MOLECULAR STUDIES IN DROUGHT TOLERANCE OF BANANA

CHANDRASHEKAR, N.
PAK 6211

DEPARTMENT OF BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BANGALORE
2008

MOLECULAR STUDIES IN DROUGHT TOLERANCE OF BANANA

CHANDRASHEKAR, N.

PAK 6211

Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfillment of the requirements for
the award of the Degree of

Master of Science (Agriculture)

in

Plant Biotechnology

BANGALORE **JULY 2008**

“Affectionately dedicated to my beloved Parents, Teachers, Sisters and Friends”

DEPARTMENT OF BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BANGALORE-560065

CERTIFICATE

This is to certify that the thesis entitled **“Molecular studies in drought tolerance of banana”** submitted by **Chandrashekar, N. ID No. PAK 6211,** in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in **Plant Biotechnology** to the **University of Agricultural Sciences, Bangalore,** is a record of bonafide research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
July, 2008

(K. V. Ravi shankar)
Major Advisor

APPROVED BY:

Chairman: ____________________________
Dr. K. V. Ravi Shankar

Members:

1. ____________________________
Dr. P. H. Ramanjini Gowda

2. ____________________________
Dr. C. K. Suresh

3. ____________________________
Dr. R. H. Laxman

4. ____________________________
Dr. A. Rekha

5. ____________________________
Dr. Sheshasayee

ACKNOWLEDGEMENT

It is always a nostalgic feeling whenever one glances back to the days of hard work, tension and the need of the hour to excel. One would not achieve without the help, encouragement and the wishes of the near and the dear ones. Teachers, parents, friends and well-wishers are an integral part of this. I owe them a lot and it always is a difficult task expressing and putting into words the sense of gratitude I feel towards them. I wish to remember and acknowledge all who made this thesis possible.

I wish to express my deep sense of gratitude and indebtedness to **Dr. K. V. Ravishankar,** Senior Scientist, Division of Biotechnology, IIHR, Hessaraghatta, Bangalore and the esteemed chairman of my Advisory Committee, without whose appreciation, valuable advice and constant encouragement, this task would not have been accomplished. I owe my heartfelt thanks for his wonderful guidance, extreme patience and intangible efforts for providing me with the atmosphere and facilities that I needed for completing this work successfully.

I am indebted to the members of my Advisory Committee, **Dr. P. H. Ramanjini Gowda**, Professor and Head, Department of Biotechnology, U.A.S., GKVK, Bangalore; **Dr. C. K. Suresh,** Professor Department of Biotechnology, U.A.S., GKVK, Bangalore; **Dr. R. H. Laxman**, Senior Scientist, Division of Biochemistry and plant physiology, IIHR, Hessaraghatta, Bangalore, **Dr. A. Rekha**, Senior Scientist, Division of Fruit crops, IIHR, Hessaraghatta, Bangalore, **Dr. M. S. Sheshasayee**, Associate professor, Department of Biotechnology, U.A.S., GKVK, Bangalore for their untiring guidance, valuable suggestions and critical evaluation of manuscript throughout my study.

I also pay my gratitude to **Dr. Shiva shankar,** Senior Scientist, **Dr. Manamohan** Senior Scientist, **Shreedhar sir, Param sir, earanna sir, chandrashekar sir, Swarupa madam, Ambika madam, Prakash sir, Savitha madam, Anju george, Sudarshini madam, Ashwini, Prabha, Giri, Srinivas, Hanumantha raju, Muniraju.**

I also have been highly fortunate in having many affectionate friends whose hands were evident at every moment of tension, anxiety and achievements. I am ever grateful to ***Chalapathi, Erayya, Gopal reddy, Naveen, Dhananjaya, Lokesh, Bhaskara reddy, Deepa, Vishwa, Kumar nag, Ranga, chandra shekar, jagdeesh, Chandrashekar, Vikram, Mahesh sir*** *for their support through out my M. Sc. Programme. I also acknowledge my seniors* ***Gireesh, Manjunath reddy, Venu, Prakash, Sumesh, giri*** *for their moral support and help during my research work.*

I also greatfull to ***Canara Bank****, Kolar branch for having provided me financial support for my PG-degree programme.*

The non-teaching staff members of IIHR, Hessaraghatta, Department of Biotechnology, UAS, GKVK, Bangalore were every bit understanding. They were kind and provided all the support they could.

Finally, I thank the eternal love of the great Almighty for gracing the peaceful atmosphere during the course of my study.

Any omission in this brief acknowledgement does not mean lack of gratitude.

Bangalore **(Chandrashekar, N)**

July, 2008

MOLECULAR STUDIES IN DROUGHT TOLERANCE OF BANANA

Chandrashekar, N.

ABSTRACT

The present study examines the genetic variation in drought tolerance among AA and BB genomic groups of banana using two characteristics i.e Leaf water retention capacity and Carbon discrimination and in this study AA genotypes recorded relatively higher water use efficiency than BB genotypes. BB genotypes had higher Leaf water retention capacity (77.73%) compared to AA genotypes (69.3 %).

From earlier studies two contrasting genotypes, *M. acuminata.* ssp *burmaniceoides* commonly recognised as 'Calcutta-4' (AA) and 'Bee hee kela' (BB) were used for physiological, biochemical and molecular studies during water stress.

The per cent reduction in photosynthetic rate (P_N) between control and stressed plants was 45.28 (%) for 'Calcutta-4' and 36.01(%) for 'Bee hee kela', Transpiration rate (E) was 30.24 (%) for 'Calcutta-4' and 22.36 (%) for 'Bee hee kela' and Stomatal conductance (gs) was 60.30 (%) for Calcutta-4 and 56.10 (%) for 'Bee hee kela', indicating BB genotypes are tolerant to water deficit conditions.

Leaf water potential (Ψ) was higher in 'Bee hee kela' (BB) both in watered (-0.913 MPa) and under stress (-1.518 MPa) situations when compared with 'Calcutta-4' (AA) control (-1.35 Mpa) and stressed (-1.824 MPa) plants. Malondialdehyde content was estimated to be high in 'Calcutta-4' than that of 'Bee hee kela' indicating higher degree of membrane damage in Calcutta-4. The two antioxidant enzymes namely Super oxide dismutase and Catalase activities were found to be higher in Bee hee kela stressed samples than 'Calcutta-4' indicating better oxidative damage withstanding capacity. 'Bee hee kela' (BB) with higher Ψ, gs, and antioxidant enzyme (SOD and Catalase) activities. 'Bee hee kela' genotype is more drought tolerant than 'Calcutta-4'.

Gene expression study using selected gene primers and cDNA revealed that drought not only changes the expression pattern of drought inducible genes but also changes the expression levels of growth and metabolism related genes.

Department of Biotechnology
University of Agricultural Sciences
GKVK, Bangalore-65
Date and Place- 26-07-2008, GKVK.

(Dr. K. V. Ravishankar)
Signature of Major Advisor

ಬಾಳೆಯ ಬರ ಸಹಿಷ್ಣುತೆ ಕುರಿತು ಅಣು ಅಧ್ಯಯನ

ಚಂದ್ರಶೇಖರ್, ಎನ್.

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಪ್ರಸ್ತುತ ಅಧ್ಯಯನವು ಬಾಳೆಯ ಎಎ ಮತ್ತು ಬಿಬಿ ಬರ ಸಹಿಷ್ಣುತೆಯಲ್ಲಿನ ವಂಶವಾಹಿರೂಪಗಳ ವೈವಿದ್ಯತೆಯನ್ನು ಎರಡು ಗುಣ ವಿಶೇಷಣಗಳಾದ ಎಲೆಯ ನೀರು ಹಿಡಿದಿಡುವಿಕೆ ಮತ್ತು ಇಂಗಾಲದ ಪ್ರತ್ಯೇಕಿಕರಣದ ಆಧಾರದ ಮೇಲೆ ಪರೀಕ್ಷಿಸಲಾಗಿದೆ. ಈ ಅಧ್ಯಯನದ ಪ್ರಕಾರ ಎಎ ವಂಶವಾಹಿರೂಪಗಳು ಬಿಬಿ ವಂಶವಾಹಿರೂಪಗಳಿಗಿಂತ ಹೆಚ್ಚು ಜಲ ಬಳಕೆಯ ಕಾರ್ಯಕ್ಷಮತೆಯನ್ನು ತೋರಿಸಿವೆ. ಬಿಬಿ ವಂಶವಾಹಿರೂಪಗಳು (77.73 %) ಎಎ ವಂಶವಾಹಿರೂಪಗಳಿಗಿಂತ (69.30 %) ಹೆಚ್ಚು ಎಲೆ ನೀರು ಹಿಡಿದಿಡುವಿಕತೆಯನ್ನು ಹೊಂದಿವೆ.

ಹಿಂದಿನ ಅಧ್ಯಯನಗಳನ್ನಾಧರಿಸಿ ಎರಡು ವ್ಯತಿರಿಕ್ತ ವಂಶವಾಹಿರೂಪಗಳಾದ ಕಲ್ಕತ್ತಾ-೪ (ಎಎ) ಮತ್ತು ಬೀ ಹೀ ಕೇಲಾ (ಬಿಬಿ) ಇವುಗಳನ್ನು ಕ್ರಿಯಾಶಾಸ್ತ್ರೀಯ, ಜೀವರಾಸಾಯನಿಕ ಮತ್ತು ಅಣು ಅಧ್ಯಯನಗಳಿಗೆ ಬರ ಒತ್ತಡದ ಸಂದರ್ಭದಲ್ಲಿ ಬಳಸಲಾಯಿತು. ದ್ಯುತಿಸಂಶ್ಲೇಷಣ ಕ್ರಿಯೆಯ ದರವು ಬರದ ಒತ್ತಡದಲ್ಲಿರುವ ಮತ್ತು ಇಲ್ಲದ ಗಿಡಗಳ ನಡುವಿನ ಶೇಕಡವಾರು ಇಳಿಕೆಯು ಕಲ್ಕತ್ತಾ-೪ ನಲ್ಲಿ (45.28%) ಆದರೆ ಬೀ ಹೀ ಕೇಲಾದಲ್ಲಿ (36.01%) ದಾಖಲಾಗಿದೆ. ಭಾಷ್ಪವಿಸರ್ಜನೆ ಕ್ರಿಯೆಯ ದರದಲ್ಲಿನ ಶೇಕಡವಾರು ಇಳಿಕೆಯು ಕಲ್ಕತ್ತಾ-೪ ನಲ್ಲಿ (30.24%) ಮತ್ತು ಬೀ ಹೀ ಕೇಲಾದಲ್ಲಿ (22.36%) ಹಾಗೂ ಪತ್ರರಂಧ್ರಗಳ ವಾಹಕತೆಯಲ್ಲಿನ ಶೇಕಡವಾರು ಇಳಿಕೆಯು ಕಲ್ಕತ್ತಾ-೪ ನಲ್ಲಿ (60.30%) ಮತ್ತು ಬೀ ಹೀ ಕೇಲಾದಲ್ಲಿ (56.10%) ದಾಖಲಿಸಿವೆ.

ಈ ಮೇಲಿನ ಅಂಶಗಳನ್ನು ಗಮನಿಸಿದಾಗ ಬಿಬಿ ವಂಶವಾಹಿರೂಪಗಳ ಬರ ಸಹಿಷ್ಣುತೆಯನ್ನು ಸೂಚಿಸುತ್ತದೆ. ಎಲೆ ನೀರು ಅಂತಸ್ಸತ್ವವು ಬೀ ಹೀ ಕೇಲಾದಲ್ಲಿ ಬರದಲ್ಲಿನ (-1.518 ಎಂಪಿ.ಎ) ಬರದಲ್ಲಿಲ್ಲದ (–0.913 ಎಂಪಿ.ಎ) ಮತ್ತು ಕಲ್ಕತ್ತಾ-೪ ನಲ್ಲಿ ಬರದಲ್ಲಿನ (–1.824 ಎಂಪಿ.ಎ) ಬರದಲ್ಲಿಲ್ಲದ (–1.35 ಎಂಪಿ.ಎ).

ಮೆಲಾನ್‌ಡೈಆಲ್ಡಿಹೈಡ್ ಅಂಶವು ಕಲ್ಕತ್ತಾ-೪ರಲ್ಲಿ ಬೀ ಹೀ ಕೇಲಾಗಿಂತ ಜಾಸ್ತಿಯಿದ್ದು ಕೋಶಭಿತ್ತಿ ನಾಶವು ಕಲ್ಕತ್ತಾ-೪ರಲ್ಲಿ ಹೆಚ್ಚಾಗಿದೆ ಎಂಬುದನ್ನು ತೋರಿಸುತ್ತದೆ. ಎರಡು ಪ್ರತಿಉತ್ಕಷ್ಟಕ ಕಿಣ್ವಗಳಾದ ಸೂಪರ್ ಆಕ್ಸೈಡ್ ಡಿಸ್‌ಮುಟೇಸ್ ಮತ್ತು ಕೆಟಲೇಸ್ ಕಿಣ್ವಗಳ ಕ್ರಿಯಾಶೀಲತೆಯು ಬೀ ಹೀ ಕೇಲಾದ ಒತ್ತಡದ ಗಿಡಗಳಲ್ಲಿ ಕಲ್ಕತ್ತಾ-೪ರ ಒತ್ತಡದ ಗಿಡಗಳಿಗಿಂತ ಜಾಸ್ತಿಯಿದ್ದು ಉತ್ಕಷ್ಟಕತೆಯ ಪರಿಣಾಮವನ್ನು ತಡೆಯುವ ಶಕ್ತಿಯನ್ನು ಹೊಂದಿದೆ. ಬೀ ಹೀ ಕೇಲಾದ ಅತಿ ಹೆಚ್ಚು ಎಲೆ ನೀರು ಅಂತಸ್ಸತ್ವ, ಪತ್ರರಂಧ್ರಗಳ ವಾಹಕತೆ ಮತ್ತು ಪ್ರತಿಉತ್ಕಷ್ಟಕತೆಯ ಕಿಣ್ವಗಳ ಕ್ರಿಯಾಶೀಲತೆಯು ಜಾಸ್ತಿಯಿದ್ದು ಬರ ಸಹಿಷ್ಣುತೆಯ ಸೂಚಕವಾಗಿವೆ. ವಂಶವಾಹಿ ತೋರ್ಪಡಿಕೆಯ ಅಧ್ಯಯನವನ್ನು ಆಯ್ದ ಪ್ರೈಮರ್‌ಗಳು ಮತ್ತು ಸಿ-ಡಿಎನ್‌ಎ ಉಪಯೋಗಿಸಿ ನಡೆಸಲಾಯಿತು. ಈ ಅಧ್ಯಯನದಿಂದ ತಿಳಿದುಬರುವುದೇನೆಂದರೆ ಬರದಿಂದ ಪ್ರಚೋದಿಸಲ್ಪಡುವ ವಂಶವಾಹಿಗಳ ತೋರ್ಪಡಿಕೆಯ ಸ್ವಭಾವವನ್ನಷ್ಟೆ ಅಲ್ಲದೆ ಇತರೆ ತೋರ್ಪಡಿಕೆಯ ಸ್ವಭಾವಗಳಾದ ಬೆಳವಣಿಗೆ ಮತ್ತು ಕಾರ್ಯಾನುಕೂಲತೆಯ ವಂಶವಾಹಿಗಳ ಸ್ವಭಾವವನ್ನು ಬದಲಿಸುತ್ತದೆ ಎಂದು ತಿಳಿದುಬಂದಿದೆ.

(ಡಾ: ಕೆ. ವಿ. ರವಿಶಂಕರ್)
ಮುಖ್ಯ ಸಲಹೆಗಾರರು

ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ವಿಭಾಗ
ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ
ಜಿ.ಕೆ.ವಿ.ಕೆ. ಬೆಂಗಳೂರು-65
ದಿನಾಂಕ: 26-07-08
ಸ್ಥಳ: ಬೆಂಗಳೂರು

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

	determination of $\Delta^{13}C$ (flash-ea) in leaf biomass at national facility for stable isotope studies in biological sciences, dept of Crop. physiology, UAS, GKVK, Bangalore.	
4.	a) Gel profile showing good quality RNA isolated from well watered and stressed banana leaf samples	
	b) Gel profile showing cDNA of well watered and stressed banana leaf samples	
5.	Gel profile showing the amplification of 9 primers using genomic DNA of Calcutta-4 and Bee hee kela	
6.	Gel profile showing PCR amplified products using from control and stressed plants	

Introduction

I. INTRODUCTION

Bananas and plantains are important sources of carbohydrate for the millions of people worldwide. Globally banana (*Musa sp*) is fourth most important commodity after rice, wheat and corn and is produced in tropical and sub tropical regions of developing economies, grown across 130 countries in an area of 8.25 mha with a production of 97.38 million tonnes (NHB Database, 2006). India leads in global banana production. The other important banana growing countries are Brazil, Philippines Indonesia, China, Ecudor, Cameroon, Mexico, Columbia and Coasta Rica. The important banana growing states of India are Maharashtra, Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Tamil Nadu, West Bengal and Orissa.

Banana ranks first in production and second in area among the fruits grown in India accounting for the production of 16.5 million tonnes annually from an area about 0.565 million hectares (FAO, 2007). Its share in total fruit production is 32 per cent from 12 per cent area under fruits. Like in many other countries, banana has dominated the Indian fruit industry contributing the highest GDP of 1.99 per cent to agriculture sector.

Owing to its multifaceted uses of various plant parts ranging from underground stem to male flower, it is referred to as “Kalpataru” (a plant of virtues). Banana has several important roles in socio-religious ceremonies in India (Amalraj *et al.*, 1993) and is considered as a symbol of good omen, fertility and prosperity. The fruit is available throughout the year. It is easily digestible, a food for people suffering from gastritis and other stomach ailments (Rao, 1984). Apart from fruits, leaves are used as plates. The male flower bud is used as a vegetable, the juice of central axis is said to be useful against kidney stones.

Water deficit is one of the principal limiting factors for crop production throughout the world. It prevents the crop plants from expressing their full genetic potential. Water deficit, which is a consequence of either intermittent or terminal period of drought, causes significant yield reduction on cultivated crops. These restrictions on yield potential are of great concern in terms of meeting food demand of increasing world population. To achieve the objectives, understanding the fundamentals of plant responses are vital for a given species of crop within a specific environment.

Drought stress induces a range of physiological and biochemical responses in plants. These responses include stomatal closure, repression of cell growth and photosynthesis, and activation of respiration. Plants also respond and adapt to water deficit at both the cellular and molecular levels, for instance by the accumulation of osmolytes and proteins specifically involved in stress tolerance. An assortment of genes with diverse functions are induced or repressed by these stresses (Shinozaki *et al.,* 2003; Bartels and Sunkar, 2005). Many drought-inducible genes with various functions have been identified by molecular and genomic analyses in *Arabidopsis*, rice and other plants, including a number of transcription factors that regulate stress-inducible genes expression.

Banana plants require uniformly warm and moist conditions for optimum growth and yield. The need to provide adequate water supply for growth and plant development is well-documented (Srikul and Turner, 1995; Milburn, 1992). This crop requires 160 mm of water per month, depletion of soil water from the root zone affects many plant physiological and biochemical processes (Bradford and Hsiao, 1982). Bananas rarely attain their full genetic potential for yield due to limitations imposed by water. Sensitivity of banana plants to moisture stress is reflected in terms of reduced growth through reduced stomatal conductance and leaf size (Kallarackal *et al.*, 1990; Turner, 1995), increased leaf senescence (Batalgia, 1980) ultimately limiting the overall photosynthesis (Robinson, 1996).

Candidate gene approach states that candidate genes are sequenced genes of known biological function. This approach has been utilized successfully to determine the biotic and abiotic characters in many crop plants. These genes associated with the manifestation of the drought resistance trait, may be structural genes or genes in a regulatory pathway or biochemical pathway, which affect trait expression.

The increasing demand for water for domestic and industrial needs has led to limited availability of good quality water for agriculture. Growing crops under such water limiting conditions is a great challenge for the scientists. Hence, understanding the response of the crops to moisture limiting conditions and using this knowledge for growing crops successfully is very much essential. Banana crop bearing a mesophyte is very sensitive to water stress conditions, most of its cultivation is under irrigation and understanding the changes in banana genotypes response to water deficit conditions is very important. Keeping above points in view, the present investigation was carried out with the following objectives:

1. To study the physiological and biochemical changes due to moisture stress in banana.

2. To study the gene expression of a few selected candidate genes under moisture stress.

Review of literature

II. REVIEW OF LITERATURE

The view of earth from space shows that most of planet's surface is covered with water, but most of it is in unusable form for animals and plants. Water is the most abundant substance in any living system, comprising up to 70 per cent or more of weight. Organisms have effectively adapted to their aqueous environments and have even evolved means of exploiting the unusual properties of water. Atmosphere being the major source of carbon dioxide for photosynthesis and simultaneously being dry makes the plant to lose large amount of water, by means of transpiration during the process of CO_2 entry. Even slight imbalance in water flow causes water deficit, which is some extent is responsible for shaping the evolution of plants.

