membrane stability, expression of aquaporins and stress proteins are vital mechanisms of drought tolerance. Aquaporins are membrane water channels that play critical roles in controlling the water contents of cells. Some studies showed that the aquaporin RWC3 probably played a role in drought avoidance in rice (Lian *et al.*, 2004).

Dehydration avoidance is considered to be an adaptive strategy whereby plants decrease transpiration and modulate water extraction in order to retain water in the tissues and in the soil (Blum, 2009). These processes are mainly co-ordinated by non-hydraulic signals, such as abscisic acid (Parent et al., 2009; Tardieu et al., 2010). The phytohormone abscisic acid (ABA) is a stress-induced plant hormone

and it has attracted much research attention as a potentially useful trait in selecting for drought tolerance in crops (Zhang *et al.*, 2006). Increasing ABA concentration leads to many changes in development, physiology, and growth of plants. ABA stimulates osmotic adjustment (Ober and Sharp, 1994), induces the synthesis of protective proteins (the *LEA* and related proteins) (Bray, 1993) and it has also been shown to induce the expression of various water stress-induced genes. Generally, drought induces metabolic changes related to protein turnover such as alterations in protein synthesis, maintaining the level of some proteins or protein degradation (Bray, 1997). In accordance with Medrano *et al.*, (1997) the amount of Rubisco protein is slightly affected by moderate and even prolonged severe drought. The chaperons are the functional class of unrelated families of protein that mediate the correct non covalent assembly of other polypeptides. When a cell is under stress the amount of molecular chaperons increased and they bind the exposed surface and protect damaged protein from aggregation and loss. Rokka *et al.*, (2001) suggest that Rubisco activase protein protects chloroplast protein synthesis from drought stress as a molecular chaperone.

Another group with chaperone-like function is the one of dehydrins (DHNs). They are a group of heat-stable plant proteins produced during late embryogenesis and believed to play a protective role during cellular dehydration (Close, 1996; Campbell and Close, 1997). Dehydrin proteins and their transcripts have been shown to accumulate during dehydrative stress conditions (drought, low temperature, and salinity) and abscissic acid synthesis and they have a potential *in vivo* role in stabilizing cells under stress (Close, 1996; Suprunova *et al.*, 2004). All observations are consistent with a hypothesis that dehydrins are surfactants capable of inhibiting the coagulation of a range of macromolecules, thereby preserving structural integrity (Close, 1997). According to Boudet *et al.*, (2006) dehydrins stabilize membranes by acting as chaperons or by other means which buffer the altered solvent properties inside water stressed cells.

Significance of heat shock proteins

The physiological response to stress has been documented in many different biological systems. A common feature of this response is the induction of a group of

proteins which were first seemed as 'heat shock protein' due to their initial discovery in cells exposed to hyperthermia. An entire family of these proteins now known as 'stress protein'. Some of these proteins are constitutive which are found in cells under normal conditions, while others are found to be expressed under variety of cellular stresses including heavy metals, high temperature, drought and high light mediated oxidative stress and ischemia. Upon exposure of an organism to elevated temperatures, cells synthesize a small set of proteins, the heat shock proteins (HSPs). HSPs are generally divided into two classes: high-molecular-mass HSPs (60–110 kDa) and low-molecular-mass HSPs (15–30 kDa) (Vierling, 1991; O'Connell, 1994). The low-molecular-mass HSPs, which are encoded by a large gene family, are the most abundant HSP class found in plants (Vierling, 1991; Waters *et al.*, 1996). All these proteins appear when the plant is under stress. The optimal conditions for HSP induction in higher plants is a drastic temperature shift to 39-41°C. HSP also can be induced if there is gradual temperature increase as 2-5°C increase per hour.

Heat shock proteins (HSPs) increase their expression when cells are exposed to high temperatures or other stresses (Lindquist, 1986). Heat shock proteins are also specified as molecular chaperones for protein molecules (Schoffl *et al.*, 1998). They perform functions in various intra-cellular processes and play an important role in protein-protein interactions, folding, assembly, intracellular localization, secretion, prevention of unwanted protein aggregation or degradation and reactivation of damaged proteins (Vierling, 1991; Parsell and Lindquist, 1993). HSPs also help in transporting proteins across membranes within the cell. Their chaperon pathways require energy in the form of ATP hydrolysis for their functioning. Morimoto and Santoro (1998) indicated that Hsps protect cells from injury due to stress and facilitate recovery and survival after a return to normal growth conditions. Heat shock proteins are present in cells under perfectly normal conditions because of their essential role in protein maintenance. They are induced when a cell undergoes various types of environmental stresses like heat, cold and oxygen deprivation (Feder and Hofmann, 1999; Kregel, 2002). According to Sorensen *et al.*, (2003) HSP family and other molecular chaperones play significant roles in relation to stress resistance. Proteins that fail to fold correctly by HSP are generally degraded.

Intracellular proteolysis might have an important role in the reorganization of plant metabolism under stress (Grudkowska and Zagdanska, 2004).

The proteolytic response under drought was found to be different from that of natural senescence (Feller, 2004; Grudkowska and Zagdanska, 2004). The contribution of cysteine proteases to total proteolytic activity increases drastically in response to water deficit in wheat (Zagdanska and Wisnievski, 1996) and some experimental evidence suggests that drought-sensitive species and varieties have higher proteolytic activity compared to the resistant ones (Roy-Macauley *et al.*, 1992; Hieng *et al.*, 2004). The combined effect of drought stress and heat shock on the induction of cyclophilin proteins was studied in 3-day-old wheat seedlings (Sharma *et al.*, 2009). In tomato plants under heat stress HSPs aggregate into a granular structure in the cytoplasm possibly protecting the protein synthesis machinery (Miroshnichenko *et.al.*, 2005).

In young plants of *Hevea* a novel chloroplast small HSP (sHSP) was identified and the amino acid sequencing was studied (Annamalainathan *et al.*, 2006). The small HSP is 23 kDa in size probably having a role in drought and high light tolerance as the protein was over expressed in tolerant clones. Plant sHsps are all encoded by nuclear and are divided into 6 classes: 3 classes of (classes CI, CII and CIII) of sHsps are localized in the cytosol or in the nucleus and the other three in the plastids, the endoplasmic reticulum and the mitochondria (CIV, CV and CVI). It has been shown that sHsps located in mitochondria and chloroplasts protect respiratory electron transport in mitochondria and PSII electron transport in chloroplast. Similar proteins have been already reported in woody plants and demonstrated that the HSP involved in the protection of PSII. In chloroplast, the sHSPs have been implicated in protecting this organelle from photo inhibitory and oxidative stress by preventing protein aggregation and stabilizing thylakoid membrane (Torok *et al.*, 2001). Among natural rubber clones RRIM 600 showed prominent expression of sHSP which is also a drought tolerant clone (Annamalainathan *et al.*, 2006).

MATERIALS AND METHODS

Plant material and growth condition

The experimental plants were raised at the Rubber Research Institute of India's (RRII) Regional Research Station at Dapchari, Maharastra which is geographically lies at 20.04°N, 72.04° E and altitude is 48 MSL. The soil is of clay loam type with pH 6.4. Agro-climatically it is North Konkan region of India and it is classified as a non-traditional area for the cultivation of rubber. Summer in this region is lasting for more than six months with practically no rain from December onwards till May. This region is characterized by fast depletion of soil moisture, high intensity of solar radiation, high temperature and very low atmospheric relative humidity. Life saving irrigation is normally practiced in this region to establish young plants.

Budded stumps of four clones of *Hevea* namely, RRII 430, RRII 414, RRII 105, and RRIM 600 were planted in large (35 x 65 cm) size polythene bags. The plants were grown under normal field conditions (twenty plants per treatment) in open sunlight. One set of plants in each clone was imposed with drought stress by withholding irrigation for ten days during the rain free April and May of the year 2011 and another set was kept as irrigated controls. For biochemical and chloroplast protein analysis leaf samples were collected after 10 days of withholding irrigation and samples were transported in dry ice to RRII, head quarters, Kottayam, immediately.

Water Potential

The water potential of the leaf was measured before sampling (for pigment analysis and photosynthesis) measurements by using Psypro water potential system-Wescor (435 752 6011). Psychrometer measures the water vapor pressure of a solution or plant sample, on the basis of the principle that evaporation of water from a surface cools the surface. The sample chambers of Wescor system were taken to the field and the collected leaf discs were immediately transferred to the chambers, transported to lab and then observations were taken.

Estimation of chlorophylls

Total chlorophyll contents were estimated by the method of Arnon (1949). The chlorophylls were extracted in Acetone: Dimethyl sulphoxide (1:1) solution. Leaf discs of 100mg were weighed and put into 1:1 ratio of Acetone: Dimethyl sulfoxide (DMSO). It was allowed to stand overnight with frequent shaking. Filtered supernatant was read at 645 and 663nm.

Calculations:

Chlorophyll *a*: $((12.7_{A663})-(2.69 \times A_{645}) / 1 \times 1000 \times wt(mg)) \times Volume$

Chlorophyll *b*: $((22.9_{A645})-) 4.68 \times A_{663)} / 1 \times 1000 \times wt (mg)) \times Volume$

Total Chlorophyll: $(20.2_{645} + 8.02_{663} / 1 \times 1000 \times wt (mg)) \times Volume$

Estimation of carotenoids

The carotenoids contents were estimated by the method of Lichenthaler (1987). The total carotenoids were extracted in Acetone: Dimethyl sulphoxide (1:1) solution. The following calculations were done using the formula:

$((1000 \times A_{470})-(1.82 \times Ca)-(85.02 \times Cb))/198$

Estimation of proteins

The protein content was estimated by the method of Lowry *et al* (1951).

The following reagents were used:

Solution A: 2% Na_2CO_3 in 0.1 N NaOH.

Solution B: 0.5% $CuSO_4$ solution in 1% NaK (Sodium Potassium Tartarate.)

Solution C: 50 ml of Sol. A + 1ml of Sol. B.

Solution D: Foiln: phenol reagent (1:1)

Procedure:

Alkaline copper reagent (Solution C) was taken in a test tube and added a known aliquot of protein in buffer or SDS. Then 0.5ml Folin phenol (1:1) reagent was added. Absorbance was read at 660nm. Protein amount was calculated by using BSA as the standard.