2.1 General effects of drought on growth and metabolism:

2.1.1 Strategies adapted by plants against stress:

Plants over years have evolved mechanisms broadly categorized as drought postponement and desiccation tolerance to combat water deficit. Most of the xerophytic plants adapt mechanisms underneath drought escape. Plants usually either function by maintaining tissue water potential by avoiding tissue dehydration or by tolerating low tissue water potential. The first mechanism that involves traits that minimize water loss and maximize water uptake. Water loss can be minimized by stomatal closure, paraheliotrophic movements reducing heat load, increase in reflectance character by dense trichomes or waxiness of leaves in addition to minimizing cuticular transpiration, decrease in canopy leaf area by shedding of older leaves and reduced growth (Larcher, 2000). Water uptake is maximized mainly by increasing root growth to deeper layers of soil (Jakson *et al.*, 2000).

The second mechanism termed tolerance, involves functioning even when tissue water potential decreases. This has been achieved by synthesizing and protecting macromolecules and accumulating substances, which can further reduce water potential, powering entry of water into cell these mechanisms operate at cellular level. Ultimately it is tolerance along with drought postponement mechanisms leading to whole plant drought tolerance per se (Mundree *et al.*, 2002).

Plants can perceive abiotic stress and elicit appropriate responses with altered metabolism, growth and development. The regulatory circuits include stress sensors, signaling pathways comprising network of protein-protein interactions, transcription factors and final product that is proteins or metabolites imparting stress tolerance. The products of stress inducible genes can be classified into two groups; (i) Those that function directly in protecting against environmental stresses also termed as functional or downstream genes; and (ii) those that regulate gene expression and signal transduction in response to stress termed as regulatory or upstream genes (Oono *et al.*, 2003). In response to environmental adversities plants have developed several strategies to cope with these challenges either by adaptation mechanisms, which follow them to survive the adverse conditions or specific growth habits to avoid stress conditions. Stress tolerant plants have evolved certain adaptive mechanisms, displayed by different degrees of tolerance, which are largely determined by their genetic plasticity. This differential stress tolerance could be due to difference in terms of perception of stress, signal transduction and appropriate gene expression patterns, or presence of novel metabolic pathways restricted to tolerant plants (Bartels and Sunkar, 2005).

2.1.2 Perception of stress:

Plants feel an extremely feeble stimulus, which is below the limit of perception, and display fast action (closure of stomata, diurnal variation in temperature and water status). It remains still an open question, how plant sense an osmotic stress or drought? Determining how plants sense water deficit is complex? There may be various signals for water stress ranging from chemical, hydraulic to electrical signals, including the primary mediators such as water status, turgor, bound water, hormones (Abscisic acid etc), alteration in cell membranes and other signals which are still under debate (Knight and Knight, 2001).

A mere perception would be of little use, unless a message is sent and effective action taken without the least delay, for which the signals have to be transduced for the cell to respond appropriately. The more important are the activities in the organism which bring about the altered physiological and biochemical response, which helping the plant to re-programme itself to the changing environment.

2.2 Physiological and biochemical changes during drought in general:

When the demand exceeds the supply, perennial plants can regulate the water use by finding additional water sources or find other ways to conserve water for both metabolism and growth needs. There is a great variety of morphological, anatomical and physiological characteristics that either look for adaptation or act as a buffer against the negative water deficit effects. In water deficit conditions, the water vapour loss occurs through two parallel ways, the stomatal and the cuticular, being the stomatal participation more significant in the transpiration loss mechanism than cuticular (Larcher, 1980).

Plants face various kinds of stresses, among which abiotic stress conditions such as drought, temperature (high and low), salinity, anorexia etc., contribute to the reduction in yield. In tropical countries like India abiotic stresses cause appreciable reduction in crop productivity. Amongst the different abiotic stresses drought is the major one, which limits the potential yield of crops to a great extent. More than 10 per cent of arable land is affected by drought and salinity, with rapid desertification and salinization, nearly more than 50 per cent in yield of crop plants occurs (Bray *et al.*, 2000). Therefore understanding plant tolerance to both drought and salinity is of fundamental importance.

Under mild drought, decrease in photosynthesis is generally considered to be the result of reduced availability of CO_2 due to stomatal closure (Mansfield and Davis, 1981). Growth and performance reduction by water stress has been very well documented (Fischer, 1980; Kriedemann and Barrs, 1981), concurring to reduce gas changes and the leaf lengthening. Both are hardening process dependent (Bradford and Hsiao, 1982).

The onset of stress may initially cause a loss of cell turgor, which in turn reduces gas exchange and leaf elongation since both are turgor-dependent processes. The result is a decrease in growth rate since this is a function of transpiration rate and leaf area (Chartzoulakis *et al.*, 1992).

Leaf water retention capacity (LWRC per cent) was considered to be a better estimation of drought resistance than stomatal conductance (Clarke and McCaig, 1982). Leaf water content and gas exchange are very sensitive to drought stress. Reductions in leaf water potential (Ψ) result in photosynthetic competence in many plant species (Bradford and Hsiao, 1982).

Decrease in net photosynthetic rate (P_N) under water stress is related to disturbances of biochemical processes of non-stomatal nature, caused by oxidation of chloroplast lipids and changes in structure of pigments and proteins (Graan and Boyer, 1996). However, increases in rate of reduction of O_2 will not be sufficient to dissipate the excess excitation energy in PS II antennae and increased down-regulation of photosynthesis will occur and minimize photo-damage to PSII reaction centers. Under prolonged mild or severe water deficit the electron transport to O_2 and down-regulation may be unable to dissipate excitation energy in PS2 antennae and, consequently, photo-damage and net loss the D1 protein (32 kDa) of PSII RCs can result (Baker, 1993).

Photosynthesis is particularly sensitive to water deficit because the stomata close to conserve water as available soil water declines. Stomatal closure deprives the leaves of carbon dioxide and photosynthetic carbon assimilation is decreased in favour of photorespiratory oxygen uptake. The process of stomatal closure and the enhancement of flux through the photorespiratory pathway increase the oxidative load on the tissues as both processes generate reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2). Hydrogen peroxide is also generated as a secondary messenger in abscisic acid (ABA)-mediated stomatal closure (Pei *et al.*, 2000).

Clarke and McCaig (1982) investigated the technique for use in wheat breeding schemes in the Americas and concluded that the leaf water loss rate was a suitable method for distinguishing between genotypes of wheat that were tolerant of drought. Drought tolerance was evaluated by comparing the yield of genotypes in rainfed plots with yields from irrigated plots.

A common effect of drought stress, similarly as of other environmental stresses, is to cause oxidative damage (Smirnoff, 1998).

Generation of reactive oxygen species (ROS) lead to lipid peroxidation (Chen *et al.*, 2000; Sreenivasulu *et al.,* 1999), protein degradation (Jiang and Zhang, 2001) and nucleic acid damages (Hagar *et al.*, 1996).

The superoxide anion radical occurs in photosystem-I (PS1) under limited supply of NADP. In photosystem-II (PSII), the occurrence of Reactive oxygen species is caused by damage of thylakoid membranes, when electrons from water are transferred to oxygen. Very sensitive to oxidative stress are chloroplasts mainly due to high concentration inside these organelles of oxygen, which as a result of irradiation is transformed into singlet oxygen (Sgherri *et al.*,1996).

Drought-stress causes increase in content of the reactive oxygen species (ROS). In response to drought induced oxidative stress plants increase activity of anti-oxidative enzymes such as superoxide dismutase (SOD), catalase, peroxidase, or glutathion reductase (Neill *et al.*, 2002). Increased ROS, damage PS-II to the extent that it can’t be repaired and inhibit synthesis of drought inducible proteins. Plants have evolved efficient non-enzymatic and enzymatic means to alleviate from oxidative damage (Kramer *et al.*, 2002). The non-enzymatic system up regulated under dehydration include ascorbate (Vitamin C), tocopheral (vitamin E), flavanoids, alkaloids, caroteinoids, glutathione. Other antioxidants usually reported under dehydration stress include enzymes such as superoxide dismutase, peroxidase, catalase and glutathione-s-transferase (Mittler, 2002). ROS usually causes lipid peroxidation, which is always accompanied by generation of highly reactive molecules such as complex aldehydes.

Production of ROS is usually a secondary response to dehydration, which include production of singlet oxygen, super oxide anion radicals, hydroxyl radicals and hydrogen peroxide. Hydroxyl radicals are the most reactive molecules, leading to oxidaton of lipids and nucleic acids (Apel and Hirt, 2004).

2.3 About banana crop:

Today, most people of the world are familiar with this delicious fruit. Edible clones of bananas and plantains, based on landraces, are derived from hybrids of the wild sub-species of *Musa acuminata* (A genome) and *M. balbisiana* (B genome). The wild bananas occur within the tropics from India to Oceania but there is a distinction between the distribution of *M. acuminata* and *M. balbisiana* within that range. *Musa balbisiana* overlaps the northern part of the range of *M. acuminata* and extends beyond it to the west and north (Simmonds, 1962).

The edible clones are now grown more widely including in the subtropics of both hemispheres. Triploids (AAA, AAB and ABB) are most common among the edible bananas with the plantains belonging to the AAB group. A special feature of the edible clones is the parthenocarpic development of sterile fruit (Simmonds, 1959).

Simmonds (1962) summarized the features of the wild bananas, based on observations in the field, as being a group of broadly opportunistic plants that are intermediate in ecological succession, distributed by animals, and requiring high temperature, humidity and light. They do not tolerate competition or poor soil drainage and tend to be short-lived. Within the wild bananas, Simmonds noted significant differences between species in their ecological demands and tolerances.

Circumstantial evidence suggests that the *Musa balbisiana* (B) genome confers greater drought tolerance to bananas and plantains than the *Musa acuminata* (A) genome. Hence the genetic makeup of bananas and plantains may affect the response of leaf gas exchange to the environment. Field data cannot be readily used to study the independent effects of environment but laboratory studies allow independent control of environmental parameters (Thomas *et al.,* 1998).

Early comments on bananas, particularly of the cv. 'Gros Michel' (AAA group) used by the international trade early in the 20th century, focus on the sensitivity of the plant to changes in the environment (Popenoe, 1941). Most of the research on the effect of environment on *Musa species* has been on members of the edible bananas. There is a need to know more about the responses of the wild species that provide the genetic basis for the edible genotypes.

2.4 Physiological and Biochemical changes during water limiting conditions in banana crop:

Banana has been known as a plant with a rapid growth rate, high consumption of water, shallow and spreading root distribution, roots with weak penetration strength into the soil, poor ability to withdraw water from soil which is drying, low resistance to drought and rapid physiological response to soil water deficit (Robinson, 1995).

Low wind speed and large leaf area increase the depth of the boundary layer, slowing the movement of water vapour from the leaf to the air. Thus, all things being equal, we could expect less evaporation per unit of leaf area from large leaves than small leaves. In reality the situation is more complex because sensible heat also diffuses across the boundary layer. Thus large leaves will be warmer than smaller leaves, as demonstrated clearly by Taylor and Sexton (1972).

The capacity of a canopy of leaves in a plantation to intercept light and fix carbon is measured by the leaf area index, LAI. The LAI includes the area of all green leaves on all shoots present. It is the area of leaf (single side) divided by the area of land occupied by the plants. It varies with location, planting density and a number of other factors, including season, and ranges from 2 to 5. This is not large compared with an apple tree that may have an LAI of 7 or more (Proctor *et al.*, 1976), but even apple trees normally have LAI within the range of 3.5 to 4.6 when the leaves are fully grown (Jackson, 2003).

Bananas (*Musa spp.*) rarely attain their full genetic potential for yield due to limitations imposed by water. The banana plants sensitivity to moisture stress is reflected in changes in reduced growth through reduced stomatal conductance and leaf size (Kallarackal *et al.*, 1990; Turner, 1995), increased leaf senescence (Batalgia, 1980) ultimately limiting the plants' photosynthesis. Robinson (1996) reported that soil drying in a healthy, previously well irrigated banana plantation to -12, -25, -53 and -70 Pa, induced a proportional reduction in photosynthetic rate amounting to 8, 18, 44 and 82 percent, respectively. Cayon *et al.* (1998) noted a 50% reduction in photosynthesis in cultivar "Dominico-Harton" under water stress compared to 30.61 μ mol CO_2 m^{-2} s^{-1} for un-stressed plants.

Ekanayake *et al.* (1994) investigated the ability of different genotypes of *Musa* to adapt to drought in the field by measuring the stomatal conductance (gs) in the morning and afternoon. In this case, the soil water content is unlikely to change significantly between measurements but the leaf-to-air vapour pressure difference will increase as temperature rises. It is therefore a strategy for detecting the sensitivity of the stomata to vapour pressure deficit, which may or may not be related to drought tolerance. They found differences among *Musa* genotypes. Those genotypes that tended to restrict stomatal opening in the afternoon were classed as 'water savers' and could therefore be expected to tolerate short periods of soil water deficit in contrast to those whose stomata remained open in the afternoon. The cvs. 'Bluggoe' (ABB) and 'Fougamou' (ABB) showed the greatest differences in leaf conductance between morning and afternoon while cvs. 'Bobby Tannap' (ABB) and 'Valery' (AAA, Cavendish subgroup) maintained their gs throughout the day. They concluded that there was variation in response to drought among the genotypes in the ABB genomic group that was worth further investigation.

Limitations of photosynthesis by stomatal as well as non-stomatal mechanisms depend not only on duration and intensity of drought-stress but also on plant species, stage of plant development, and leaf age (Kicheva *et al.*, 1994). Some of the observed changes in leaf water status and gas exchange are reversible and subside after finishing exposure to drought. They may be irreversible and remain even at sufficient water supply (Sestak and Shiffel, 1997).

Robinson (1996) points out that bananas have rapid physiological responses to soil water deficit and this is the feature that is most likely to determine the response of the crop to irrigation. The most sensitive indicator of soil water deficit in banana is the rate of emergence of the new leaf (Kallarackal *et al.*, 1990; Hoffmann and Turner, 1993; Turner and Thomas, 1998). If the soil dries rapidly, the leaf may stop emerging after 2 to 10 d and if it dries slowly, leaves may stop emerging after 23 days. In the experiments of Hoffmann and Turner (1993) a 21 kPa reduction in soil water potential halved the rate of leaf emergence but a 40 kPa reduction in soil water potential was needed to halve transpiration rate. Thus the rate of emergence of the new leaf is a sensitive indicator of drying soil, more so than the closing of stomata. When drought is prolonged, a decrease of photosynthesis is controlled by “non-stomatal” mechanisms of gas exchange connected with damages of mesophyll cells, membranes, and chloroplasts, decrease in chlorophyll content, and disturbances in assimilate synthesis and transport (Keutgen *et al.* 1997).

Transpiration from sections of leaves is often measured with a gas analysis system where a small section of leaf is enclosed in a chamber through which there is a flow of air (Thomas and Turner, 2001). The boundary layer in the chamber is determined by the flow rate of the air stream and the size of the chamber. It is standardized so that stomatal conductance can be estimated. The boundary layer in these chambers is very different in size to that encompassing a whole leaf that influences evaporation from the whole leaf or plant.

These factors indicate that banana is sensitive to even slight variations in soil water content and that irrigation scheduling is critical. The water holding capacity of the soil, effective rooting depth of banana, and the percentage of depletion of total available water allowed before irrigation determine the amount of water to apply, while crop coefficient together with the evapo-transpiration data determine the irrigation interval (Robinson, 1995).

Bananas remain highly hydrated even when the soil is dry and so there needs to be a mechanism by which this occurs. Thomas (1995) grew bananas cv. 'Williams' (AAA, Cavendish subgroup) with a split root system and showed that if half of the roots were exposed to drying soil, then the stomata closed, even though the leaves were well hydrated. Severing the roots that were exposed to the dry soil opened the stomata, indicating a signal from roots to shoots that closed them. By this mechanism the banana would be able to sense drying soil and begin to close its stomata. This would reduce water loss from the leaves, but it does not explain why the plant remains highly hydrated because the data of Bananuka *et al.* (1999) show that if a leaf is severed from a banana plant then it will lose from 24 to 76% of its weight (primarily water) after 48 h of drying, depending on the genotype. This is a high rate of water loss considering that in the field the loss of water from intact leaves on banana plants (cv. 'Dwarf Cavendish', AAA, Cavendish subgroup) was only 10 per cent even though they were subjected to a reduction of 70 per cent in available water in the soil over ten days (Shmueli, 1953). This implies that the root system has an important role to play in keeping the plant hydrated even when the soil is drying. The mechanism by which this might occur is that once the stomata are closed, root pressure becomes the dominant force supplying water to the shoot. Bananas have strong root pressure (Davis, 1961). With this drought tolerance mechanism the banana is able to survive long periods of soil water deficit but the disadvantage is that production is very sensitive to soil drying. Whether this mechanism applies across the genetic diversity of *Musa species* needs to be established. If genetic variation can be detected then there is the possibility of developing cultivars that are less sensitive to soil drying, from the point of view of production, but which might not survive long periods of drought. From a practical perspective it would be useful to determine the relationship between soil water potential and stomatal conductance for a range of cultivars in different environments.

Thomas *et al.* (1998) used controlled conditions to separate the effects of *vapour pressure deficit* and temperature on stomatal conductance (gs) of cvs. 'Williams' (AAA, Cavendish subgroup), 'Lady Finger' (AAB) and 'Bluggoe' (ABB). Stomatal conductance of cv. 'Williams' was much more sensitive to leaf to air vapour pressure difference than that of cv. 'Bluggoe', decreasing as the vapour pressure deficit increased. At a vapour pressure difference of 5 kPa, there was no difference in gs between either cultivar with differences increasing under more humid conditions and They showed that the stomata of bananas respond to the aridity of the air as well as to soil drying and that there is genetic variation in this trait.

Lu *et al*. (2002) measured the water use of whole banana plants cv. 'Williams' (AAA, Cavendish subgroup) by using gravimetric and sap flow techniques. Water flowing from the root system to the leaves must flow through the corm and sap flow sensors placed there can give an estimation of the water use by the whole plant. In this case the boundary layer of the leaves is not modified by the instrumentation used to measure transpiration, such as it is when a gas exchange system is used. They compared the maximum sap flux density they measured in the corm of banana plants at 15 g cm $^{-2}$ h^{-1} with published values for spruce (19 g cm $^{-2}$ h^{-1}), mango (35 g cm $^{-2}$ h^{-1}) and tropical rainforest tree species (40 g cm $^{-2}$ h^{-1}). They pointed out that the value for banana was lower than for other species and they attributed this to the low leaf area/sapwood area ratio in banana compared with other species. Thus large leaves can be misleading as banana has large leaves but the sap flux density in the corm is less than that measured for tree species with small individual leaves. They suggesting that bananas are remarkably tolerant of soil water deficit and can evaporate less water than other crops

High leaf area index, shallow root systems or the poor ability of banana roots to extract water from the soil can be invoked to account for the sensitivity of the crop to soil drying. However, the sensitivity of the root system to drying soil, the ability of the plant to send this information to the leaves so that stomata close and once the stomata are closed, the capacity of root pressure to maintain the plant in a hydrated state are consistent with the widely observed sensitivity of the plant to soil drying. In addition to pointing to a physiological mechanism that might be modified in the plant to produce cultivars more adapted to dry conditions, knowing that the banana plant itself is not substantially different from other plants in its use of water leads to questions about the technology of applying water to bananas. (Lu *et al*., 2002) They also pointed out the large difference between the amount of water evaporated by the plant compared with the amount of water recommended for irrigation at Darwin, Australia. They suggested that water-use efficiency at the level of the plantation could be improved by paying attention to the technology of irrigation

A canopy with a high Leaf area index (LAI) could be expected to evaporate more water than a smaller canopy with lower LAI. In the longer term, the amount of water evaporated from a leaf canopy will be proportional to the amount of radiation intercepted because energy is needed to convert water from a liquid to vapour. About 90 per cent of the incoming radiant energy is intercepted at an LAI of 4.5 to 5.0 for bananas, although under protected cultivation this may reach 94 per cent (Tanny *et al.,* 2006). Thus LAI beyond about 5.0 is unlikely to contribute greatly to increased evaporation. The LAI of banana plantations is similar to that of other fruit crops.

2.5 General effects of drought on molecular aspects:

2.5.1 Transcriptional regulation of gene expression

2.5.1.1 Cis acting elements:

ABA Response Elements (ABREs): Two ABA dependent pathways are known to mediate gene expression in plants during water deficit stress. The distinction is largely based on cis-elements that exists in the promoters of ABA-inducible genes. The ABA- dependent pathways are thought to mediate gene expression through an ABRE-element and b-ZIP transcription factors (Busk and Pages, 1998), while the other pathway is through a MYC and MYB elements and transcription factors (Yamaguchi-Shinozaki and Shinozaki, 1993). Many cis-regulatory elements known as ABA-responsive elements (ABREs) have been identified. Among them(C/T)ACGTG(G/T) C motifs have been reported to function as ABREs in many genes (Ingram and Bartels, 1996; Busk and Pages, 1998).hte core element of the V is the CAGGTG motif (G-box motif, which functions in the regulation of plant genes stimulated by variety of environmental signals (Michel *et al.*, 1994).

Dehydration response element (DRE): The investigation of dehydration-induced genes in *Arabidopsis* has also revealed ABA-independent signal transduction pathways (Shinozaki and Yamaguchi-Shinozaki, 2000). In aba (ABA deficient) and abi (ABA insensitive) mutants, several genes are induced by dehydration indicating that these genes do not require ABA for their expression under drought conditions. The *A. thaliana* genes rd29A and rd29B are differentially induced under conditions of dehydration, salt, cold stress, or ABA treatment. This multiple expression Pattern requires at least two cis-acting elements for the rd29A gene. The 9-base pair direct repeat (TACCGACAT), termed the dehydration responsive element (DRE), functions in the initial rapid response of rd29A to dehydration, salt, or low temperature. The slower ABA response is mediated by another promoter fragment that contains an ABRE. The DRE is an essential cis-acting element for the regulation of rd29A induction in the ABA independent response to dehydration in Arabidopsis (Yamaguchi-Shinozaki and Shinozaki, 1993).

2.5.1.2 Transcription factors:

Basic region leucinen-zipper proteins (bZIP): Basic region leucinen-zipper proteins (bZIP) contain a DNA binding domain rich in basic residues and adjacent to a leucine zipper dimerizatiuon domain. bZIPS are a large family of transcription factors in plants and are represenred by 75 members in Arabidopsis(Jacoby *et al*, 2002). All plant bZIP proteins bind to an ACGT core sequence, but the sequences flanking the core sequence affect the precise DNA binding.

The bZIP transcription factors are refered to as ABRE binding (AREB) proteins or ABRE binding factors (ABFs). They were isolated using the yeast hybrid screening method (Choi *et al.*, 2000). Among these AREB/ABF proteins, expression of AREB1/ABF2, AREB2/ABF4, and ABF3 was upregulated by ABA, dehydration, and high salinity stresses.

ABF3 and ABF4 over expression in transgenic *Arabidopsis* plants resulted in enhanced drought tolerance accompanied by decreased transcription, suggesting that ABF3 and ABF4 are involved in stomatal closure mediated by ABSA. These transgenic plants also showed improvement of drought stress tolerance and the expression of some ABA responsive genes, such as LEA class genes (rd29B and rab18), cell cycle regulator genes (ICKL), and protein phosphotase 2C genes (abi1 and abi2), suggesting that AREB/ ABF proteins are involved in ABA response and stress tolerance in plants (Kang *et al.*, 2002)

There are 75 members of bZYP proteins in *Arabidopsis*, several of which bind to ABRE cis element. Candidate genes coding for bZIP have been cloned, that are induced by osmotic stress and or ABA. The ABFs are known to respond to dehydration either at transcriptional or post transcriptional level (Jacoby *et al.*, 2002). ABF3 and ABF4 involved in stomatal closure have shown to enhance drought tolerance of transgenics over expressing them (Kang *et al.*, 2002).

2.5.1.3 Homeodomain-Leucine zipper proteins (HD-ZIPP): HD-ZIP genes encode proteins that have only been identified in plants so far and are thought to regulate developmental processes and responses to environmental cues. HD-ZIP proteins are characterized by the presence of a DNA binding homeodomain with a closely linked Leucine zipper motif. The activity of HD-ZIP residues primarily in the specific DNA binding property of the homeodomain and the ability of the leucine zipper to mediate protein-protein interaction with other HD-ZIPs. In *Arabidosis* three HD-ZIP genes (ATHB-6, ATHB-7 and ATHB-12) are shown to be responsive to dehydration (Lee and Chun, 1998).

2.5.1.4 Zn-Finger proteins: A zinc finger motif represents the sequence in which cysteines and/or histidines coordinate a zinc atom(s) to form local peptide structures that are required for their specific functions. The zinc finger motifs are classified based on the arrangement of the zinc binding aminoacids. They play critical roles in interactions with other molecules. The cDNA clone for Alfin-l was isolated by differential screening of salt tolerant alfalfa cells grown on NaCl. Overexpression of the Alfinl gene in transgenic plants increaserd the MsPRP2 transcript levels in root band enhanced NaCl tolerance, induicating that the Alfinl gene product regulates MsPRP2 expression *in- vivo* (Vinicov and Bastola, 1999).

2.5.1.5 AP2/ERE-Type transcription factors: AP2/ERE domain proteins include the DREB or CBF proteins binding to dehydration response elements (DRE) or C repeats. The *Arabidopsis* genome encides

145 DREB/ERF related proteins (Sakuma *et al.*, 2002). A major transcriptional regulatory system is represented by DRE/C repeat promoter sequences in stress activated gene DREBs/CBF factors that control stress gene expression (Liu *et al.*, 1998).

2.5.1.6 Myb-Like proteins: The Myb-motiff comprises three imperfect repeats forming a helix-tern helix- related motif. Each repeat contains three conserved tryptophan residues every 18 to 19 amino acids, which promotes a secondary structure with a functional Myb domain. In plants, the first tryptophan of R3 is substituted by phenylalanine or isoleucine (Villalobes *et al.,* 2004). The Myb gene family is represented by 190 genes in Arabidopsis (Riechmann *et al.,* 2000). Three additional Arabidopsis Myb-like transcription factors were identified as upregulated by dehydration, high salinity, or cold stress in a microarray study (Seki *et al.,* 2002).

2.5.1.7 Myc-Like Proteins: Myc-Like Proteins contains the basic helix-loop-helix (bHLH) domain, which is composed of two sub domains, the basic regions found in bZIPs) responsible for DNA binding and the HLH9 (helix-loop-helix) region for dimerization with interacting proteins. The *Arabidopsis* rd22BPl gene encodes a Myc-like transcription factor and is induced by dehydration, high salt conditions, and ABA. It activates the downstream rd22 gene by binding to the Myc promoter motif (Abe *et al.*, 1997).

2.5.1.8 NAC transcription factor: *Arabidopsis* RD26 encodes a NAC protein and is induced not only by dehydration but also by ABA. Microarray analysis of transgenic plants over expressing the NAC genes revealed that several drought-inducible genes were upregulated in the transgenic plants. These plants also exhibited significantly increased drought tolerance. Around more than 5 per cent of the *Arabidopsis* genome codes for more than 1500 transcription factors, which are themselves regulated by reversible phosphorylation or by de-novo synthesis. Many of these are reported to be up regulated under different kinds of stress and it has been difficult to identify all the target genes of the transcription factor under study. A good number of transcription factors are also reported to be regulated under different kinds of stresses and it has been difficult to identify all the target genes of the transcription factor under study. A good number of transcription factors are also reported to be regulated by dehydration and are classified into different categories based on structure, motif, DNA binding core sequence etc, (Ramanjulu and Bartels *et al.*, 2002).

Majority of the transcription factors up regulated by osmotic or dehydration stress belong to the largest group of AP2/ ERF family of DNA binding proteins, which include DREB and CBFs. CBFs, referred to as DREBs, were reported initially based on studies of dehydration stress genes (Liu *et al.,* 1998). DREB1A, DREB1D/CBF4, DREB2A and DREB2B are induced by salt and dehydration (Sakuma *et al.,* 2002; Nakashima et al., 2000), in *Arabidopsis*. Similarly rice AP2 transcription activators, OsDREB1A, OsDREB1B, OsDEREB1C abd OsDREB2A have been isolated and it was found that OsDREB1A and 2A are involved in dehydration tolerance (Dubouzet *et al.*, 2003). One novel DNA binding factor DBF1 from maize induced by dehydration (Kizis and Pages, 2002).

Though a number of functional and regulatory genes involved in drought stress and signaling have identified, not many have been well characterized. Attempts in recent years have been to isolate genes from various species, with emphasis on tolerant species, to identify genes probably playing role in stress tolerance.

With the complete genome sequence of *Arabidopsis* available, candidate tolerant plants have been those that are closely related to *Arabidopsis* family or species. Highly salt tolerant *Thellungiella hapliphila* (Amtamann *et al.,* 2005) and High metal accumulator *Thalapsi caerulescence* (Cosio *et al.,* 2005); *Sporobolus stapfianus* (Phillips *et al.*, 2002). Have been the choice of study. At the same time resurrection plants like *Creterostigma plantagenium* (Noorwood *et al.*, 2003). *Sporobolus stapfianus* (*Phillips et al.,* 2002) and tolerant crop plants have been explored to identify stress responsive genes. Though the probable candidates involved in signal transduction or perception has been difficult to identify, efforts are carried out to identify stress responsive genes (Zhu, 2002). Most of the molecular genetic studies performed with *Arabidopsis thaliana*, have helped in dissection of stress response pathways at molecular level (Zhu, 2002; Shinozaki *et al.,* 2003).

2.6 Upstream regulatory genes and Signal transduction:

In contrast to signal perception various components of signal transduction have been identified. It is largely unknown, where they are positioned in the complex signaling network and how the various different molecules interact with each other and how the signal is transduced. Signaling molecules like Ca^{2+}, Reactive Oxygen Species (ROS), phosphoproteins etc, help in amplification of the signal (Bartels and Ramanjulu, 2005).

2.6.1 SNF-1-Like Kinases:

Another family of protein kinases are the SNF/AMP activated protein kinases, which were first analyses in yeast (SNF=sucrose-non-fermenting). These kinases may sense the ATP/AMP ratio and thus control fluxes between anabolism and catabolism via transcription of genes encoding enzymes related to carbohydrate metabolism. In plants some members of this grop of kinases areexpressed in response to dehydration or ABA. In rice, 10-SnRK2 protein kinases were reported (Kobayashi *et al.*, 2004) all family members atre activated by huperosmotic stress and three of them are also activated by ABA.

2.6.2 Phosphatases:

Phosphatases are classified according to their substrate specificity. There are two major types of phosphatases: phosphoprotein (serine/threonine). Phosphatases(or PPases) and phosphotyrosine(protein tyrosine phosphatases or PTPases). Protein phosphatases 2Cs are serine/threonine phosphatases and their involvement in stress is well studied in fungal models. In yeast PP2C interacts with a MAP kinase cascade that controls osmolyte biosynthesis (HOG pathway). PP2C has been described by *abi1* and *abi2 Arabidopsis* mutants defective in a PP2C isoform (Leung *et al.,* 1994), which seems to function as a regulator in a pathway that mediades resposes to environmental stresses involving ABA.

2.7 Functional genes:

Number of genes with wide range of functions are up regulated by drought, these can be broadly classified as those involved in osmotic adjustment- sugars and osmolyte chaperones or protective proteins, proteins involved in detoxification and genes down regulated under stress.

2.7.1 Sugars and osmolytes

Compatible solutes are non-toxic molecules and range from amino acid to cyclic alcohols. The exact mechanism of protection provided by these is not understood, as accumulation is not often sufficient to account for osmotic adjustment. Yet a number of other roles have been proposed (Ramanjulu and Bartels; 2002). These molecules may have a primary role in turgor maintenance, but may also be involved in stabilizing proteins and cell structures (Yancey *et al.*, 1982). Though the accumulation per se may not be important, their metabolic pathways are predicted to have adaptive value under osmotic stress (Hasegawa *et al.*, 2000). It is further hypothesized that they may be involved in scavenging ROS (Chen and Murata, 2002). Sugars are proposed to act as osmolytes or protect specific macromolecules and stabilizing membranes by forming glass like structure (Black and Pritchead, 2002). The ressurection plant *Craterostigma plantagineum* accumulates, an unusual 8-carbon sugar, octulose, which is rapidly converted to sucrose during dehydration (Norwood *et al.*, 2003). Substantial amount of trehlose was identified in *Myrothamus flabellifolia* and *Sporobolus stafianus*, ressurection plants (Phillips *et al.,* 2002). Other polyols, such as pinnitol or ononitol have frequently been reported in response to drought (Streeter et al., 2001). Proline the most studied osmolyte has been proposed to function in various way, besides osmotic adjustment, as a sink of energy or reducing power, protectant of plasma membrane, a source of carbon and nitrogen or hydroxyl radical scavenger (Hong *et al.*, 2000).

Apart from other osmolytes, Glycine betaine is known to marginally improve osmotic stress tolerance. The ability of osmolytes to enhance stress tolerance, have been studied in over expressing transgenic plants (Bartels and Sunkar, 2005).

2.8 Protective proteins and chaperons:

This broad category comprises of proteins known to play role of chaperons such as LEAs and HSPs, proteases and proteinase inhibitors (proteins required for peotein homeostasis), water channel proteins like Aquaporin and polyamines.

Late embryogenesis abundant (LEA) and heat shock protein encoding genes comprise a diverse group of protective proteins, which are not normally expressed in vegetative tissue under normal condition but are induced by environmental stress such as drought (Campalans *et al.*, 2001; Ingram and Bartels, 1996). Based on the conserved structure LEAs have been classified into 6 groups and they are correlated with dehydration based on its response dehydration and ABA (Dure, 1993). Although the biochemical tolerance function of LEA proteins has not been proven, positive evidences of its function as protective molecules has been proposed based on over expression studies in both plants and animals. The probable function of group 1 Lea seems to be in bindung or replacement of water. Group 2 or dehydrins and 4 contribute to maintenance of protein and membrane structure where as group 3 and 5 are suggested to form dimer with coiled-coil structure capable of sequestration of ions, accumulated during water depletion (Ramanjulu and Bartels, 2002). Recently, for the first time, it was shown that LEA proteins exhibit anti- aggregation activity under water stress. A model was proposed where by LEA proteins might act as a novel molecular chaperons or molecular shield preventing formation of damaged protein aggregate during water stress (Tunnacliffe *et al.*, 2005).

Heat shock protein (HSP) family encompasses many chaperons, which function in folding. Assembly of proteins during synthesis, in removal, disposal of non- functional and degraded proteins. Arabidopsis mutants with reduced HSP expression wre found to be sensitive to desiccation (Wehmeyer and Vierling, 2000). HSPs are generally induced by water stress, reported in several plants. The cytosolic HSPs function as molecular chaperons by preventing thermal aggregation of substrate proteins and facilitate subsequent reactivation (Lee *et al.,* 1995). The abundance of small HSPs and their characterization function, suggests the importance of HSPs in stress tolerance (wang *et al.,* 2004). Water stress is also known to induce chaperone-binding proteins (BiPs) in plants (Cascardo *et al.*, 2000).

Not much work has been done on candidate gene expression studies in drought tolerance of banana.

Carbon Isotope Discrimination:

Plants discriminate against the heavy isotope of carbon (^{13}C) during photosynthesis resulting in the depletion of the ^{13}C content in the biomass (O'Leary, 1981). This deviation of the carbon isotopic ratio ($^{13}C/^{12}C$) of biomass from that of air, called discrimination ($\Delta^{13}C$) is related to the ratio of the partial pressures of CO_2 inside the leaf to that in ambient air (Pi/Pa) (Farquhar *et al.,* 1982; Farquhar *et al*, 1989; Hubick and Farquhar,1989) as follows;

$$\boldsymbol{\Delta^{13}C = a+(b-a)\ (Pi/Pa),}$$

Where, **a** and **b** are fractionation against ^{13}C ($\delta^{13}C$) during diffusion through stomata; carboxylation by RuBisCO respectively. Since WUE is also related to the CO_2 partial pressures, at a given VPD, a strong inter-relationship between Δ^{13} C and WUE is expected (Hubick and Farquhar, 1989).

The $\Delta^{13}C$ in plant samples is generally determined using a sophisticated analytical instrument called Isotope Mass Spectrometer (IRMS) specially designed for high precision measurements of the ratio R, defined as:

$$R={}^{13}CO_2/{}^{12}CO_2$$

$$\delta^{13}C= R_{sample} - R_{standard}/R_{standard} * 1000$$

Since the organic sample has less R-value than the standard, δ^{13}Cof organic material is more negative, i.e., less ^{13}C content hence more discrimination and vice versa (O'Leary, 1984).

Importance and biochemical basis of Δ^{13} C and the relationship of Δ with WUE have extensively been studied (Farquhar *et al.,* 1982; Codon *et al.*, Read *et al.,* 1991). The range of Δ values across the crop types, having different photosynthetic pathways is given below:

Range of δ^{13}C composition

1.Atmosphere (Air) = -6.4 to –7.0 %o
2.C_3 plants = -22 to –44 %o
3.C_4 plants = -9 to –19 %o
4.CAM plants = -11 %o (approximate)

Carbon isotope discrimination (Δ^{13}C) at different steps during photosynthesis

The fractionation of carbon isotope during photosynthesis involves several distinct biochemical and physical processes. These processes have different tendencies to discriminate between ^{12}C and ^{13}C , and the overall discrimination of a particular plant will be a function of the mechanism it uses for CO_2 fixation and the relative balance of the processes that participate in photosynthesis. During photosynthesis, CO_2 must diffuse from the atmosphere to the chloroplast stroma. Since $^{12}CO_2$ diffuses faster than $^{13}CO_2$ several fractionation processes occurs along this diffusion path, so that the CO_2 available at the sites of carboxylation is always significantly depleted in ^{13}C compared to the atmosphere (Table 1) (Farquhar *et al.*, 1989; Bragnoli and Farquhar, 1998).

Table 1: Fractionation of carbon isotope during photosynthesis.

Process	Discrimination (%)	Reference
Diffusion of CO_2 in air through the stomatal pore	4.4	Craig, 1953
Diffusion of CO_2 in air through the boundary layer to the stomata	2.9	Farquhar, 1983
Diffusion of dissolved CO_2 through water	0.7	O'Leary, 1984
Fixation of gaseous CO_2 by RuBisCo	29	Guy and Hoering, 1987

The principal components of photosynthesis that influence discrimination are diffusion of CO_2 through the stomata and the carboxylation process mediated by RuBisCo (O'Leary, 1988,1993). Irrespective of the photosynthetic sub component-determining discrimination, ^{13} C is related to Pi as following relationship suggests:

$$\Delta = \{a+(b-a)\ Pi/Pa-d\}$$

Where a, b are constants for the discrimination against $^{13}CO_2$ during diffusion of CO_2 in the leaf and carboxylation, respectively and d is a component contributed from respiration, diffusion of dissolved CO_2 and Pi & Pa are the intercellular and ambient CO_2 partial pressures.

$\Delta^{13}C$ and WUE –Relationship

An inverse relationship between A/gs and $\Delta^{13}C$ (Meinzer *et al.*, 1990; Richards and Tieszen, 1993) and a positive relationship between Pi/Pa and $\Delta^{13}C$ signify that Pi determines the variability in $\Delta^{13}C$ (Hubick *et al.*, 1988; Gutterrez and Meinzer, 1994).

Although WUE and $\Delta^{13}C$ are related through the ratio of Pi/Pa, as well as with A/gs (Condon *et al.*, 1990), because of diurnal and seasonal fluctuations in 'A' and gs, these parameters will not give an integrated estimate of WUE over a period of time (Hall *et al.*, 1993 and Udayakumar and Prasad, 1994). From this context, $\Delta^{13}C$ is a dependable parameter as it is a reflection of tim integrated estimate of carbon gain per unit transpiration, especially in C_3 plants. $\Delta^{13}C$ in whole plant dry matter appears to be reliable indicator of plant WUE in pot grown sunflower and negative relationship was obtained between these two traits in structural carbon both in well watered and drought conditions (Johnson *et al.*, 1993). In wheat, as in other C_3 species, genetic variability in $\Delta^{13}C$ is reflected in variation in WUE at both the leaf and at the whole-plant level (Condon and Richards, 1993).