Assay of quantum yield of PS II

The chlorophyll fluorescence measurements were made following standard technique as proposed by Schreiber *et al.*, (1998). Chlorophyll fluorescence parameters namely, maximal fluorescence under light exposure (Fm), steady state fluorescence at any given time (Φ PSII) and minimal fluorescence immediately after light exposure (Fo), effective PSII quantum yield(Φ PS II) efficiency of excitation energy capture by open PS II reaction centre were measured by using PAM 2000 (Walz Germany), (Schreiber *et al.*,1998).

Isolation of Chloroplasts

Type II broken chloroplast were isolated by the method of Reeves and Hall (1973). Fresh leaf sample was ground with liquid nitrogen in a mortar and pestle. The powdered leaf sample was added with 5 ml of grinding buffer and transferred to a centrifuge tube. The homogenate was centrifuged at 500g for 2 minutes. The pellet represented unbroken cells and tissue was removed and the supernatant was spun at 3500g for 5 minutes and the resulting pellet was suspended in 1 ml of Tris buffer as chloroplast suspension.

Protein preparation for SDS-PAGE:

Chloroplasts were precipitated with 10% TCA and left on ice for 30 min before centrifugation to collect the pellet. A trace of TCA left behind in the pellet was removed by three washing in ice cold acetone. The final pellet was air dried and solublised in a small amount of 10% of SDS to which equal volume of sample buffer was added. The samples were boiled for 2 min and centrifuged at 3000 x g for 5min to remove unsolublised materials. Chloroplast proteins were dissolved in 10% SDS and quantified by the method of Lowry *et al.* (1951).

SDS-PAGE analysis of proteins

Analysis of chloroplast protein was carried out by SDS-PAGE according to the method of Laemmli (1970) using a 10% linear gel. The composition of the various solutions is as follows.

a. Sample buffer (for 10ml)

0.5 M Tris-Hcl, pH 6.8	2.5 ml
Beta-mercaptoethanol	2.5 ml
Glycerol	2.5 ml
1% bromophenol blue	1.25 ml
Distilled water	1.25 ml

b. Separation gel buffer (for 30ml)

Acrylamide (30%)	12 ml
0.5 M Tris-HCl, pH 8.8	7.2 ml
Distilled water	10 ml
10% SDS	0.3 ml
10% APS	0.15 ml
TEMED	10 µl

c. Stacking gel buffer (for 10ml):

Acrylamide (30%)	1.35 ml
0.5 M Tris-HCl, pH 6.8	3.0 ml
Distilled water	5.5 ml
10% SDS	0.1 ml
10% APS	0.05 ml
TEMED	5 µl

d. Acrylamide stock (30%):

Acrylamide	30.0 g
N, N-methylene bisacrylamide	1.6 g
Distilled water added to make up to	100 ml

e. Running buffer:

50 mM Tris-Hcl, pH 8.3	3.0 g
Glycine	14.3 g
SDS	1.0 g

Preparation of separating gel:

A linear gel of 1.5 mm thickness was prepared by adding 30% of acrylamide solution followed by 0.5 M Tris-HCl, distilled water, 10% SDS, 10% APS and TEMED.

Preparation of stacking gel:

The stacking solution was layered over the separating gel after inserting a comb and was allowed to polymerize. Protein samples were mixed with equal volume of sample buffer and heated to 100 ^{0} C for 3 min. After cooling to room temperature the samples were centrifuged at 10, 000 g for 2 min. The supernatant was loaded on the gel and was run at 50 V till the samples cross the stacking layer. Then the voltage was increased to 120 V. Electrophoresis was carried out at 20 ^{0} C.

Staining and Destaining:

The gel after electrophoresis was immersed in staining solution. The stain was prepared by dissolving 500 mg of coomassie brilliant blue (sigma) in 80 ml of methanol, 100 ml of distilled water and 20 ml of glacial acetic acid. The gel was stained for 6 h and destained with 40% methanol and 10% acetic acid mixture for 12 h. The destained gel was preserved in 7% acetic acid solution.

The destained gel was documented with the help of Bio Imaging system. The relative intensity of the stress protein bands in the drought samples were compared with respective control chloroplast samples.

RESULTS AND DISCUSSION

With an objective of elucidating the drought tolerant potential of modern *Hevea* clones such as RRII 400 series, a study was conducted at Regional Research Station (RRS) Dapchari, Maharashtra. This station is situated in the west coast region of North Konkan India. The study was conducted during summer season of 2011. *Hevea* clones such as RRII 430, RRII 414, RRII 105 and RRIM 600 were raised in big size polybags. Drought was imposed for 10 days by with holding irrigation during April-May, 2011.

Drought effect on plant morphology

The peak summer season of North Konkan region is during April and May. The maximum temperature at noon time was always above 37°C and it was more than 40°C for a few days (Table 1). The minimum temperature during night time was recorded above 26°C. The afternoon relative humidity (RH) was very low during summer season and it was around 40%. The potential evapo transpiration of this area during summer period was very high compared to the traditional area. It was 7.7 mm during May 2011. It indicated that a very high atmospheric vapour pressure deficit is prevailing during dry spell in this region. There was absolutely no rainfall during summer months in North Konkan region. Hence, this region was considered as a severe drought prone area.

Table: 1 Agro-meteorological data of Regional Research Station, Rubber Research Institute of India, Dapchari, Maharashtra for the months of April and May 2011.

Monthly mean	**Temp. (^{0} C)**		**RH (%)**		**Sunshine hrs**	**Potential Evaporation (mm)**	**Rainfall (mm)**
	Max.	Min.	6:30 am	2:40 pm			
April	38	29.5	84	41.5	9.5	6.7	Nil
May	37.5	26.5	79.5	43	9.9	7.7	Nil

1(a) 1(b)

Figure: 1 Young Hevea plants (clone RRII 430)grown in poly bags with irrigation 1(a).Drought was imposed by withholding irrigation for 10 days 1(b).

2(a)

2(b)

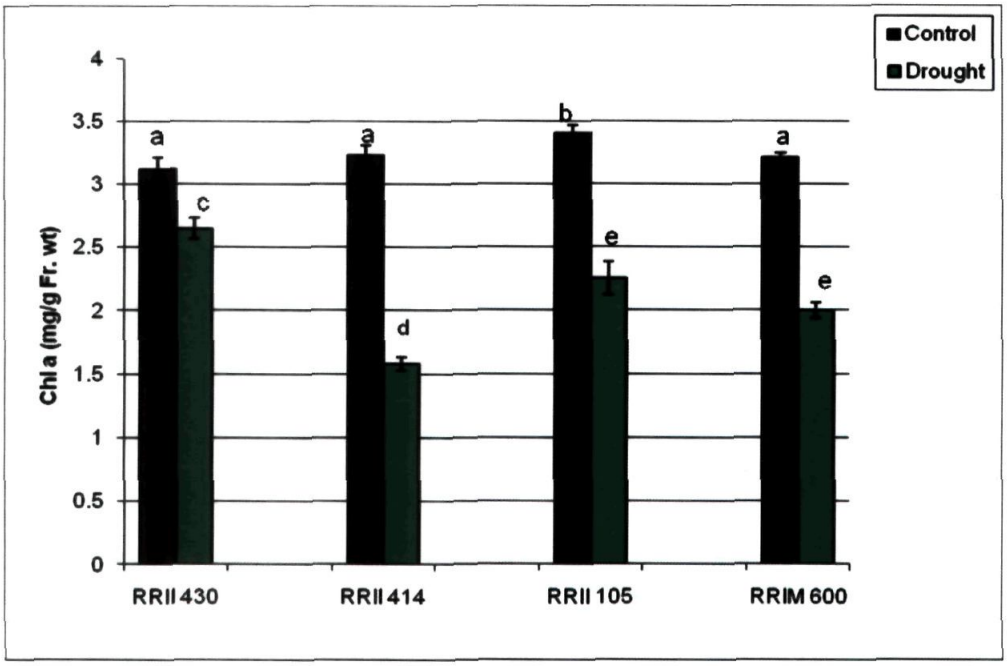
Figure:2 Young *Hevea* plants (clone RRII 414)grown in poly bags with irrigation 2(a).Drought was imposed by withholding irrigation for 10 days 2(b).

The drought imposed plants showed typical morphological symptoms in leaf such as discoloration and a slight degree of leaf lamina drying (Fig.1 (b) and Fig.2 (b)). Among the four clones RRII 414 was affected drastically under water deficit stress as evidenced from growth reduction, more yellowing, relatively high degree of leaf lamina scorching and leaf shedding. The clones RRII 430 and RRIM 600 are seemed to be drought tolerant as these plants are visibly not much affected except for some degree of yellowing and leaf tip drying. A forenoon water potential of -1.7 and -2.2 MPa was recorded in irrigated and unirrigated plants, respectively. The water potential between irrigated and drought imposed plants was significantly different. However, there was no significant difference among the clones.

Further certain physiological and biochemical parameters were studied to find out the relative drought tolerant potential of these modern clones.

Photosynthetic Pigment Contents

The photosynthetic pigments namely chlorophyll *a*, chlorophyll *b* and carotenoids were estimated in irrigated and drought imposed plants. When compared to irrigated plants, drought imposed plants showed a drastic reduction of chlorophyll *a* in clones *RRII 414*, *RRII 105* and *RRIM 600*. Chlorophyll *a* reduction was very small in RRII 430 (Fig 3a). Chlorophyll *b* content also drastically reduced in RRII 414, RRIM 600 and RRII 105. On the controversy there was no significant reduction of chlorophyll *b* in RRII 430 (Fig 3b). The reduction in chlorophyll *a* and *b* content in drought imposed plants were revealed in total chlorophyll contents also. Degradation of total chlorophyll content was drastic in all the clones except in RRII 430 (Fig 4). Photosynthetic pigments like chlorophyll *a* and *b* are more sensitive to water deficit and high solar light stress conditions. In North Konkan region the solar radiation was more than1800$\mu m/m^2$/sec during noon time. Drought stress coupled with high temperature and high light condition resulted in photo oxidation of pigments. Drought mediated oxidative stress and production of reactive oxygen species (ROS) and free radicals inflict lipid peroxidation and bleaching of pigments in photosynthetic apparatus (Smirnoff 1993, Asada 1999).