Such a relationship between $\Delta^{13}C$ and WUE in several crop species as depicted was not altered even plants were subjected to abiotic stresses. Maintenance of the relative ranking of genotypes in control and stress implies that for WUE and $\Delta^{13}C$, genotype and environment interaction is low and the broad sense heritability is high (Hubick *et al.*, 1998). Due to these distinct advantages, $\Delta^{13}C$ appear to be a very reliable parameter for the identification of variability in WUE. This led to the initiation of several breeding programs to improve WUE using carbon isotope discrimination technique (Hall *et al.*, White, 1993).

Materials and Methods

III. MATERIALS AND METHODS

Water deficit is one of the principle limiting factor for crop production through out the world. Water deficit which is a consequence of either intermittent or terminal period of drought causes significant yield reduction on cultivated crops. These restrictions on yield potential are of great concern in terms of meeting food demand for accelerated increasing world population. The problem becomes more complicated by the fact that supplies of suitable irrigation water are dwindling rapidly therefore, efforts are needed for better watershed management and integrated approaches to water conservation. Understanding the fundamentals of plant responses is vital within a specific environment. Keeping this in view, the main objective of this study was to understand the physiological and biochemical changes and the expression of a few selected candidate genes during drought in banana. To study the above mentioned parameters the following experiments were conducted.

3.1 Screening of AA and BB Banana Genotypes:

The banana accessions belongs to AA and BB genomic groups (Table-2) maintained at IIHR field were screened for drought tolerance using the following parameters.

1. Leaf water retention capacity

2. Carbon isotope discrimination analysis

3.1.1 Screening of banana genotypes for the Leaf Water Retention capacity:

1. The fully opened third leaf from the banana plants belongs to AA and BB genomic groups were collected (Table-2) early in the morning (9:0 AM).

Table-2: Banana genotypes used for screening drought tolerance using Leaf water retention capacity

AA Genotypes	BB Genotypes
Musa acuminata Wild	Amtur kela
Matti	Ram kela
Sanna chen kadali	Wild-2
Anaikomban	*Musa balbisiana* ssp *tani*
Kadali	*Musa balbisiana*-1
M. acuminata. ssp *burmaniceoides* (Calcutta-4)	Fruit less variety
Erachi vazhai	*Musa balbisi*ana-2
Annara kannan	Sirsi wild
Pisang linin	Bee hee kela
CV Rose	*Musa species*
Pisang jari buya	Betta bale
Tongat	Bhimaitia
Madhu sagar	Wild-5
Musa acuminata ssp. *banksii*	
Musa acuminata ssp *malaccensis*	

2. Fresh weight was recorded and they were hanged to the rope under room temperature to record the loss of water (Plate-2).

3. Weight was recorded again after 48 hours.

4. The leaves were then kept in the hot air oven at 80^0 C to get dry weight of the leaves.

The formula used to calculate the LWRC (%) was calculated using the formulae,

$$\text{LWRC (\%)} = \frac{\text{Moisture content after 48 hour drying}}{\text{Total leaf moisture content}} \times 100$$

Moisture after 48 hour = Fresh weight – weight after 48 hour

Total leaf moisture content= Fresh weight – dry weight

(Bananuka *et al.*, 1999)

3.1.2 Screening of banana genotypes for water use efficiency using carbon isotope discrimination.

During the fixation of carbon by photosynthesis, the naturally occurring heavy stable isotope, ^{13}C, is discriminated against. Therefore, plants have a smaller ratio of ^{13}C to ^{12}C than the air. Farquhar *et al.* (1982) developed the theory, which predicted that discrimination would be least in those C_3 plants that fix the most carbon per unit amount of water transpired i.e. in those that have the greatest water use efficiency. Ever since then, the Δ^{13}C technique has been widely adopted as a powerful estimate of WUE.

. 3.1.2 .1 Determination of Δ^{13}C in leaf samples

The carbon isotope discrimination (Δ^{13} C) was determined with an objective to study genetic variability in WUE in banana from the genotypes of AA and BB group accessions.

1. Third fully expanded leaf was collected.

2. Kept at 80° C for three days to completely dry the samples.

3. Dried samples were finely powdered in a mortar and pestle. Care was taken to prevent any contamination by washing the pestle and mortar with alcohol after grinding each sample.

The Δ^{13} C of the leaf samples was determined using continuous-flow Isotope Ratio Mass Spectrometer (IRMS) at the National facility for stable isotope studies in Biological Sciences, Department of Crop Physiology, U.A.S, G. K. V. K., Bangalore. The details of the methodology are described below (Plate-3).

3.1.2.2 Mass Spectrometric Analysis

Carbon isotope discrimination is measured as the deviation of the molar ratio between ^{13}C to ^{12}C from an international standard – PDB (Pee Dee Delmnite). Carbon isotopes are fractionated due to diffusion through stomata denoted as 'a' and at the carboxylation site by RuBisCo denoted as 'b'. The total isotope discrimination is the deviations of these fractions from the isotopic ratio in air therefore the discrimination Δ^{13} C computed as follows.

$$\Delta^{13}C = \delta a - [a + (b - a)\, Ci/Ca]$$

Where a=4.4%, b=29%, Ci and Ca respectively are the CO_2 concentration inside the leaf of the plant organic sample from that of the ambient air, as follows:

$$\Delta^{13}C\ \% = \frac{\delta a - \delta b}{1 + \delta p / 1000}$$

where ,

$\delta a = \{(R_{air} - R_{PDB}) / R_{PDB}\} X\ 1000$

$\delta p = \{(R_p - R_{PDB}) / R_{PDB}\} X\ 1000$

$R = {}^{13}C / {}^{12}C$

Finely powdered leaf samples were accurately weighed in the range 0.8-1.2 mg into silver capsules. The crimped capsules with the sample were placed sequentially in the carousel of the autosampler. The samples were dropped at precise times along with an injection of pure O_2 into the oxidation reactor.

The combustion (oxidation) reactor contains chromium oxide and silvered cobaltous-cobaltic-oxide in a quartz column heated to1080^0 C. The biomass is completely oxidized to produce CO_2, N_2O and H_2O these gases are swept into the reduction furnace using Helium carrier gas (purity 99.995%). The reduction furnace contains reduced copper in quartz tube heated to 680^0 C. In this reaction the N_2O is reduced to N_2 and the excess O_2 is absorbed. The resultant gases are then flushed through scrubbers to trap water. The pure CO_2 and N_2 gases are then passed through a GC column. The N_2 gas elutes faster through the GC and hence gases can be effectively separated before introduction into the ion source of the IRMS. At the ion source, CO_2 is ionized by electron impacts ionization to produce molecular radicals (CO_2). These CO_2 radicals are accelerated under the influence of high voltage potential 3000 volts through a strong magnetic field. When accelerated radicals pass through a strong magnetic field, the radicals are deflected with the radius of deflection being proportional to the molecular mass of the radicals. Faraday cups collect these deflecting ${}^{12}CO_2$ and ${}^{13}CO_2$ and the signal is amplified and transmitted to the computer.

3.2 Location of the drought experiment: Banana field, Indian Institute of Horticultural Research, Hessaraghatta Bangalore-89.

3.2.1 Experimental material selected for the planting: We have selected two genotypes for this investigation.

1. *M. acuminata.* ssp *burmaniceoides* commonly recognised as

   Calcutta-4 (AA Genomic group)

2. Bee hee kela (BB Genomic group)

3.2.2 Preparation of structures for planting:

Two structures having dimensions of 12 X 8 X 3 feet were constructed using cement bricks and they were filled with equal quantity of Soil: Sand: FYM mixture.

The healthy uniform suckers of the genotypes Calcutta-4 (AA) and Bee hee kela (BB) were selected from the field and treated with bavistin 1% solution for 30 minutes before planting and planted in structures with even spacing.

3.2.3 Crop management: Recommended dosage of fertilizers was applied; irrigation was given once in two days. Prophylactic measures were taken to control pests and diseases by spraying plant protection chemicals periodically.

3.2.4 Stress imposition: Two treatments were imposed i.e., control and stress. Irrigation was with held 120 DAP (Days After Planting) for a period of twelve days for stress treatment and control plants were regularly watered (Plate-1).

Plate-1: Drought experimental structures, IIHR, Hessaraghatta, Bangalore.

Plate-2: Technology for Screening of banana genotypes for Leaf Water Retention Capacity at room temperature

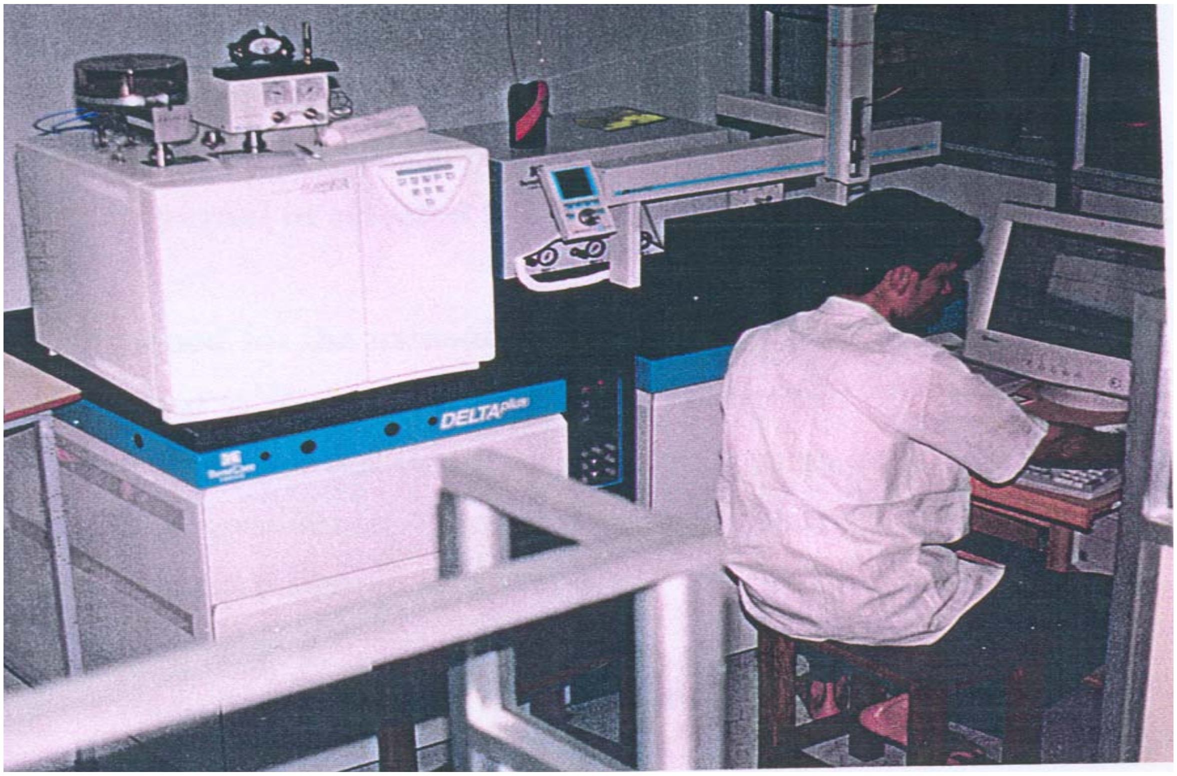


Plate-3: Isotopic ratio mass spectrometer (IRMS) for the determination of $\Delta^{13}C$ (flash-ea) in leaf biomass at national facility for stable isotope studies in biological sciences, Dept of Crop. physiology, UAS, GKVK, Bangalore.

3.3.1 Observations recorded:

Gas exchange parameters *viz.*, Photosynthesis rate (P_N), Transpiration rate (E) and Stomatal conductance (gs) were measured from the third fully expanded leaf using portable photosynthetic system Infra red gas analyser (IRGA) (Model-LCA-3) ADC Company. All observations were recorded during bright sunlight hours (10AM-12 Noon).

3.3.1 .1 Principle of IRGA:

Infra Red Gas Analysers (IRGA) are used for the measurement of a wide spectrum of hetero atomic gas molecules including CO_2, H_2O, NH_3, CO, SO_2, N_2O, NO and gaseous hydro carbons like CH_3. Hetero atomic molecules have characteristeric absorption spectra in the infra red region: therefore absorption of radiation by a specific hetero atomic molecule is directly proportional to its concentration in a given air sample.

3.3.2 Determination of leaf water potential (Ψ)

Leaf water potential (Ψ) was measured with psychrometer *HR 33T* (*Wescor,* USA) in the mode “dew point” equipped with sample chamber C-68 and C-79.

Procedure followed:

1. The leaf material from the middle portion of the fully (third leaf) expanded collected and folded in aluminium foil immediately and then brought to laboratory.
2. The leaf discs were prepared using paper punch of 0.5 cm diameter and kept in the disc chamber and closed and left for 4minutes.
3. Readings were recorded and expressed in -Mpa.

$$\textbf{Leaf water potential} = \frac{\text{Dew point reading}}{\text{Chamber range}} \times 10 \quad (\text{-Mpa})$$

3.3.3 Determination of soil moisture content:

The soil from the 45 cm depth was collected using Auger in 4-5 spots in each structure and pooled. Halving technique was followed to get desired quantity of soil sample. Soil sample was immediately placed in polythene cover and initial weight was recorded. Dry weight was taken after keeping the soil sample in oven at 80^0 C for two days and percentage soil moisture content was determined on dry weight basis.

$$\text{Moisture content of soil} = \text{Initial soil weight} - \text{Dry weight of soil}$$

$$\%\ \text{SMC} = \frac{\text{Moisture content in soil}}{\text{Dry weight of the soil}} \times 100$$

3.4 Biochemical analysis:

3.4.1 Estimation of lipid peroxidation of membranes by quantifying melondialdehyde content:

Estimation of melondialdehyde content:

The estimation was done as per the methods by Draper and Hadly (1990).

3.4.1.1 Reagents required:

1. Five per cent aqueous trichloro acecitic acid (TCA):- 5g of TCA in 100ml of distilled water.
2. Methanolic Butylated Hydroxy toluene (BHT) 0.5 per cent: - 0.5 g of BHT in 100ml 0f methanol.
3. Saturated Thiobarbituric Acid Solution (TBA).

3.4.1.2 Extraction method: - One gram of leaf tissue was homogenised thoroughly with 5 ml of 5 per cent aqueous trichloro acetic acid solution and 0.5 ml of methanolic BHT. The resulting homogenate was heated for 30 min in boiling water bath. Then the sample was cooled and centrifuged at 1000g for 10 min. The volume of the resulting supernatant was make up to 10ml with the distilled water.

3.4.1.3 Estimation method: - To one ml aliquot of the supernatant sample one ml of saturated TBA solution was added and the tubes were incubated in boiling water bath for 30 minutes. Tubes were cooled and the absorbance was read at 532 nm against a blank solution and expressed as OD change/ ml of aliquot.

3.4.2 Estimation of the activities of antioxidative enzymes

Assay of super oxide dismutase (SOD) activity: The activity was assayed following the method by Zhanyuan Du *et.al.* (1994).

3.4.2.1 Reagents required:

1. 50 mM Na/K phosphate buffer pH 7.8
    a) 0.2 M $NaH_2PO_4.2H_2O$ – 15.6 gm was dissolved in distilled water and made up to 500ml(stock solution).
    b) 0.2 M $Na_2HPO_4.2H_2O$ - 17.7gm was dissolved in distilled water and made up to 500ml (stock solution)

For pH 7.0 – 39ml of a + 61 ml of b), diluted to 200ml with distilled water.
For pH 7.8 – 8.5 ml of a + 91.5 ml of b), diluted to 200ml with distilled water.

2. Magnesium chloride 0.3M solution ($MgCl_2$):- 6.099 g of $MgCl_2$ in 100 ml of distilled water.

3. EDTA 10 mM solution:- 0.372 g of EDTA in 100ml of distilled water.

4. L- Methionine 39mM solution:- 0.5818 g of methionine in 100ml of distilled water.
5. Nitroblue tetrazolium chloride 450 mM solution (NBT):- 0.03679 g of methionine in 100ml of distilled water.

6. Riboflavin12µM solution: - 0.45 mg of Riboflavin solution in 100ml of distilled water.

3.4.2.2 Enzyme extraction method: Exactly two grams of leaf tissue was weighed and homogenised using a chilled mortar and pestle in 10ml of 50 mM phosphate buffer pH 7.0, 0.1ml of $MgCl_2$ solution, 0.1ml of EDTA solution and 0.2 per cent insoluble poly vinyl pyrrolidone. The homogenate was centrifuged at 11,000g for 15 minutes and the supernatant was used for enzyme assay. All the steps in preparation of the extract were carried out at 0-4^0 C. Enzyme activity was expressed as units per gram fresh weight.

3.4.2.3 SOD enzyme assay method:

SOD activity was assayed by measuring ability of SOD to inhibit the photochemical reduction of Nitroblue tetrazolium chloride. The assay mixture contained 0.8ml of buffer pH-7.8, 0.1M EDTA solution, 1.0 ml of Methionine solution, 0.5ml of Riboflavin solution, 0.5ml of NBT solution and 0.1ml of enzyme extract. The assay mixtures were mixed thoroughly and placed 50cm below a white light source consisting of eight 15V fluorescent lamps. The reaction was started by switching on the lights and was allowed to proceed for ten minutes. The reaction was stopped by switching off the lights, and the absorbance of the assay mixture was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme that inhibited the

NBT photoreduction by 50 per cent under assay conditions and expressed as U gm^{-1} FW.

3.4.2.4 Assay of catalase activity: The activity was assayed following the method by Masia (1998).

3.4.2.5 Reagents required: -

1) Sodium phosphate buffer 100mM, pH–7.0:- 39ml of 0.2 M $NaH_2PO_4.2H_2O$ and 61ml of 0.2 M $Na_2HPO_4.2H_2O$ and pH was adjusted to 7.0.

2) Sodium phosphate buffer 50mM, pH-7.0:- 39ml of 0.2 M $NaH_2PO_4.2H_2O$ and 61ml of 0.2 M $Na_2HPO_4.2H_2O$ diluted to 200ml and pH was adjusted to 7.0.

3) Hydrogen peroxide10mM solution (H_2O_2):- 0.3ml of 30 per cent H_2O_2 diluted to 30ml with 50mM sodium phosphate buffer pH-7.0.

3.4.2.6 Enzyme extraction method: - One g of frozen leaf tissue was taken in pre chilled pestle and morter and ground with liquid nitrogen and then homogenized with 10ml of 100mM Sodium phosphate buffer pH – 7.0, filtered through four layers of muslin cloth and centrifuged at 11,000g for 20 minutes at 4 ^{0}C. The resulting supernatant solution was immediately used for enzyme assay. All the steps in preparation of the enzyme extract were carried out at 0-4 ^{0}C.

3.4.2.7 Enzyme assay:- Catalase activity was measured at 20 ^{0}C according to Aebi (1984). The assay mixture contained 1.5 ml of 50 mM Sodium phosphate buffer pH -7.0, 0.5 ml of hydrogen peroxide solution (H_2O_2) and 0.3 ml of crude enzyme extract. Catalase activity was measured by the decrease in the absorbance of hydrogen peroxide at 240 nm and was expressed as units per gram fresh weight per min. One unit of enzyme is defined as amount of enzyme which produces a decrease of 0.01 OD per min.

3.5 Molecular Analysis:

3.5.1 Sample Collection and RNA isolation.

The leaf samples from experimental (both control and stressed) plants were collected using sterile blade and wearing gloves. The leaf was cleaned with dilute ethanol immediately after collection and sterilised leaf sample were cut into pieces. One to two gram tissue for each sample was kept in aluminium foil immersed in liquid nitrogen in the field itself to reduce the phenolic secretions and stored at -80^0 C for further use.

3.5.2 Isolation of total RNA from control and water stressed banana leaf tissues

RNA was isolated from the leaf samples using the modified version of the method (Liu *et al.,* 1998).

3.5.2.1 DEPC treatment:

The materials and glassware used for isolation of RNA were treated overnight with DEPC (diethyl pyrocarbonate) water, and then autoclaved, this is to inhibit RNAase activity.

Glassware were baked at 180^{0}C for 8 hours and then rinsed with choloroform. Glassware were immersed in 0.1% DEPC which is strong but, not absolute inhibitor of RNAase. DEPC filled glassware or plasticware were allowed to stand for 2 hrs at 37 ^{0}C and then rinsed several times with sterile water. These were then heated at 100 ^{0}C for 15 minutes or autoclaved for 15 minutes at 15 lbs pressure, which is known to remove traces of DEPC that might otherwise modify residues in RNA carboxymethylation. The efficiency of transformation is very low in cell free synthesis of carboxymethylated RNA.

3.5.2.2 RNA extraction protocol (leaf tissue)

Reagents required:

Extration buffer:

2 % CTAB,
2 % PVP,
100 mM Tris Hcl (pH 8.0),
25 mM EDTA,
2M NaCl and
2 % 2- Mercaptoethanol

Chloroform: Isoamyl alcohol (49:1)
10 M $LiCl_2$
3 M $LiCl_2$
Absolute alcohol
DEPC water (1%)

3.5.2.3 Preparation of solutions:

All the solutions required for RNA extraction were prepared with 0.1% DEPC treated autoclaved Milli Q water and P^H was adjusted for required solutions and then autoclaved.

3.5.2.4 Protocol:

1. 1.5 g of frozen tissue was taken into mortar and pestle and ground into powder by pouring liquid nitrogen with out rash grinding.
2. 150 µg of polyvinylpyrrolidone was added and ground with liquid nitrogen for two times.
3. 10 ml extraction buffer was added and ground well for two minutes.
4. 200 µl of 2-mercaptoethenol was added and ground for one minute.
5. Solution was taken into 50 ml Oakridge tubes and kept for incubation for 20 minutes at 65 ^{0}C.
6. Immediately cooled and centrifuged for 30 min at 10000 rpm.

7. Carefully pipetted out supernatant to new oakridge tubes and added equal volumes of chloroform: Isoamyl alcohol (49:1).
8. Centrifuged for 20 min at 10000 rpm.
9. The supernatant was taken out carefully to avoid interface and equal volumes of chloroform: Isoamyl alcohol (49:1) was added and centrifuged for 15 min at 10000rpm.
10. The above step was repeated again and supernatant was taken out carefully and 1/4th volume of 10 M $LiCl_2$ was added and kept for overnight precipitation at – 80 ^{0}C.
11. Here the solutions was centrifuged for 30 minutes at 10000 rpm, the pellet position was marked and supernatant was pipetted out properly without disturbing a pellet.
12. Pellet was washed with 80% DEPC alcohol by centrifuging at 10000 rpm for 15 min.
13. Washed pellet was air dried for 5minutes, and then pellet was dissolved in 400µl of DEPC water this was transferred to 2 ml eppendorfs tube to this 1 ml of absolute alcohol and 10µl 0f 5 M sodium acetate was added and kept for precipitation at -20 ^{0}C for 2 hrs.
14. The solution was centrifuged for 15 minutes, discard the solution and pellet was air dried properly and dissolved in 50µl of RNAse free water, stored at-80 ^{0}C.

    Isolated RNA was checked for integrity after each isolation by denaturing gel electrophoresis containing formaldehyde.

3.5.2.5 Reagents (Gelelectrophoresis of RNA)

1. 5X Formaldehyde gel running buffer- 40 mM sodium acetate, 0.2 m MOPS (pH 7.0) 0.5 M EDTA (pH 8.0)
2. Formaldehyde
3. Formamide
4. Agarose
5. Sterile DEPC treated water

6. Formaldehyde gel loading buffer- 50% glycerol, 1 mM EDTA (pH 8.0), 0.25% bromophenol blue and 0.25% xylene cyanol
7. Ethidium bromide (0.1%)

3.5.2.6 Electrophoresis tanks:

Electrophoresis tanks were cleaned with detergent solution, rinsed in water and dried with ethanol and then filled with 3% H_2O_2 solution. After ten minutes. The tanks were rinsed with water that had been treated with 0.1% DEPC. DEPC reacts rapidly with amines and can't be used to treat solutions containing buffers such as tris borate or Tris Acetate.

3.5.2.7 Preparation of denaturing gel

1. The following components were added

Agarose	- 0.36g (1.2 %)
DEPC water	- 18.75 ml
Formaldehyde (Final conc. 2.2 M)	- 5.3ml
5X gel running buffer (Final conc.1X)	-5.9 ml
TOTAL	- 30ml

7. The above solution, which contains agarose, was boiled until melting
8. The gel was cast by pouring into the unit cleaned with sterile DEPC water after cooling down to 60 ^{0}C

3.5.2.8 Preparation of RNA sample for loading

1. Add the following components:

RNA	-5µl
5X gel running buffer	-2.0µl
Formaldehyde	-3.5µl

Formamide - 10.0 µl

2. The above mixture was boiled at 65 ^{0}C for 15 min to denature the secondary structure of RNA
3. Then it was kept in ice fort 5 sec; to this 2.5µl formaldehyde gel loading buffer and 2.5µl ethidium bromide were added
4. Spun at top speed for 30 seconds.
5. Then the samples were loaded on to the denaturing gel to check the integrity of RNA.

3.5.2.9 Checking the Integrity of RNA

The gel was submerged in 1X formaldehyde gel- running buffer. Electrophoresis was carried out at 5 Volt per cm for two to three hours. The integrity of RNA was checked by observing the 28S and 16S RNA bands.

3.5.2.10 RNA Quantification:

Concentration of RNA in the sample was estimated by recording absorbance at 260 nm in UV spectrophotometer. Good quality RNA samples in required quantity were immediately used for cDNA synthesis and stored at -80 ^{0}C.

3.5.2.10.1 Protocol:

1. 995 micro litre of DEPC water was taken in quartz cuvette and 5 micro litre RNA Sample was added.
2. The absorbance was measured at 260 nm.
3. RNA concentration was calculated using the relationship

1 OD at 260nm= 40 µg /µl of RNA.

$$\underset{(\mu g/\mu l)}{\text{Concentration of RNA}} = \frac{\text{OD at 260 nm X 40 X dilution factor}}{1000}$$

$$\text{Dilution factor} = \frac{1000}{\text{Volume of sample taken.}}$$

3.6 Protocol for RNA clean up before proceeding to cDNA preparation using Rneasy Mini Kit [taKaRa clontech].

Buffer RLT and ethanol were added to the sample to create conditions that promotes selective binding of RNA to the RNeasy membrane. The sample was then applied to the RNeasy Mini spin column. Total RNA binds to the membrane, contaminants were efficiently washed away, and high-quality RNA was eluted in RNase-free water.

1. Sample was adjusted to a volume of 100 µl with RNase-free water. Added 350 µl Buffer RLT, and mixed well.
2. Added 250 µl ethanol (96–100 %) to the diluted RNA, and mix ed well by pipetting.
3. Transfered the sample (700 µl) to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Closed the lid gently, and centrifuged for 15 s at 8000 x g (10,000 rpm). Discarded the flow-through.
4. 500 µl Buffer RPE to the RNeasy spin column was added. Close the lid gently, and centrifuged for 15 s at 8000 x g (10,000 rpm) to wash the spin column membrane. Discarded the flow-through.
5. Added 500 µl Buffer RPE to the RNeasy spin column. Closed the lid gently, and centrifuged for 2 min at 8000 x g (10,000 rpm) to wash the spin column membrane.
6. Placed the RNeasy spin column in a new 2 ml collection tube (supplied), and discarded the old collection tube with the flow-through. Closed the lid gently, and centrifuged at full speed for 1 min.
7. Placed the RNeasy spin column in a new 1.5 ml collection tube (supplied). Added 30–50 µl RNase-free water directly to the spin

column membrane. Closed the lid gently, and centrifuged for 1 min at 8000 x g (10,000 rpm) to elute the RNA.

Buffer RLT contains guanidine thiocyanate
Buffer RLC contains guanidine hydrochloride

3.7 cDNA synthesis:

cDNA was synthesised using TaKaRa mRNA Selective PCR Kit (AMV).The procedure is as follows:

First in a tube the reaction mixture was prepared by combining the reagents in the proportions as shown below by doing all operations by keeping vessels on ice.

Reagents	**volume**	**Final concentration**
2x mRNA Selective PCR Buffer I or II	25 µl	1x
25 mM $MgCl_2$	10 µl	5mM
dNTP/analog mixture	5 µl	1 mM
RNase Inhibitor (40 units/µl)	1 µl	0.8 units/µl
AMV RTase XL (5 units/µl)	1 µl	0.1 units/ µl
Oligo dT primer, or Random 9 mers, Or specific primer (20µm)	1 µl	
Total RNA	1µl(1 µg)	
RNase free distilled water	6 µl	
Total volume	50 µl	

Then the following reaction condition is performed using PCR,

$$\left.\begin{array}{ll} 30\,^{o}C \longrightarrow & 10\ \text{min} \\ 42\text{-}50\,^{o}C \longrightarrow & 15\text{-}30\ \text{min} \\ 5\,^{o}C \longrightarrow & 5\ \text{min} \end{array}\right\} \text{One cycle}$$

3.8 Primer designing:

Using IDT (Integrated DNA Technology sogtware) we have designed primers (Farward and Reverse) for nine candidate genes having relevance in drought, growth and metabolism and gene regulation, to carryout Real time PCR.

3.8.1 Primer synthesis: Primer synthesis was done at MWG Biotech Pvt Ltd, Bangalore. (Table-3)

3.9 PCR conformation for the amplification of primers designed

3.9.1 Polymerase Chain Reaction:-

3.9.1.1 Instruments required

Thermocycler, microfuge tubes, auto pipettes of range 2-200µL, 20 - 200µL and 200-1000µL, electrophoresis unit with power supply, deep freezer of –20 ^{0}C, refrigerator, and laminar airflow.

3.9.1.2 Reagents

1. Reaction buffer (10X)

   750 mM Tris-HCL pH 8.8 at 25C
   200 mM $(NH_4)_2\ SO_4$
   0.1 % Tween 20
   15 mM $MgCl_2$

2. Primers (Farward and Reverse) – stock 5 pM
3. Taq DNA polymerase –Stock 3 U/µl

Table-3: List of primers used to study gene expression during water stress and control conditions

Gene name	**Product size (bp)**	**Primer**	**Primer sequence**
Catalase	156	F	5^{1}-CCATCGTCACTGGCAAGCGTGAG-3^{1}
		R	51-GGCTCATAGCTGACCTTTGGGTG-3^{1}
Peroxidase	147	F	5^{1}-5^{1}GAGGTTGTCAGCACGAATGGC-3^{1}
		R	5^{1}-CACACTGTGGCATCATCAGTTGG-3^{1}
bZIP	135	F	5^{1}-CAAGCTCGTCGAGATGGCAATGG-3^{1}
		R	5^{1}-GGCAATGTCTGCACCTTGTGTTCC-3^{1}
Elongation factor	183	F	5^{1}-CCATGAAGCTCTCCAAGAAGCCG-3^{1}
		R	5^{1}-CATTGCTAATTTGGCCTGGGTGG-3^{1}
Heat shock protein	193	F	5^{1}-CAGCGAACAAAGCGGTCTCAAAGG-3^{1}
		R	5^{1}-GGGCAGGTACGACATCAACTTCC-3^{1}
Rubisco activase	152	F	5^{1}-CGAAGTGAGGAAGTGGGTGGAAAC-3^{1}
		R	5^{1}-GCTCCTGTTCCTTCACCAGCATG-3^{1}
Mitotic inhibitionf actor	114	F	5^{1}-CGGATGAATGAAGCTAGAGGCGGC-3^{1}
		R	5^{1}-GGGCGACACTGTCACTTTGGTTTC-3^{1}
RNA polymerase	183	F	5^{1}-GCGTCTCTTATCGGGCAATCCG-3^{1}
		R	5^{1}-GCGGGTATAATCCCACCCAGAAC-3^{1}
Chaperon D K	191	F	5^{1}-GTTCAGACCTCCTTGACAGGCTC-3^{1}
		R	5^{1}-GGCAACAACCTCATCAGGGTGAC-3^{1}

4. Template cDNA – Stock 50 ng/µl
5. dNTPs – Stock 2 mM
6. Bromophenol Blue- Stock solution: 0.25% in 50 % glycerol Working solution: 200µl stock + 200µl of 50% glycerol.

3.9.1.3 PROTOCOL

1. The Thermocycler was switched on at least 15 minutes earlier.
2. The master mix was prepared for samples of DNA and cDNA and 20µl of the reaction mixture was prepared for earlier amplified four primers (Peroxidase, Rubisco activase, Mitotic inhibition factor and RNA polymerase) by adding the following components.

   Reaction buffer (10X) - 2µl
   Farward Primer -2µl
   Reverse Primer- 2µl
   DNTPs (1mM)-2µl
   Taq DNA polymerase (3U/µl)- 0.33µl
   Template cDNA (50 ng/µl)- 2µl
   Water (20- (2+2+2+2+0.33+2))- 9.67µl
   Total reaction mixture - 20µl.
3. 18µl of the master mix was transferred to sterile tubes.
4. 2µl of cDNA (control and stressed) of Calcutta-4 and Bee hee kela samples was added to different tubes and centrifuged at top speed for 15 seconds.
5. The tubes were placed firmly in the wells of the Thermocycler and the following temperature programme was set.

   Step 1- 94 ^{0}C for 3 min [Denaturation]
   Step 2 – 94 ^{0}C for 1 min [Denaturation]
   Step 3 – 55 ^{0}C for 1 min [Annealation]
   Step 4 – 72 ^{0}C for 1 min [Extention]
   Step 5- repeat steps 2 to 4: 30 times
   Step 6 – 72 ^{0}C for 5 min [Extended polymerization]
   Step 7- refrigerate at 4 ^{0}C.

At the end of the run the tubes were taken out, 2.5µl of diluted bromophenol blue was added and spun for 2-5 seconds at top speed in microfuge. The tubes were stored at 4 ^{0}C till electrophoresis.

3.9.1.4 Electrophoresis and visualization of amplified products

20 µl of the amplified product was run along with 100bp ladder on 1.5 % agarose gel and visualized under UV light.

Electrophoresis is a widely used technique to separate macromolecules and to test the homogeneity of nucleic acids and proteins. Separation is based on the electrical charge carried by the compound. When these charged molecules are subjected to an electrical field they move towards opposite charge. DNA molecules carry a net negative charge and therefore when placed in an electrical field, they migrate towards the positive electrode (anode). Electrophoresis is usually carried out in solid matrix like agarose or polyacrylamide gels. In a gel, the shape and size of the DNA fragment to be separated and the concentration of the agarose used will influence the migration rate. Smaller the DNA fragment, higher the concentration of the agarose to be used in the gel for good separation. The DNA fragments are resolved based on their molecular weight using agarose gel electrophoresis. The size of the fragment is determined by comparing mobility of DNA fragments of known size and mobility, such as 100 base pair DNA ladder (marker).

Materials required

Electrophoresis gel unit, Power pack

3.9.1.5 Reagents required

1. Agarose powder
2. 1X TBE buffer
3. Ethidium bromide
4. Bromophenol dye

3.9.1.6 Casting of agarose gel:

Prepared 0.8 % agarose solution in 1X TBE buffer for 30ml [1.5% agarose solution]. Heated to dissolve completely. Cooled to 40°C, add ethidium bromide solution (0.5 μg/ml), poured into the cast.

3.9.1.7 Running the gel

Pipette out 20 μl of the above DNA solution into a microfuge tube. To that add 1.0 μl of bromophenol dye and centrifuged at top speed for a few seconds in order to mix them well.

1. Filled the gel tray with 0.5X TAE buffer (running buffer) and loaded the samples using 20 μl pipettes.
2. Ran the gel at 50 volts for one and a half to two hours.
3. Allowed the bromophenol blue dye to travel less than 2/3rd of the length of the gel, before removing the slab
4. Examined the slab under UV Light

4.10 Quantification of band intensity:

The concentration of band intensity for amplified four primers (Peroxidase,Rubisco activase, Mitotic inhibition factor and RNA polymerase) in control and stressed samples of Bee hee kela and Calcutta-4 was calculated by comparing 100bp ladder molecular weight and its concentration by using Gene analysis software(Syngene company, Genius model).

Experimental Results

IV. RESULTS

Water availability is perhaps the most important constraint to achieve potential crop productivity. Crop losses to water shortage may exceed those from all other causes combined. Sustaining productivity under water limited conditions and saving irrigation water are the two most important aspects that need to be addressed immediately if agriculture is to feed the burgeoning population of the country.

Though plants have naturally evolved several stress adaptive strategies most of them pertain to the survival of plants under stress. However, from agricultural point of view, any stress adaptation strategy would be use full only if it is associated with superior crop growth rates under a given stressful environment.

The present investigation carried out on the behaviour of banana to water stress that helps to understand a few physiological, biochemical and molecular aspects during irrigated and water deficit conditions. The results obtained in this study are presented below.

4.1.1 Screening of banana genotypes for the Leaf Water Retention capacity and water use efficiency:

The screening of fifteen genotypes belonging to AA genomic group and thirteen genotypes belonging to BB genomic group using leaf water retention capacity showed that, values for AA genotypes were ranged from 57.03 to 81.75 per cent and the values for BB genotypes ranged from 71.5 to 83.44 per cent. The average leaf water retention capacity of AA and BB genotypes were 69.3 per cent and 77.73 per cent, respectively where as the values on $\Delta^{13}C$ for AA genotypes were ranged from15.91 to 21.03 and for BB genotypes the values were ranged from 17.13 to 20.93. The average $\Delta^{13}C$ values for AA and BB genotypes were 17.86 and 18.6 respectively.

Correlation between per cent LWRC and $\Delta^{13}C$ values are Non-significant (at P=0.05) for both AA and BB genotypes, but per cent LWRC and $\Delta^{13}C$ values for AA genotypes are positively correlating with each other where as in BB genotypes it is negatively correlating with each other. (The per cent LWRC and $\Delta^{13}C$ values are presented in Table-4 and Figure-1)

Based on present and earlier experiments two genotypes Bee hee kela belonging to BB genome and Calcutta-4 belonging to AA genome were selected as tolerant and susceptible genotypes for further study.

4.2.1 Net Photosynthetic rate (P_N):

The observations recorded on photosynthesis rates under well watered and water stressed conditions showed significant variations. Average photosynthetic rates of irrigated and stressed plants of Calcutta-4 genotype showed to be 8.76 ± 0.41 and 4.75 ± 0.87 μ mol m^{-2} sec^{-1}, respectively, and values for Bee hee kela (BB) under well watered and stressed conditions showed 12.03 ±1.67 and 7.70 ± 0.6 μ mol m^{-2} sec^{-1}, respectively (Table-5).

Under water stressed conditions the genotype Culcutta-4 (AA) showed high per cent reduction in photosynthetic rate (45.77 per cent) when compared with the genotype Bee hee kela (BB) (36.01 per cent) (Figure-2a).

4.2.2 Transpiration rate (E):

Data on transpiration rates of two genotypes Bee hee kela and Calcutta-4 under irrigated and water stressed conditions exhibited differences, the genotype Bee hee kela (BB) exhibited transpiration rates of 12.30 ± 0.35 m mol m^{-2} sec^{-1} and 9.55 ± 0.15 m mol m-^{2} sec^{-1}, under irrigated and water stressed conditions, respectively. The genotype Calcutta-4 noticed transpiration rates of 11.97 ± 0.13 m mol m^{-2} sec^{-1}

Table-4: Per cent leaf water retention capacity and Δ^{13}C of AA and BB genotypes

Genome	Genotype	Per cent LWRC	Average LWRC (per cent)	Δ13C Value	Average Δ13C
AA	*Musa acuminata* Wild	81.75	69.3±1.87	19.275	17.86 ±0.34
	Matti	78.67		18.283	
	Sanna chen kadali	76.94		16.916	
	Anaikomban	76.27		17.255	
	Kadali	71.90		17.293	
	M. acuminata. ssp *burmaniceoides* (Calcutta-4)	71.74		16.418	
	Erachi vazhai	71.10		17.763	
	Annara kannan	67.84		21.032	
	Pisang linin	67.51		19.140	
	CV Rose	66.82		18.626	
	Pisang jari buya	66.23		15.905	
	Tongat	65.31		18.132	
	Madhu sagar	61.39		16.913	
	Musa acuminata ssp. *banksii*	60.45		18.483	
	Musa acuminata ssp *malaccensis*	57.03		16.512	
BB	Amtur kela	83.44	77.73 ± 1.23	17.820	18.6 ± 0.29
	Ram kela	82.39		17.731	
	Wild-2	82.19		19.136	
	Musa ssp *tani*	82.14		18.627	
	Musa balbisiana-1	81.13		18.724	
	Fruit less variety	80.22		18.467	
	*Musa balbisi*ana-2	76.17		19.227	
	Sirsi wild	76.16		17.825	
	Bee hee kela	75.85		19.598	
	Musa species	75.54		17.127	
	Betta bale	72.13		17.320	
	Bhimaitia	71.68		20.927	
	Wild-5	71.50		19.280	

Figure-1: Average leaf water retention capacity (per cent) and $\Delta^{13}C$ values for AA and BB genotypes.