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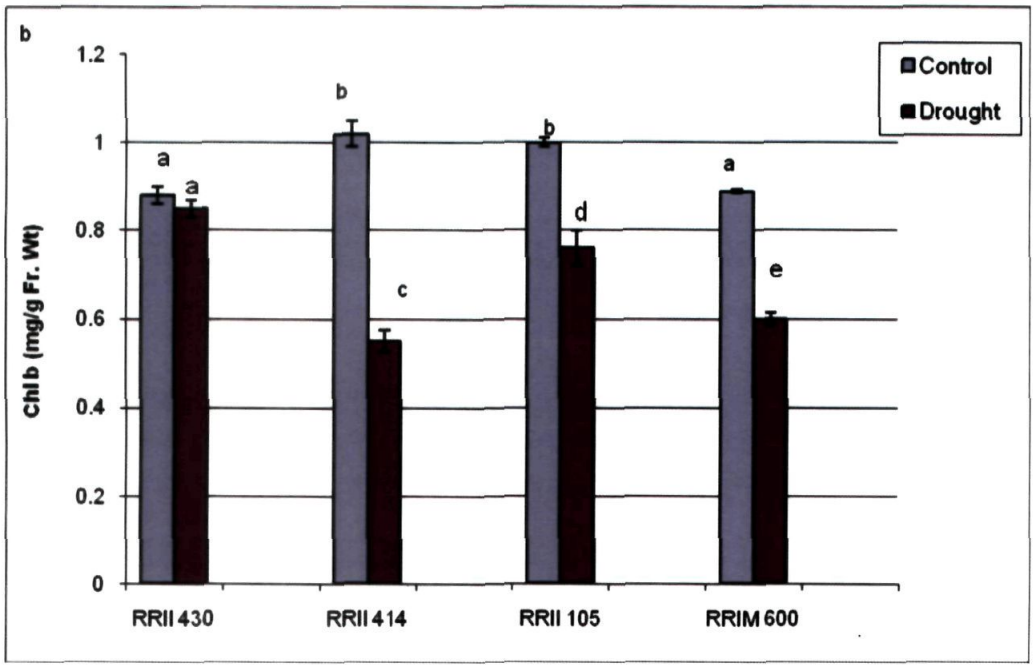

Fig 3. Leaf chlorophyll *a* (Fig 3a) and chlorophyll *b*(Fig 3b) contents of young plants of *Hevea* grown under irrigated and drought conditions (with holding irrigation for 10 days) at RRS, Dapchari, Maharashtra. Different alphabets indicate significant difference at5% level.

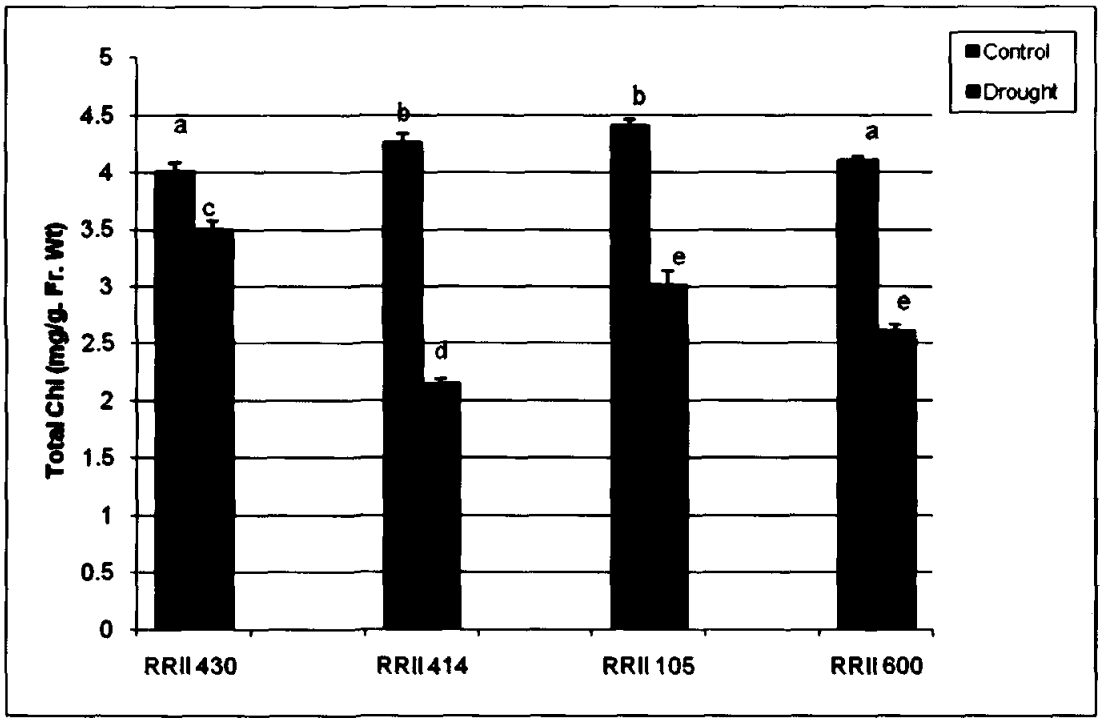

Fig 4. Total chlorophyll contents of young plants of *Hevea* grown under irrigated and drought conditions (withholding irrigation for 10 days) at RRS Dapchari. Different alphabets indicate significant difference at 5% level.

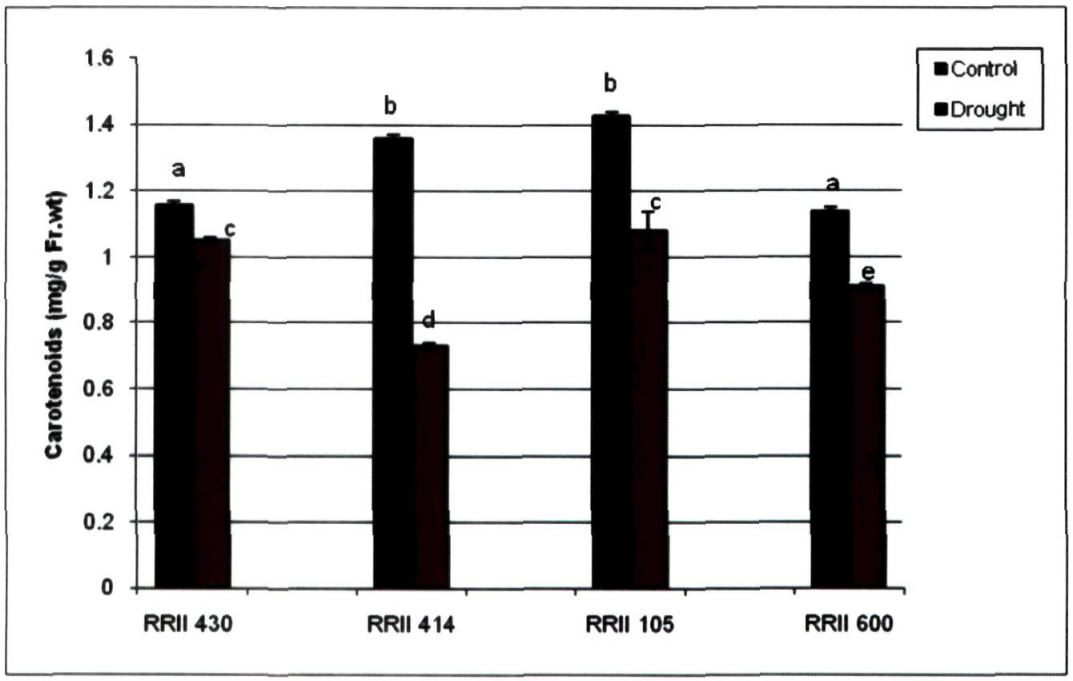

Fig.5. Carotenoids content of young plants of *Hevea* grown under irrigated and drought conditions (with holding irrigation for 10 days) at RRS, Dapchari, Maharashtra. Different alphabets indicate significant difference at 5% level.

The Fig. 5 showed total carotenoid contents of young plants belong to four clones of *Hevea* with and without irrigation. Among the irrigated plants RRII 414 and RRII 105 recorded significantly higher level of carotenoids. After imposing drought for 10 days the clones such as RRII 414 and RRII 105 recorded a drastic reduction in carotenoids content, while RRII 430, RRIM 600 showed a small reduction (Fig.5). Carotenoids are the important accessory pigments of photosystem involved in light harvesting and photo protection. First, they act as light-harvesting pigments, in coordination with chlorophylls effectively harvesting the solar light and funneling photons to the reaction centres of photosynthetic apparatus. Secondly, they perform an essential photo protective role by quenching triplet state chlorophyll molecules and scavenging singlet oxygen and other toxic oxygen species formed within the chloroplast (Young, 1991). Carotenoids play a major role in dissipation of excess electron as non photochemical quenching (NPQ) through xanthophyll cycle (Demmig-Adams and Adams 1992). The mechanism of xanthophyll cycle involves the enzymatic removal of epoxy groups from xanthophylls to create so-called de-epoxidised xanthophylls. This reduces the amount of energy that reaches the photosynthetic reaction centers. Non-photochemical quenching is one of the main ways of protecting against photoinhibition. In higher plants there are three carotenoid pigments that are active in the xanthophyll cycle: violaxanthin, antheraxanthin and zeaxanthin. During light stress violaxanthin is converted to zeaxanthin via the intermediate antheraxanthin, which plays a direct photo protective role acting as a lipid-protective antioxidant and by stimulating non-photochemical quenching within light harvesting pigment-proteins. This conversion of violaxanthin to zeaxanthin is done by the enzyme violaxanthin de-epoxidase, while the reverse reaction is performed by zeaxanthin epoxidase (Wright *et al.*, 2011).

In the present study the degradation of carotenoids were comparatively lesser in RRII 430 and RRIM 600 indicated the relative stability of photosystems in these clones there by providing better photo protection. Interestingly the reduction in chlorophyll *b* and carotenoids are very less in RRII 430. This clone was seemed to be a drought tolerant.

PSII Activity

The effective quantum yield of PS II is a crucial parameter to determine the drought tolerance or susceptible nature of young rubber plants. The Φ PS II or apparent quantum yield of PSII in irrigated plants were almost same in all clones (Fig.6). At 10^{th} day of drought imposition the Φ PS II was drastically inhibited in all clones. However, in RRII 430 the level of reduction of PS II activity was smaller than other clones. PSII often declines concomitantly under water stress with high solar radiation, suggesting that the activity of the photosynthetic electron chain is finely tuned to that of CO_2 uptake (Genty *et al.*, 1989; Loreto *et al.*, 1995). The PSII, thylakoid membranes and electron transport components are the main targets of photo inhibition due to the formation of excess active oxygen species during adverse climatic conditions (Halliwell and Gutteridge 1999).