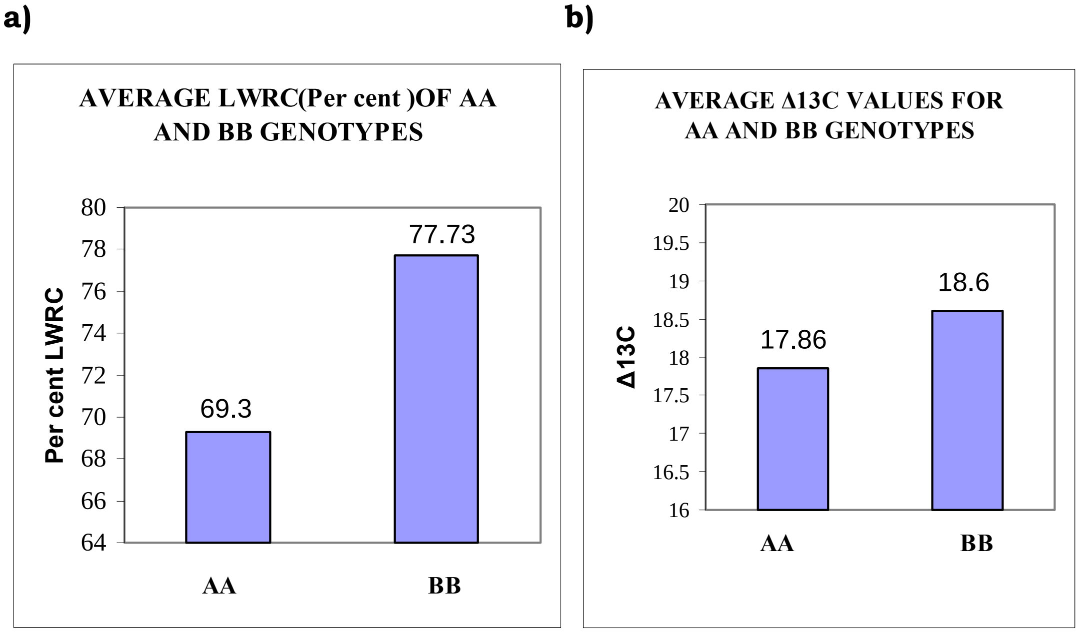
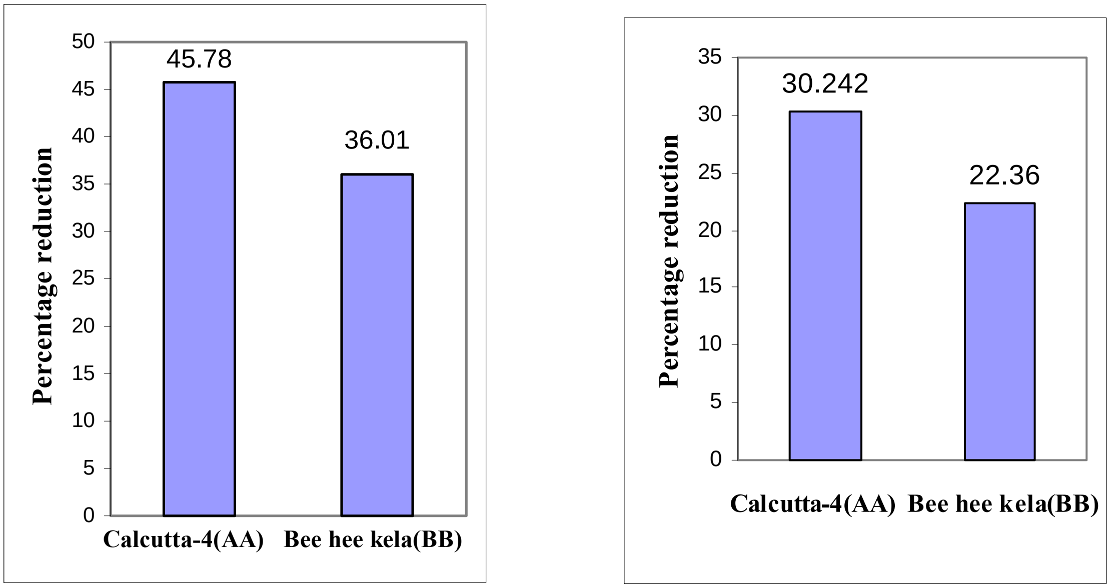


Table: 5. Gas exchange characteristics in two banana genotypes under well watered and stress conditions.

Genotype	**Treatments**	**P_N (µ mol m^{-2} sec^{-1})**	**E (m mol m^{-2} sec^{-1})**	**gs (m mol m^{-2} sec^{-1})**
Calcutta-4 (AA)	Well watered	8.76	11.97	866
	Water stressed	4.75	8.35	343
Bee hee kela (BB)	Well watered	12.03	12.3	1060
	Water stressed	7.70	9.55	465

P_N: Net photosynthetic rate
E: Transpiration rate
Gs: Stomatal conductance

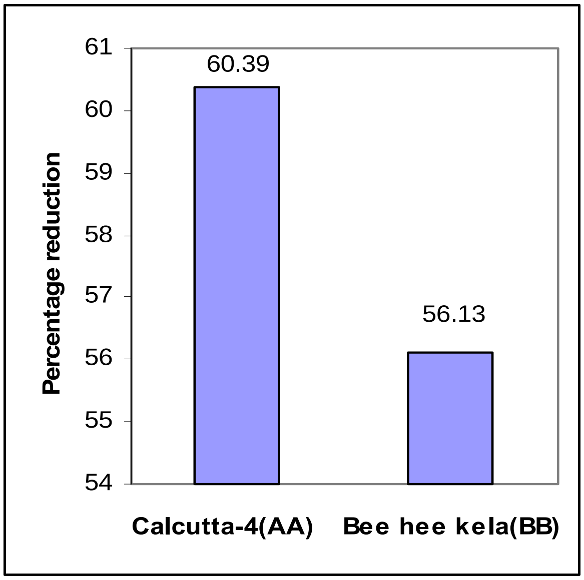
Figure-2: Per cent reduction in gas exchange characteristics of banana genotypes due to water stress



a) Photosynthetic rate

b) Transpiration rate

c) Stomatal conductance



and 8.35 ± 0.15 m mol m^{-2} sec^{-1} under irrigated and water stressed conditions, respectively (Table-5).

The per cent reduction in the rate of transpiration was noticed to be high for the genotype Calcutta-4 (30.24 per cent) and low for the genotype Bee hee kela (22.36 per cent) under water stressed conditions (Figure -2b).

4.2.3 Stomatal conductance (gs):

Effect of water stress showed significant changes in the stomatal conductance of both the banana genotypes. The stomatal conductance of the genotype calcutta-4 under well watered and water stressed conditions was shown to be 866 ± 18.87 m mol m^{-2} sec^{-1} and 343 ± 49.1 m mol m^{-2} sec^{-1}, respectively. The genotype Bee hee kela recorded stomatal conductance of 1060 ± 180 m mol m^{-2} sec^{-1} and 465 ± 5 m mol m^{-2} sec^{-1} under well watered and water stressed conditions, respectively (Table-5).

Per cent reduction in stomatal conductance (gs) after twelve days stress was recorded high for the genotype Calcutta-4 (60.39 per cent) genome when compared with Bee hee kela (56.13 per cent) (Figure-2c).

4.2.4 Leaf water potential (Ψ):

From the data obtained on leaf water potential showed differences in two genotypes of banana, the genotype Bee hee kela recorded water potentials of -0.930 (MPa) and -1.518 (MPa) under irrigated and water stressed conditions and the genotype Calcutta-4 noticed water potentials of -1.350 (MPa) and -1.824 (MPa) under irrigated and water stressed conditions (Table 6a).

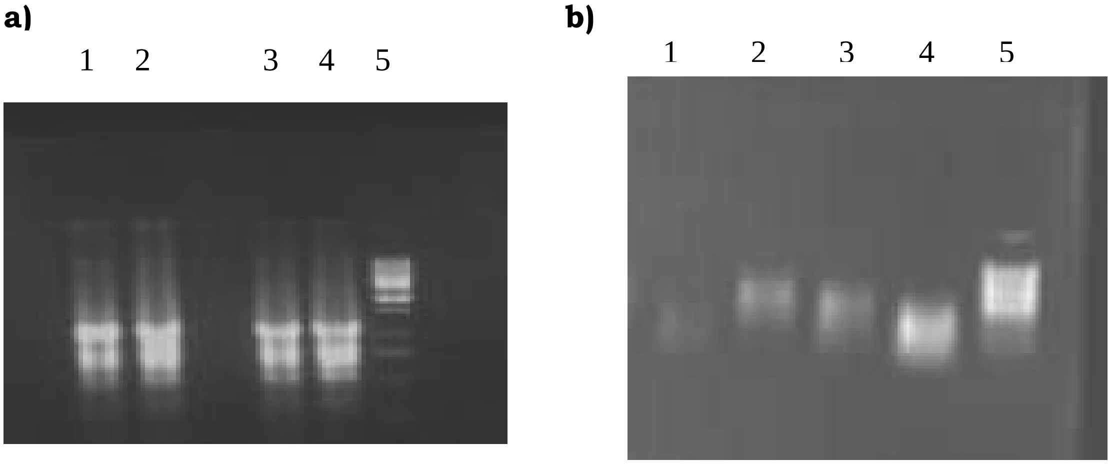
Table: 6 a) Leaf water potential changes in watered and stressed banana genotypes

Genotype	Treatments	Leaf water potential (Mpa)
Calcutta-4 (AA)	Well watered	-1.35
	Water stressed	-1.824
Bee hee kela (BB)	Well watered	-0.913
	Water stressed	-1.518

b) Per cent soil moisture content in control and stressed structures

Treatments	Per cent soil moisture content
Well watered	18.97
Water stressed	13.2

4.2.5 Soil moisture content: Soil moisture content after twelve days of withholding irrigation was 13.2 per cent in case of stressed structures as compared to 18.97 per cent in case of well-watered structures (Table 6b).



Total RNA

cDNA

Plate-4:

a) Gel profile showing good quality RNA isolated from well watered and stressed banana leaf samples

b) Gel profile showing cDNA of well watered and stressed banana leaf samples

Description:

1. Calcutta-4 control
2. Calcutta-4 stressed
3. Bee hee kela control
4. Bee hee kela stressed
5. Ladder

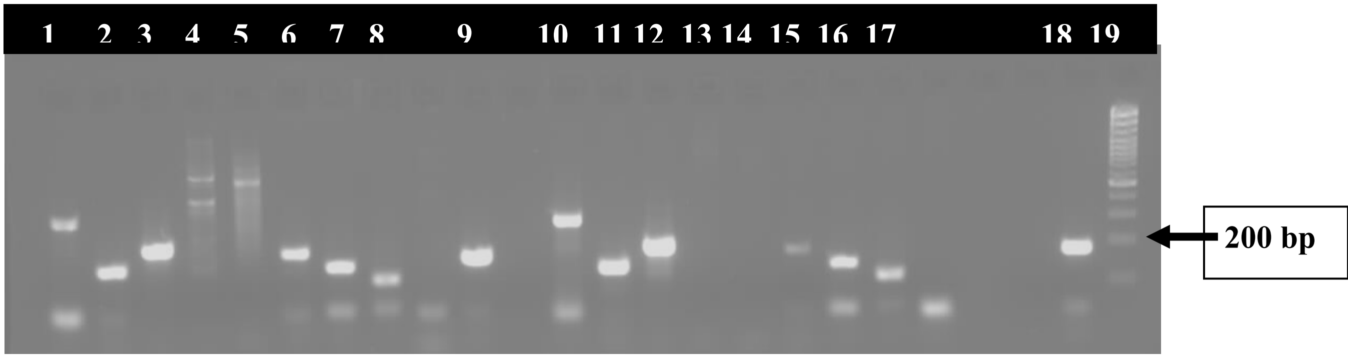


Plate-5: Gel profile showing the amplification of 9 primers using genomic DNA of Calcutta-4 and Bee hee kela.

Description:

Product length of primers: Less than 200 bp

1-9 (Calcutta-4), 10-18 (Bee hee kela)

Peroxidase (Amplified) (1, 10)
Catalase (Not amplified) (2, 11)
bZIP (Not Amplified) (3, 12)
Elongation factor (Not amplified) (4, 13)
Heat shock protein (Not amplified) (5, 14)
Rubisco activase (Amplified (6, 15)
Mitotic inhibition factor (Amplified) (7, 16)
Chaperon (Not amplified) (8, 17)
RNA polymerase (Amplified) (9, 18)

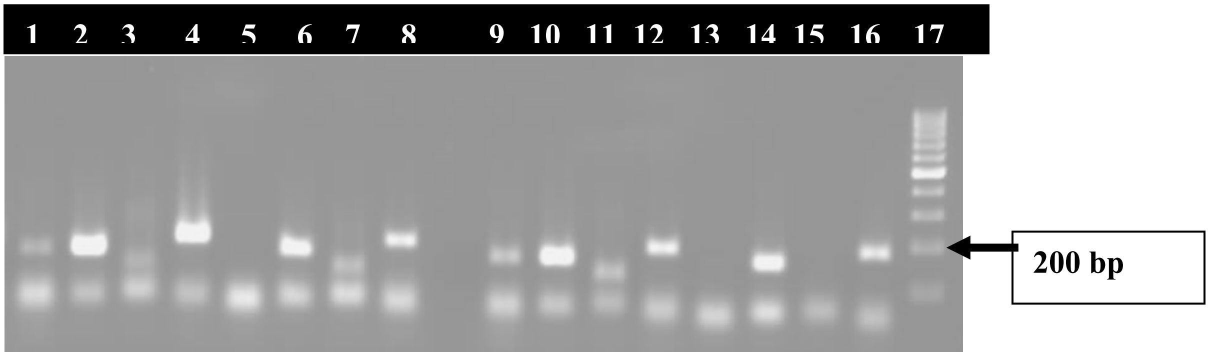


Plate-6: Gel profile showing PCR amplified products using from control and stressed plants.

Description:

1-4 (Control cDNA (Bee hee kela), 5-8 (stressed cDNA (Bee hee kela), 9-12 (Control cDNA (Calcutta-4), 13-16 (stressed cDNA (Calcutta-4).

Peroxidase (1, 5, 9 and 13)
Rubisco activase (2,6,10 and14)
Mitotic inhibition factor (3,7,11 and 15)
RNA polymerase (4,8,12 and16)
100bp Ladder (17)

4.3 Biochemical analysis:

4.3.1 Peroxidation of membrane lipids:

4.3.1.1 Malondialdehyde content: The data on malondialdehyde content, which shows the extent of lipid peroxidation of membranes, in Calcutta-4 OD change at 532 nm was 0.2431 and 0.4540 for watered and stressed leaf samples, respectively. In Bee hee kela was 0.1983 and 0.3436 under watered and stressed conditions respectively (Figure-3c).

In Calcutta-4 OD change between watered and stressed plants was 0.2109 but in Bee hee kela it was 0.1453, indicating that membrane damage was high in Calcutta-4 than Bee hee kela.

4.3.2 Super oxide dismutase (SOD) activity under drought:

The data on the activity of SOD in two genotypes of banana showed that, under water stressed condition the enzyme activity was high in Bee hee kela (30.593 U gm^{-1} FW) when compared with Calcutta-4 (22.945 U gm^{-1} FW). The activity of SOD between control and stressed plants was compared, there was an increase of more than two fold in Bee hee kela (BB) as compared to less than 1.5 fold in Calcutta-4 (AA).
(Table-7, Figure-3a)

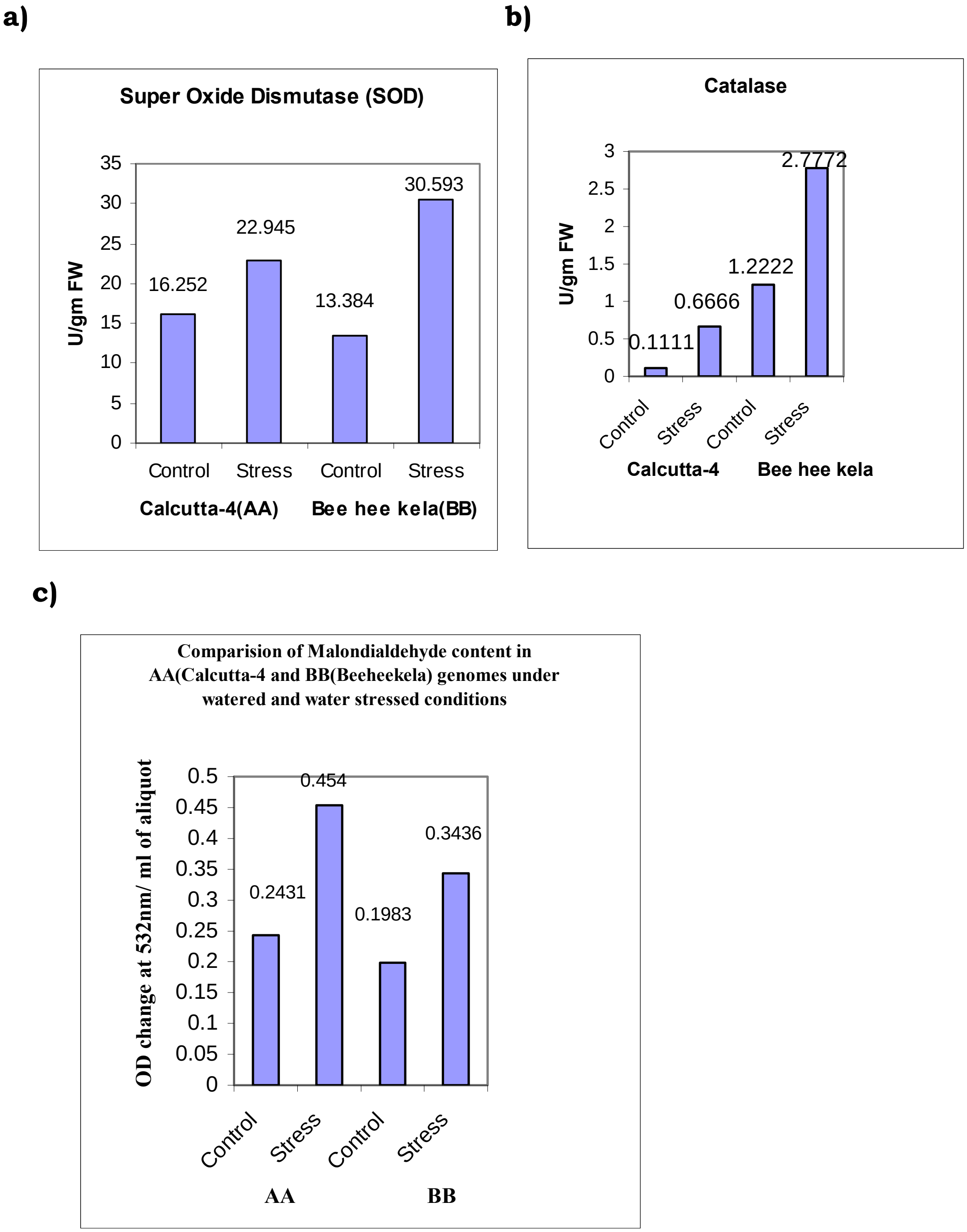
4.3.3 Catalase activity:

The results obtained on the activity of catalase showed that, there was more than two fold increase in the activity between control and stressed plants of both Calcutta-4 and Bee hee kela but this was recorded higher activity (2.7772 U gm^{-1} FW) than that of Calcutta-4 (0.6666 U gm^{-1} FW) under water stressed conditions (Table-7, Figure-3b).

Table-7: The activity of Catalase and super oxide dismutase in banana genotypes under well watered and stressed conditions.

Genotype	**Genomic group**	**Treatments**	**Catalase activity (U gm^{-1} FW)**	**SOD activity (U gm^{-1} FW)**
Calcutta-4	AA	Well watered	0.3201	16.252
		Water stressed	0.6666	22.945
Bee hee kela	BB	Well watered	1.2222	13.384
		Water stressed	2.7772	30.593

Figure-3: Activity of Super oxide dismutase, Catalase enzymes and Content of Malondialdehyde depicting extent of lipid peroxidation in banana genotypes under drought.