So the present result indicated RRII 430 was superior than clones such as RRII 414 and RRII 105 in terms of drought tolerance. RRIM 600 is a known drought tolerant clone at RRS Dapchari conditions as reported in previous studies (Alam et al 2005). However, in the present study considering the degree of reduction in photosynthetic pigments and F PS II under drought condition, RRII 430 seems to be a better drought tolerant clone than RRIM 600.

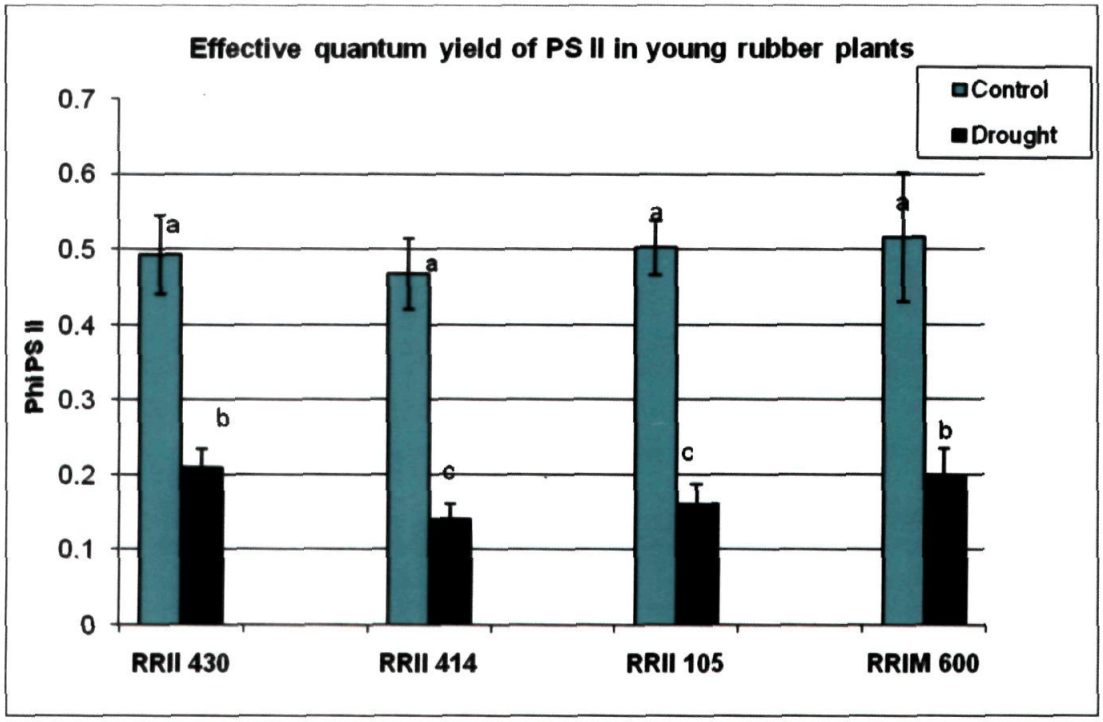

Fig. 6. Effective quantum yield of PSII (ϕ PS II) in four clones of *Hevea* grown under irrigated and drought conditions.The drought was imposed ny withholding irrigation for 10 days in polybags at RRS Dapchari,Maharashtra. Different alphabets indicate significant difference at 5% level

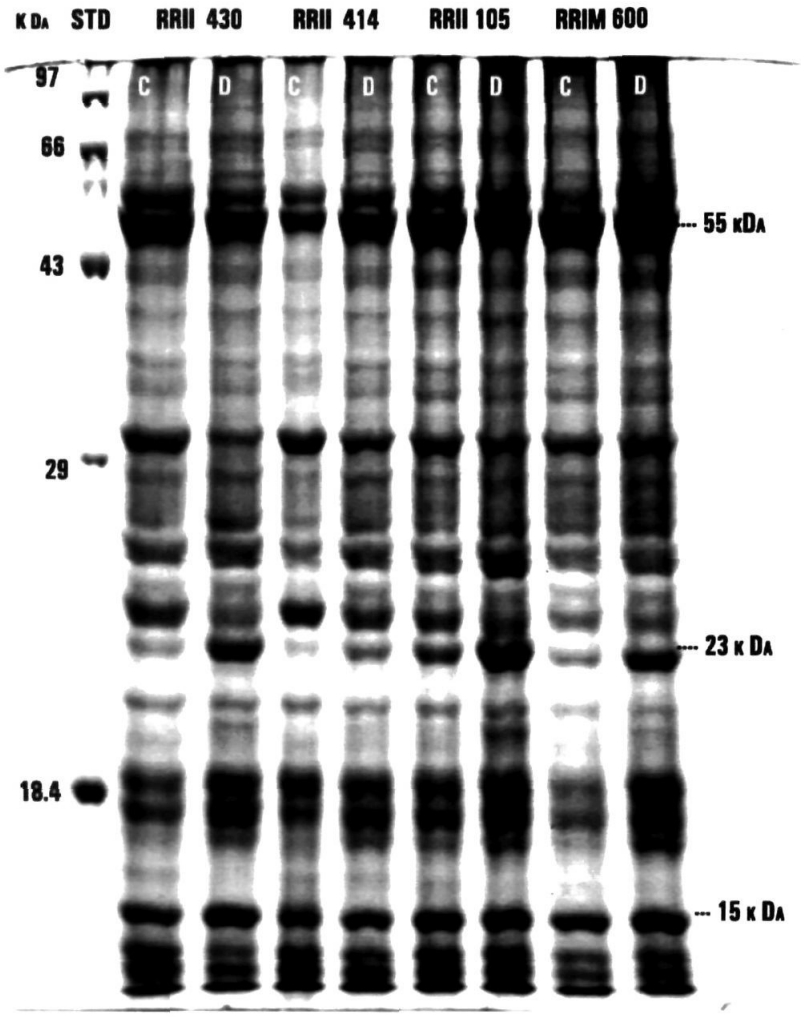

Figure: 7 Expression of 23 kDa chloroplast small heat shock protein (sHSp).The plants were grown with (control) or without (drought) irrigation .Drought condition was imposed by withholding irrigation for 10 days.The 23 kDa chloroplast small HSP is indicated in drought imposed plants