4.4 Isolation of RNA from leaf samples of watered and stressed Banana plants:

Total RNA was isolated from the leaf samples following the protocol outlined in the Materials and Methods (3.5.2). Isolated RNA was checked for integrity after each isolation by denaturing gel electrophoresis containing formaldehyde (Plate-4a).

Yield of RNA obtained was found to be in the range of 22.1-33.3 µg g^{-1} of leaf tissue. Quality of RNA was found to be good with an A_{260}/A_{280} ratio around 1.87-1.95 (Table-8).

4.5 cDNA synthesis: Here the two step RT-PCR reaction was followed as mentioned in the Materials and Methods (3.7), because the oligo-dT primers constitutes Thiamine residues (9 mers) it got bind to the mRNA poly A tail and the Reverse transcription enzyme converted it to single stranded cDNA in the first step and we were used this cDNA as template for the confirmation of amplification of the designed primers (Table-2) in the next step. The gel profile showing cDNA is shown in the Plate 4b.

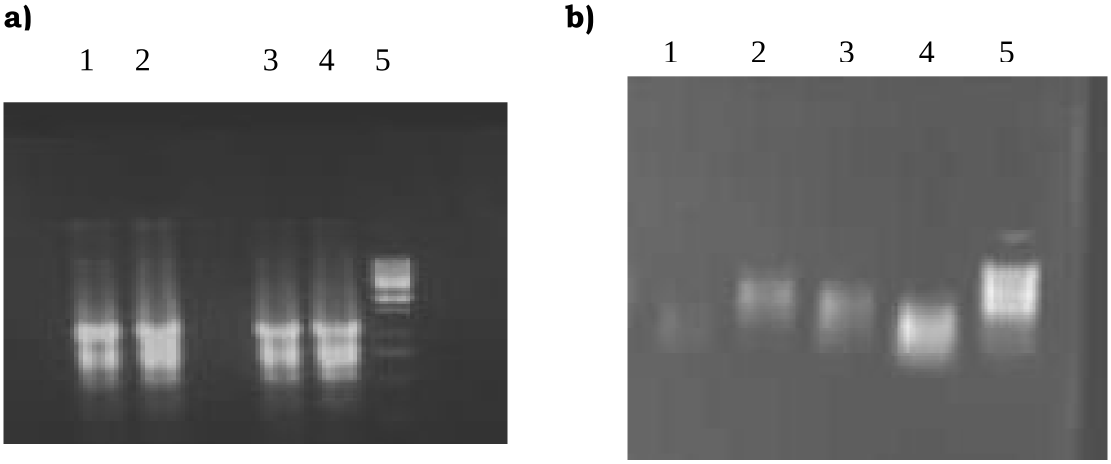
4.6 PCR conformation for the amplification of designed primers in cDNA pool:

The PCR was carried out by using cDNA as a template, by following the Materials and Methods (3.9).

The gel profile showed in Plate-5 was done by using genomic DNA of our experimental material to screen for the designed primers, out of nine primers used, four were amplified namely Peroxidase, Rubisco activase, Mitotic inhibition factor and RNA polymerase. Among nine primers five were drought related (Peroxidase, catalase, bZIP, Chaperon and Heat shock protein) out of these only one primer (Peroxidase) amplified in genomic DNA and others are related to growth, metabolism and gene regulation. After screening with genomic DNA the above-

Table-8: Quantification of RNA samples isolated from watered and stressed leaf samples of banana genotypes.

		A_{260}	**A_{280}**			
Treatments	**Genotypes**	**A_1**	**A_2**	**A_1/A_2**	**µg/µl**	**µg / g of leaf tissue**
Control	Calcutta-4	0.122	0.066	1.95	2.3	30.666
	Bee hee kela	0.125	0.066	1.90	2.5	33.333
Water Stressed	Calcutta-4	0.085	0.045	1.93	1.66	22.133
	Bee hee kela	0.096	0.053	1.87	1.66	24.533



Total RNA

cDNA

Plate-4:

a) Gel profile showing good quality RNA isolated from well watered and stressed banana leaf samples

b) Gel profile showing cDNA of well watered and stressed banana leaf samples

Description:

1. Calcutta-4 control
2. Calcutta-4 stressed
3. Bee hee kela control
4. Bee hee kela stressed
5. Ladder

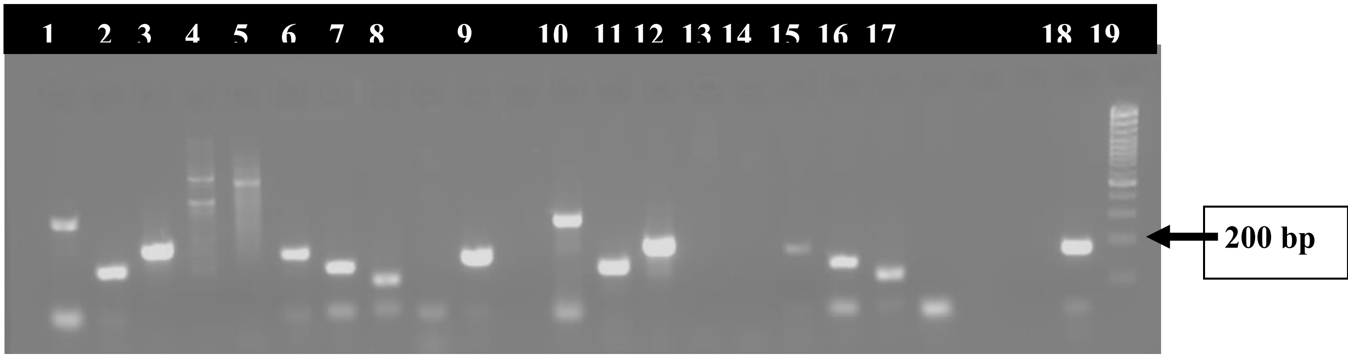


Plate-5: Gel profile showing the amplification of 9 primers using genomic DNA of Calcutta-4 and Bee hee kela.

Description:

Product length of primers: Less than 200 bp

1-9 (Calcutta-4), 10-18 (Bee hee kela)

Peroxidase (Amplified) (1, 10)

Catalase (Not amplified) (2, 11)

bZIP (Not Amplified) (3, 12)

Elongation factor (Not amplified) (4, 13)

Heat shock protein (Not amplified) (5, 14)

Rubisco activase (Amplified (6, 15)

Mitotic inhibition factor (Amplified) (7, 16)

Chaperon (Not amplified) (8, 17)

RNA polymerase (Amplified) (9, 18)

amplified four primers were used for amplification using cDNA from well watered and stressed samples. Here Peroxidase was amplified in control samples but not found in stressed samples but other three (Rubisco activase, Mitotic inhibition factor and RNA polymerase) primers showed amplification in all watered and stressed cDNA pool. The PCR reactions were carried out for different annealing temperatures for different primers but finally above-mentioned four primers got amplification at 55^0 C annealing temperature. The gel profile showing amplification of four primers using cDNA is shown in Plate 6.

4.7 Quantification of band intensity for the PCR amplified primers:

The values on concentrations of band intensity produced by four PCR amplified primers (Peroxidase, Rubisco activase, Mitotic inhibition factor and RNA polymerase) were calculated using Gene analysis (Syngene company Genius model) software showed that:

1. The concentration of band intensity for Peroxidase was 28.57 ng in control sample of Calcutta-4 and band was absent in stressed sample and it was 21.69 ng in control sample of Bee hee kela and band was absent in stressed sample.
2. The concentration of band intensity for Rubisco activase was 76.87 ng and 63.51 ng in control and stressed samples of Calcutta-4 respectively and it was 92.80 ng and 64.31 ng in control and stressed samples of Bee hee kela respectively. The per cent reduction in concentration of Rubisco activase between control and stressed samples was lower in Calcutta-4 (17.4 per cent) when compared with Bee hee kela (30.7 per cent).
3. The concentration of band intensity for Mitotic inhibition factor was 21.26 ng and 29.70 ng in control and stressed samples of Bee hee kela respectively and it was 29.80 ng in control sample of Calcutta-4 but band was absent in stressed sample.

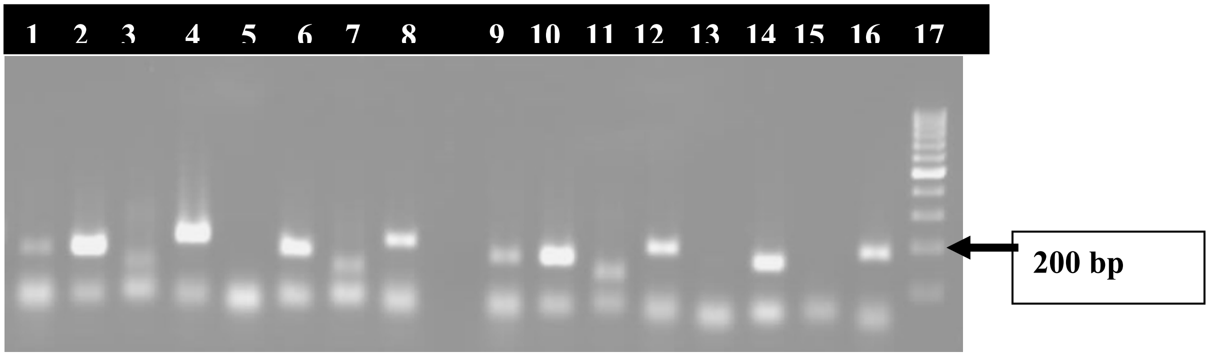


Plate-6: Gel profile showing PCR amplified products using from control and stressed plants.

Description:

1-4 (Control cDNA (Bee hee kela), 5-8 (stressed cDNA (Bee hee kela), 9-12 (Control cDNA (Calcutta-4), 13-16 (stressed cDNA (Calcutta-4).

Peroxidase (1, 5, 9 and 13)
Rubisco activase (2,6,10 and14)
Mitotic inhibition factor (3,7,11 and 15)
RNA polymerase (4,8,12 and16)
100bp Ladder (17)

4. The concentration of band intensity for RNA polymerase was 46.10 ng and 66.50 ng in control and stressed samples of Calcutta-4 respectively and it was 10.54 ng and 49.9 ng in control and stressed samples of Bee hee kela respectively. The per cent increase in concentration of RNA polymerase band intensity between control and stressed samples was lower in Calcutta-4 (30.7 per cent) when compared with Bee hee kela (78.8 per cent) (Table-9).

Table-9: Quantification of Band intensity for the PCR amplified primers in control and stressed samples in terms of nano-gram concentration using Gene analysis software

Primers	**Calcutta-4**			**Bee hee kela**		
	Control (ng)	Stress (ng)	Per cent change	Control (ng)	Stress (ng)	Per cent change
Peroxidase	28.57	Not amplified	-	21.69	Not amplified	-
Rubisco activase	76.87	63.51	- 17.4	92.80	64.31	- 30.70
Mitotic inhibition factor	29.80	Not amplified	-	21.26	29.70	+ 28.40
RNA polymerase	46.10	66.50	+ 30.70	10.54	49.90	+ 78.80

Discussion

V. DISCUSSION

Most climate change scenarios suggest an increasing pattern of aridity in many areas of the world, along with high temperature and radiation, posing most important environmental constraints for plant survival and productivity (Chaves *et al.*, 2003). A substantial amount of time and effort has gone in this field to understand plant strategies including plant biochemical and physiological processes underlying plant responses to water deficit. Recent trend has been to uncover the molecular processes involved in tolerance and resistance to stress. Nevertheless, there is a still insufficient knowledge on the physiological and molecular mechanisms underlying plant responses with in natural habitats, where complex interactions occur due to occurrence of multiple stresses simultaneously. (Mittler *et al.*, 2002)

Present investigation was carried out,

1. To study the physiological and biochemical changes due to moisture stress in banana.
2. To study the gene expression of a few selected candidate genes under moisture stress.

The experiments were conducted at IIHR fruit crops field by planting two contrasting genotypes Calcutta-4 (AA) and Bee hee kela (BB) in two structures built by cement bricks having three replications based on leaf water retention capacity and $\Delta^{13}C$ analysis of present and earlier experimental results. For one structure irrigation was with held 120 days after planting for a period of twelve days for stress treatment and other structure was maintained at 100 per cent field capacity.

Banana is an important fruit crop grown worldwide; it requires abundant and constant supplies of water (Popenoe, 1941). On the other hand, physiological investigations suggest that bananas are remarkably tolerant of soil water deficit (Kallarackal *et al.*, 1990) and transpirational loss of water is less than other crops (Lu *et al.*, 2002).

5.1 Screening of banana genotypes for the Leaf Water Retention Capacity and Water Use Efficiency:

In this study we have screened a few AA and BB genotypes for LWRC and also for $\Delta^{13}C$ analysis. As the per cent LWRC increases the ability of a genotype to withstand water-limiting conditions also gets increases. The BB genotypes showed better per cent LWRC (77.73 per cent) indicating their tolerance to water deficit conditions and AA genotypes showed lower per cent LWRC (69.3 per cent) indicating their susceptibility to drought. (Table-4, Figure-1)

The average values on $\Delta^{13}C$ shown to be little lower for AA genotypes (17.86) indicating higher WUE but low drought tolerance capacity but the BB genotypes showed little higher average value (18.6) indicating low WUE and high drought tolerance capacity. The statistical analysis showed that the correlation between per cent LWRC and $\Delta^{13}C$ for both AA and BB genotypes was non significant ($P=0.05$), but they showed positive and negative correlation in case of AA and BB genotypes respectively.

As the $\Delta^{13}C$ increases the water use efficiency decreases. As the WUE increases drought tolerance capacity decreases (Hubic *et al.,* 1998).

According to Bananuka *et al.* (1999) leaf water retention capacity of different cultivars exhibited different capacities to retain moisture after 48 hours of drying at room temperature. Cultivars having B (*M. balbisiana*) genomic composition showed low leaf water retention capacity than the cultivars having A (*M. acuminata*) genomic composition.

In present study in contrast to the above findings the genotypes having B (*M. balbisian*a) genomic composition showed high leaf water retention capacity after 48 hours of drying at room temperature as compared with the genotypes having genomic composition of A (*M. acuminata*) however our findings are on far with the statement, *M. bulbisiana* showed characteristic feature of drought tolerance capacity than *M. acuminata* (Stover and Simmonds, 1987).

5.2 Physiological studies:

In the present investigation the gas exchange parameters (Photosynthesis rate (P_N), Transpiration rate (E) and Stomatal conductance (gs)) were recorded by using portable photosynthetic system (ADC company, Model LCA-3). The leaf water potential was determined using dew point micro voltmeter (Wescor, USA. Model HR 33T).

Average photosynthetic rates of irrigated and stressed plants of Calcutta-4 genotype showed to be 8.76 ± 0.41 and 4.75 ± 0.87 μ mol m^{-2} sec^{-1}, respectively, and values for Bee hee kela (BB) under well watered and stressed conditions showed 12.03 ±1.67 and 7.70 ± 0.6 μ mol m^{-2} sec^{-1}, respectively (Table-5).

The per cent reduction in photosynthesis rate was minimal for Bee hee kela (BB) (35.01 per cent) than the Calcutta-4 (AA) (45.77 per cent) under water limiting conditions indicates, the susceptibility of Calcutta-4 and tolerance of Bee hee kela genotypes under water stressed conditions (Figure-2a).

According to Bananuka *et al.* (1999) reduction in assimilation rate of more than 50 per cent was recorded in all cultivars, with exception of 35.40 per cent in case of French Plantain (AAB) by the end of 4th week after with holding irrigation. Cultivars Lep Chang Kut (BBB) and Nfuuka (AAA-EA) recorded the highest reduction in assimilation rate (93.1 and 73.8 per cent), respectively. Cultivars

French Plantain (AAB), Gros Michel (AAA) and Sukalindizi (AB) recorded and maintained significantly ($P \leq 0.05$) low rates of reduction in assimilation rate, indicating their resistance to drought stress. This indicates the better performance of *M. acuminata* genotypes than *M. bulbisiana* genotypes.

Above findings are contradictory to the present results on photosynthesis rates of two genotypes, indicating Bee hee kela (BB) can maintain high photosynthesis rate even under water stressed environments when compared with Calcutta-4 (AA) it may be due to lower sensitivity of stomatal conductance and net photosynthesis to leaf to air vapour pressure difference of cultivars containing more B genomes A genomes, is consistent with the view that the B genome contributes to drought tolerance in *Musa sp* (Thomas *et al.*, 1998).

From the above findings we can conclude that the genotypes having genomic composition of both *A* and *B* will provide better tolerance to drought along with improved yield potential.

Drought stress reduced per cent transpiration rate in all cultivars. Cultivar FHIA-02 (AAAA) showed high per cent reduction in transpiration rate (67.3 per cent), while cultivar Nfuuka (AAA-EA) indicated an increase in transpiration rate (52.2 per cent at 4th week) with increasing water stress (Bananuka *et al.,* 1999).

The genotype Bee hee kela (BB) exhibited transpiration rates of 12.30 ± 0.35 m mol m^{-2} sec^{-1} and 9.55 ± 0.15 m mol m^{-2} sec^{-1}, under irrigated and water stressed conditions, respectively. The genotype Calcutta-4 (AA) noticed transpiration rates of 11.97 ± 0.13 m mol m^{-2} sec^{-1} and 8.35 ± 0.15 m mol m^{-2} sec^{-1} under irrigated and water stressed conditions, respectively (Table-5).

In the present study also per cent reduction in transpiration rate was occurred in both the genotypes but it was high for Calcutta-4 (30.24 per cent) indicating its susceptibility to water deficit conditions but the genotype Bee hee kela (22.36 per cent) showed less per cent reduction in transpiration rate indicating its tolerance to water limiting conditions (Figure-2b).

According to Mohmood *et al.* (2000), Stomatal conductance was sensitive to the onset of water deficit, the stomatal conductance of stressed banana plants grown under protected structure, started to decline as soon as the water stress was imposed (after two days) and continued to decline rapidly up to day 20 where it displayed complete stomatal closure (From 500 m mol m^{-2} sec^{-1} to almost zero m mol m^{-2} sec^{-1}). Stressed plants under field condition started to decline on the onset of water stress treatments and remained almost lower than the control plants through out the experiment, the marked differences found at severe drought conditions (two months after with holding irrigation).

According to Bananuka *et al.* (1999), The drought stress influenced significant reduction in stomatal conductance in all cultivars studied during the second and third week of stress. After the third week, cultivar Nfuuka (AAA-EA) maintained a higher stomatal conductance than the other cultivars until the fourth week when all cultivars showed a sudden fall. The maintenance of high stomatal conductance by Nfuuka explains its increase in transpiration rate.

In the present study also the stomatal conductance of stressed plants was lower when compared with control plants. The stomatal conductance of Calcutta-4 was 866 (m mol m^{-2} sec^{-1}) in control plants where as in stressed plants (12 Days after with holding irrigation) it was 343 (m mol m^{-2} sec^{-1}) and in Bee hee kela 1060 (m mol m^{-2} sec^{-1}) in control plants and 465 (m mol m^{-2} sec^{-1}) in stressed plants. Per cent reduction in stomatal conductance was more in Calcutta-4(60.39 per cent) than Bee hee kela (56.13 per cent) indicating stomatal conductance in Bee hee kela was less affected compared to Calcutta-4 under water stress conditions (Figure-2c, Table-5).

In the present study also noticed that the stomatal conductance of BB genomic group (Bee hee kela) was higher than AA genomic group and this was correlating with the $\Delta^{13}C$ of BB genotypes this is fine and it was expected and it proves the drought tolerance capacity of BB genomic group is better than AA genomic group.

Leaf water potentials was ranged from –0.1 to –0.5 Mpa occur in banana plants cv. Grand Nain (AAA, Cavendish sub group) where the soil had dried sufficiently to stop leaf emergence and leaf gas exchange (Kallarackal *et al.*, 1990).

In the present study genotype Bee hee kela (BB) recorded water potentials of -0.930 (MPa) and -1.518 (MPa) under irrigated and water stressed conditions respectively and for genotype calcutta-4 recorded water potentials of -1.350 (MPa) and -1.824 (MPa) under irrigated and water stressed conditions respectively (Table-6a).

Calcutta-4 (AA) showed lower leaf water potential compared to Bee hee kela under water stress conditions, indicating that Bee hee kela has got capacity to maintain higher water potential even under water stress conditions.

Soil moisture content after twelve days of withholding irrigation was 13.2 per cent in case of stressed structures as compared to 18.97 per cent in case of well-watered structures (Table- 6b).