Chloroplast protein profile

The chloroplast protein profile of four clones of *Hevea* grown in irrigated and unirrigated conditions was analysed in SDS-PAGE electrophoresis. The profile showed (fig.7) induction of certain low molecular weight proteins where as decline in the level of certain enzymatic proteins under drought conditions. A consistently over expressing 23 k Da protein was observed in RRII 430, RRIM 600 and RRII 105. The level of protein was very low in RRII 414 under water deficit condition. This is typical example of clonal difference in the expression of stress protein. The expression of this protein was reported as sHSP in *Hevea* (Annamalainathan *et al.*, 2006).A few Enzymatic proteins like subunits of Rubisco and other soluble proteins showed a reduction in their level under drought conditions.

In the present study the level of expression of sHSP was greater in those clones which show comparatively tolerance to water deficit condition. This indicated that sHSP has a role in stress protection, most probably protection of thylakoid membrane against water deficit induced oxidative stress and membrane damage. Those clones with higher level of stress protein also had high level of carotenoids and chlorophyll *b* content. However exceptionally a drought susceptible clone RRII 105 also showed prominent expression of this stress protein, most probably due to extreme climatic condition prevailed at Dapchari, during summer season. Heat shock proteins (Hsps) and other stress proteins have been known to protect cells against deleterious effects of stress (Feder *et al.*, 1999, Young, *et al.*, 2002). Hsps and their cognates are found in every organism at ordinary growth temperature and play an important role in cellular functions related with growth (Lindquist *et al.*, 1988, Waters *et al.*, 1996). The major stress proteins occur at low to moderate levels in cells that have not been stressed but accumulate to very high levels in stressed cells (Young, *et al.*, 2002). Hsps are characterized as structurally unstable proteins. They serve important physiological functions in plants. These functions of Hsps are closely related to resistance to heat and the other stresses (Ray *et al.*, 1999, Iba *et al.*, 2002). In all organisms, the induction of Hsps is remarkably rapid and intense under abiotic stress conditions. Plants probably synthesize middle level Hsps at mild heat stress conditions at first, but if heat stress continues they synthesize more Hsps

(Ahn, *et al.*, 2004). A decrease of Hsp expression level after induction is observed with age. The main reason seems to be a lower capacity to up regulate expression at an older age. A chloroplastic 22 kDa Hsp from *Chenopodium album*, which is localized in thylakoid lumen, interacts specifically with the thermo labile oxygen evolving complex of PSII. Therefore protecting it from heat stress damage but fails to reactivate the heat denatured PSII (Heckathorn, *et al.*, 1999, Sun *et al.*, 2002). After the heat stress has been released, the sHsps are quite stable with half-lives of 30-50 h, suggesting that sHsps may be important for recovery as well.

The chloroplast sHSPs are a subclass of the sHSP family, with subclasses also present in endoplasmic reticulam, mitochondrion and cytosol. In general chloroplast sHSPs have been reported in a variety of plant species, including 26 k Da sHSP from tobacco (Lee *et al.*, 1998) and 21 kDa HSP from tomato, Arabidopsis and soyabean (Suzuki *et al.*, 1998). The HSPs are present within chloroplast as large oligomers containing 9 more subunits and are actively synthesized during heat stress (Suzuki *et al.*, 1998). Heckathorn *et al.*, 2004 have reported that this protein is involved in the protection of PSII when the plants experience abiotic stresses. In chloroplast the sHSPs have been implicated in protecting this organelle from photo inhibitory and oxidative stress by preventing aggregation and stabilizing the thylakoid membrane (Torok *et al.*, 2001). It has been demonstrated that the chloroplast sHSPs plays a direct role in stabilizing the photo system II (PS II) oxygen evolution complex (OEC) proteins during heat stress and there by promotes the maintenance of PSII electron transport. This protein was also implicated in protective mechanism in plants experiencing oxidative stress by undergoing oxidation dependent conformational changes in the molecular structure. Thus sHSPs are appear to be general stress proteins in chloroplast that are involved in maintaining function and survival of this organelle during stress or facilitating recovery from stress.

Concluding Remarks

The present study was conducted to assess the drought tolerance capacity of young plants of a few modern clones of *Hevea* in an extreme drought prone area and the data revealed that the drought tolerant clones such as RRII 430 and RRIM 600 maintained better photosystem II activity with stable level of photosynthetic pigments, most probably such plants operate efficient ROS and free radical scavenging mechanisms. There was certain level of protection of photosynthetic activity (ΦPS II) in drought tolerance clones from the adverse effects of environmental stresses and it was implicated that the over expression of sHSPs may have a protective role.

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