5.3 Biochemical analysis:

Drought induces oxidative stress which in turn induces the production of Reactive Oxygen Species (ROS) such as super oxide radical (O_2^-), Hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), all of these activated oxygen species are extremely reactive and cytotoxic which can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in the plasma lemma or intercellular organells. The lipid peroxidation of membranes leads to the leakage of cellular contents, rapid desiccation and cell death, Lipid peroxidation process yields a mixture of reactive carbonyl compounds and Malondialdehyde is one such carbonyl product that can be used to guage the relative extent of lipid peroxidation.

To prevent or alleviate injuries from Reactive Oxygen Species (ROS), plants have evolved an antioxidant defense system that includes non-enzymic compounds like ascorbate, glutathione, tocopherol, carotenoids, flavonoids and enzymes such as superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POX), ascorbate peroxidase (APX), Gluthatione reductase (GR) and Polyphenol oxidase (PPO) (Agarwal and Pandey, 2004). Co-operation among these enzymes is essential for the effective protection from ROS (Scebba *et al.,* 1998).

In the present study we made an attempt to quantify the Malondialdehyde content, activity of Super oxide dismutase and Catalase enzymes in two genotypes of banana under water stressed condition.

As OD change at 532nm increases the Malondialdehyde content also gets increases, in Calcutta-4 OD change at 532 nm was 0.2431 and 0.4540 for watered and stressed leaf samples, respectively. In Bee hee kela it was 0.1983 and 0.3436 under watered and stressed conditions (Figure-3c). In culcutta-4 OD change between watered and stressed plants was 0.2109 but in Bee hee kela it was 0.1453, indicating that membrane damage was high in Calcutta-4 than Bee hee kela.

Under water stressed condition the enzyme Super Oxide Dismutase (SOD) activity was high in Bee hee kela (30.593 U gm^{-1} FW) when compared with Calcutta-4 (22.945 U gm^{-1} FW). The activity of SOD between control and stressed plants was compared, there was an increase of more than two fold in Bee hee kela (BB) as compared to less than 1.5 fold in Calcutta-4 (AA) (Table-7, figure-3a).

Under watered condition Catalase activity was high in Bee hee kela (1.2222 U gm^{-1} FW) than Calcutta-4 (0.3201 U gm^{-1} FW), Catalase activity was increased with increasing water stress in both the genotypes but the activity of Catalase under stress was high in case of Bee hee kela (2.7772 U gm^{-1} FW) when compared to Calcutta-4 (0.6666 U gm^{-1} FW) (Table-7, Figure-3b).

According to Fazeli *et al.* (2007), MDA content in stressed leaf samples of *Sesamum indicum*, cultivar Darab-14 (8.23 n mol gm^{-1} FW) was higher than the stressed leaf samples of the cultivar Yekta (6.00 n mol gm^{-1} FW). The lower level of lipid peroxidation in leaves of Yekta suggests that, this cultivar is better protected from oxidative damage under drought stress than Darab - 14. Super oxide dismutase (SOD) activity of the cultivar Yekta was higher (25.42 U gm^{-1} FW) in stressed leaf samples than Darab-14 (17.30 U gm^{-1} FW) The sesame leaf catalase activity noticed higher in case of Yekta (8.33 U gm^{-1} FW) when compared with Darab-14 (5.66 Ugm^{-1} FW) under drought conditions indicating cultivar Yekta has got better defense mechanism than Darab-14 under stressed conditions.

Reactive oxygen species are responsible for stress-dependent peroxidation of membrane lipids (Ratnayaka *et al.,* 2003, Upadhyaya and Panda, 2004). Lipid peroxidation is often used as an indicator of increased oxidative damage (Jagtap and Bhargava, 1995).

According to Celina *et al.* (2004), The effect of decreasing soil water content on leaf catalase activities was noticed to be doubled in case of stressed wheat plants than the watered plants.

The Malondialdehyde content was found to be high in case of Calcutta-4 (AA) than that of Bee hee kela (BB) suggesting that, BB genotypes are better protected from oxidative damage under drought stress than AA genotypes.

The activities of super oxide dismutase and Catalase were found to be more under water deficit conditions than well watered conditions in both the genotypes but the activity in Bee hee kela (BB) was high under drought when compared with the Calcutta-4 (AA), indicating presence of good defense mechanism in Bee hee kela (BB), this also maintained better Leaf water potential under stress also had low Malondialdehyde content under stress. All these parameters indicate Bee hee kela can withstand water stress better than Calcutta-4 (AA).

5.4 Molecular aspects:

In the present study, leaf material collected from the stressed and non-stressed banana genotypes was chosen as source material for the extraction of RNA. The first step in obtaining cDNA is to choose or develop a suitable protocol for isolation of high quality RNA. Here we have chosen Liu *et al.* (1998), modified method of RNA extraction protocol and we have standardized to some extent and used for the extraction of total RNA from the banana leaves of well watered and water stressed genotypes Calcutta-4 (AA) and Bee hee kela (BB) (Plate-4a). The same method was used for the extraction of RNA was reported by Eng-Chong Pua *et al.* (2000), where the isolated RNA was used in studying the expression of a UDP glucose pyrophosphorylase cDNA during fruit ripening of banana (*Musa acuminata*).

The OD values for RNA ranged from 1.87 to 1.95. Yield of RNA obtained ranged from 22.1 to 30.6 µg per gram of leaf tissue (Table-8). Based on OD values obtained, it was concluded that RNA obtained was of good quality.

5.4.1 RNA clean up: The total RNA obtained was cleaned by using Rneasy mini kit (QIAGEN USA) to get still good quality RNA. According to the instructions given this clean up protocol helped to reduce content of genomic DNA and some other impurities otherwise genomic DNA may interfere with the RT-PCR conditions, as it is very sensitive.

5.4.2 cDNA synthesis: cDNA was synthesised using TaKaRa mRNA Selective PCR Kit (AMV) as mentioned in materials and methods(3.7). the gel profile shown in the Plate (4b). cDNA of control and stressed leaves of Beeheekela and Calcutta-4 was synthesized using total RNA by oligo-dT primers, and this cDNA was used for the PCR confirmation of selected candidate genes.

5.5 Primers used:

Nine primers namely- Catalase, Peroxidase, bZIP, Enongation factor, Heat shock protein, Rubisco activase, Mitotic inhibition factor, RNA polymerase and Chaperon D K were designed for Real time PCR using Integrated DNA technology software. Out of nine primers Peroxidase, Catalase, Heat shock protein and Chaperon D K are drought related genes, remaining are involved in gene regulation, growth and metabolism.

5.6 PCR confirmation for the amplification of real time PCR primers:

Before going for real time PCR this technology one need to make sure that PCR amplification for the confirmation of their presence and also we need to standardise for the primer concentrations otherwise primer dimers will also give C_t value. So in our study first we have used genomic DNA of our experimental material to screen for the primers amplification, after several gradient annealing temperatures, out of nine primers four got amplification at 55^{0}C namely Peroxidase, Rubisco activase, Mitotic inhibition factor and RNA polymerase. Among them only one gene was related to drought tolerance (Peroxidase) remaining (Rubisco activase, Mitotic inhibition factor and RNA polymerase) primers are related to gene regulation, growth and metabolism. (Plate-5) and the amplified four primers were used for their presence in cDNA pool from control and stressed leaves of Bee hee kela (BB) and Calcutta-4 (AA). After PCR the gel profile showed presence of three genes (Rubisco activase, Mitotic inhibition factor and RNA polymerase) related to gene regulation, growth and metabolism in all the cDNA samples but the drought related gene (Peroxidase) was found only in control cDNA samples but not found in stressed cDNA samples of both the genotypes (Plate-6). In general peroxidase activity will be higher in stressed situation than in the non-stressed situation, but here we found its presence in control and absence in stressed situation.

5.7 Quantification of band intensity for the PCR amplified primers:

The concentration of band intensity for amplified four primers (Peroxidase,Rubisco activase, Mitotic inhibition factor and RNA polymerase) in control and stressed samples of Bee hee kela and Calcutta-4 was calculated by comparing 100 bp ladder molecular weights and its concentrations by using Gene analysis software (Syngene Company, Genius Model). The values on concentrations of band intensity produced by four PCR amplified primers (Peroxidase, Rubisco activase, Mitotic inhibition factor and RNA polymerase) showed that:

The concentration of band intensity for Peroxidase was more in control sample of Calcutta-4 (28.57 ng) than Bee hee kela (21.69 ng).

The concentration of band intensity for Rubisco activase was 76.87 ng and 63.51 ng in control and stressed samples of Calcutta-4 respectively and it was 92.80 ng and 64.31 ng in control and stressed samples of Bee hee kela respectively. The per cent reduction in concentration of Rubisco activase between control and stressed samples was lower in Calcutta-4 (17.4 per cent) when compared with Bee hee kela (30.7 per cent).

The concentration of band intensity for Mitotic inhibition factor was 21.26 ng and 29.70 ng in control and stressed samples of Bee hee kela respectively and it was 29.80 ng in control sample of Calcutta-4 but band was absent in stressed sample.

The concentration of band intensity for RNA polymerase was 46.10 ng and 66.50 ng in control and stressed samples of Calcutta-4 respectively and it was 10.54 ng and 49.9 ng in control and stressed samples of Bee hee kela respectively. The per cent increase in concentration of RNA polymerase band intensity between control and stressed samples was lower in Calcutta-4 (30.7 per cent) when compared with Bee hee kela (78.8 per cent) (Table-9).

Here there was no clear pattern of gene expression observed due to various reasons like poor cDNA quality, non annealing of primers etc.

Water stress in banana not only changes the activities of drought related genes but also changes the activities of other gene regulators, growth and metabolism related genes.

The results obtained with the physiological studies and biochemical studies are significant. As per the literature available BB genome has got better drought tolerant capacity than that of AA genome accordingly in our study the BB genotype (Bee hee kela) was found to have better mechanisms to cope with the drought situation than the AA genotype (Calcutta-4).

Summary

VI. SUMMARY

Banana is an important fruit crop grown across the world including tropics and subtropics where water availability is a constraint to potential yield. Hence there is a need to reduce water requirements by banana and other crops as they require constant supplies of abundant water. Understanding the physiological, biochemical and molecular basis of water stress tolerance is goes a long way in the endeavor.

In the present investigation AA (*M. acuminata*) and BB (*M.balbisiana*) genotypes were involved. All the present day cultivated bananas are derived from these genomic groups. An attempt also made to understand the physiological and biochemical changes and also the expression pattern of a few selected candidate genes during drought stress in banana.

Thirteen genotypes belonging to AA genomic group and fifteen genotypes belonging to BB genomic groups were screened using leaf water retention capacity and $\Delta^{13}C$ to evaluate for their tolerance to drought. The AA genotypes recorded low leaf water retention capacity where as BB genotypes recorded higher leaf water retention capacity. The average $\Delta^{13}C$ values were high for BB genotypes (18.60) than AA genotypes (17.86). Two genotypes Calcutta-4 and Bee hee kela belonging to AA and BB genomic groups respectively were selected based on earlier experiments in addition to present experiment and planted in the cement structures and allowed to grow for 4 months. Water stress was imposed by withholding irrigation for twelve days and the following findings are summarized below:

6.1 Physiological studies:

a) The per cent reduction in photosynthesis rate (P_N) between control and stressed conditions was found to be high in Calcutta-4 (45.78 %) (AA) than the Bee hee kela (36.01 %) (BB) indicating BB genomic group is tolerant to water deficit conditions.
b) The per cent reduction in transpiration rate (E) between control and stressed conditions was less in Bee hee kela (BB)(22.36 per cent) than Calcutta-4 (AA) (30.24 per cent). In general, if the transpiration rate decreases such a plant will be a water saver however that will not assure to give potential yield. The per cent transpiration reduction is low then the stomata is still open even under drought ensuring good photosynthetic rate and better yield during water limited conditions.
c) The per cent reduction in stomatal conductance (gs) was found to be high in Calcutta-4 (AA) (60.3 per cent)than that of Bee hee kela (BB)(56.10 per cent).
d) $\Delta^{13}C$ and stomatal conductance was in Bee hee kela (BB) than Calcutta-4 (AA) proved that drought tolerance capacity of BB genomic group over AA genomic group.
e) The leaf water potential (Ψ) was high in Bee hee kela (BB) both in watered (-0.913 MPa) and stressed (-1.518 MPa) situations when compared with control (-1.35 MPa) and stressed (-1.824 Mpa) conditions in Calcutta-4 (AA) indicating Bee hee kela (BB) maintained tissue hydration better than Calcutta-4 (AA) under water stress.

6.2 Biochemical studies:

1. The malondialdehyde content was determined in the leaf samples of stressed and non-stressed Bee hee kela and Calcutta-4

genotypes and found that the extent of membrane damage was high in Calcutta-4 (AA) than that of Bee hee kela (BB).

2. Under water stressed condition the enzyme SOD activity was high in Bee hee kela (30.593 U gm^{-1} FW) when compared with Calcutta-4 (22.945 U gm^{-1} FW). The increased activity of super oxide dismutase in stressed leaf samples compared to non stressed leaf samples was recorded in Bee hee kela (BB); this was contrasting in Calcutta-4 (AA genotype).
3. The activity of Catalase under stress was high in case of Bee hee kela (2.7772 U gm^{-1} FW) when compared to Calcutta-4 (0.6666 U gm^{-1} FW). The activity of Catalase was found to be high in Bee hee kela (BB) than Calcutta-4 in both stressed and non-stressed conditions.

The above findings conclude that the Bee hee kela (BB) has good tolerance mechanism to act with reactive oxygen species generated during drought than Calcutta-4 (AA).

6.3 Molecular studies:

1. The total RNA was isolated from the stressed and non stressed leaf samples of the genotypes Calcutta-4 (AA) and Bee hee kela (BB) using modified version of the method (Liu *et al.* 1998*)* with some extent of standardization in case of handling sample, grinding and centrifugation speed.
2. RNA was checked for its integrity by running on a denaturing gel electrophoresis containing formaldehyde, it showed to be 28S and 16S were intact, quality and concentration of RNA was estimated by recording absorbance at 260 nm in UV spectrophotometer, it was shown to be good quality in both the genotypes with sufficient RNA concentration.

3. The cDNA for control and stressed samples of Bee hee kela and Calcutta-4 was synthesized using TaKaRa mRNA selective PCR kit (AMV)
4. Concentrations of band intensity for four PCR amplified genes indicated that drought not only changes the expression pattern of drought inducible genes but also changes the expression levels of growth and metabolism related genes.

Future line of work:

Identification of banana genotypes tolerant to drought within the genomic group (both in AA and BB) and understanding the relationship between drought tolerance and water use efficiency. It is also necessary to identify the phenotypic characteristics like root, leaf thickness, waxiness etc which contribute to the drought tolerance. A study on gene regulation during drought onset and maintenance of cellular activity is also required.

References

VII. REFERENCES

ABE, H., YAMAGUCHI-SHINOZAKI, K., URAO, T., IWASAKI, T., HOSAKAWA, D. AND SHINOZAKI, K. 1997, Role of *Arabidopsis* MYC and MYBhomologs in drought and abscisic acid regulated gene expression. *Plant Cell*, **9:** 1859-1868.

AEBI, H., 1984, Catalase in vitro. *Methods Enzymology*, **105**: 121-126.

AGARWAL, S. AND PANDEY, V., 2004 Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia. Biol. Plant.*, **48**: 555–560.

AMALRAJ, V. A., VELAYUDHAN, K. C., AGARWAL, R. C. AND RANA, R. S., 1993, Banana genetic resources. Scientific monograph. NBPGR (ICAR) New Delhi).

AMTMANN, A., BOHNERT, H. J. AND BRESSAN, R. A., 2005. Abiotic Stress and Plant Genome Evolution. Search for New Models. *Plant Physiol.,* **138:** 127-130.

APEL, K. AND HIRT, H., 2004, Reactive Oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.*, **55**: 373-399.

BAKER, N.R., 1993, Light-use efficiency and photoinhibition of photosynthesis in plants under environmental stress. – In: Smith, J.A.C., Griffiths, H. (ed.): Water Deficits. Plant Responses From Cell to Community. *BIOS Scientific Publ.*, Oxford. Pp. 221-235.

BANANUKA, J. A., RUBAIHAYO, P. R. AND TENYWA, M. M., 1999, Reactions of musa genotypes to drought stress. *African Crop Science Journal,* **7(4):** 333-339.

BARTELS, D. AND SUNKAR, S., 2005, Drought and salt- tolerance in plants, *Crit Rev Plant Sci.,* **24**:23-58.

BATALGIA, O. C., 1980, Effects of nitrogen-water relations on maize productivity. Ph.D. Dissertation, University of California, Davis (Diss. Abstr. 81 05370).

BLACK, M. AND PRITCHARD, H. W., 2002, Dsiccation and survival in plants: drying without dying. Wallingford, UK: CABI Publishing.

BRADFORD, K. J., HSIAO, T. C., 1982, Physiological responses to moderate water stress. – In: Lange, O.L., NOBEL, P.S., OSMOND, C.B., ZIEGLER, H. (ed.): *Physiological Plant Ecology II.* Pp. 263-324. Springer-Verlag, Berlin – Heidelberg – New York.

BRAGNOLI, E. AND FARQUHAR, G.D., 1998, Photosynthetic fractionation of carbon isotopes. In photosynthesis: physiology and metabolism, advances in Photosynthesis(Eds). DEKONECHKOWSKY AND LARHER, F.). SFVP Universite de Renne. CONDON, A. C. AND HALL, A. C. 1997.

BRAY, E. A., BAILEY-SERRES, J. AND WERETILNYK, E. 2000, Responses to abiotic stresses. In: *Biochemistry and Molecular Biology of Plants.* GRUISSEM, W., BUCHNNAN, B. AND JONES, R. Eds. *American Society of Plant Physiologists*, Rockville*,* MD, 1158-1249.

BUSK, P. K. AND PAGES, M., 1998, Regulation of abscisic acid-induced transcription. *Plant Mol Biol.,* **37**: 734-746.

CAMPALANS, A., PAGES, M. AND MESSEGUER, R., 2001, Identification of differentially expressed genes by the cDNA-AFLP technique during dehydration of almond (Prunus amygdalus). *Tree physiol*, **21**:633-643.

CASCARDO, J. C. M., ALMEIDA, R.S., BUZELI, R.A.A.,CAROLINO,S. M. B., OTONI, W. C AND FONTES, E. P. B., 2000, The phosphorylation state and expression of soybean BiP isoforms are differently regulated following abiotic stresses. *J Biol Chem.*, **275**: 14494-14500.

CAYON, M. A., EL-SHARKAWY, M. AND MEIJA DE FAFUR, S., 1998, Physiological responses to water stress in the Dominico-Harton plantain clone. (*Musa* AAB Simmonds). *IFOMUSA,* **17**: 12-14.

CELINA, M., LUNA GABRIELA, M., SIMON, D., KARIN, G., STEPHANIE, B. CHARTZOULAKIS, K., NOITSAKIS, B. AND THERIOS, I., 1992, Photosynthesis, plant growth and dry matter distribution in kiwifruit as influenced by water deficits. *Irrigation Science*, **14**:1-5.

CHAVES, M. M., MAROCO, J. P. AND PEREIRA, J. S., 2003, Understanding plant response to drought-from genes to the whole plant. *Fun Plant Biol.*, **30**: 239-264.

CHEN, T. H. H. AND MURATA, N., 2002, Enhancement of tolerance of abiotic stress by metabolic engineering of betains and other compatable solutes. Curr *Opin Plant.Biol.*, **5:** 250-257.

CHEN, W. P., LI, P. H. AND CHEN, T. H. H., 2000, Glycine-betaine increases chilling tolerance and reduces chilling-induced lipid peroxidation in *Zea mays* L. *Plant Cell Environ.*, **23**: 609-618.

CHOI, H. I., HONG, J. H., HA, J. O., KANG, J. Y. AND KIM, S. Y. 2000, ABFs, a family of ABA-responsive element binding factors. *J Biol Chem.*, **275**: 1723-1730.

CLARKE, J. M. AND MCCAIG, T. N., 1982, Evaluation of techniques for screening for drought resistance in wheat. *Crop Science,* **22:** 503-506.

CONDON, A. G. AND RICHARDS, R. A., 1989, Carbon isotope fractionation and plant WUE. In stable isotope in Ecological research. (Eds. P. W. RUNDEL, J. R. EHLERINGER AND K. A. NAGY) Pp. 21-40 (Springer- verlag: New York).

CONDON, A. G. AND RICHARDS, R. A., 1993, Exploiting genetic variations in transpiration efficiency in wheat. An agronomic view. In: stable isotopes and plant carbon/ water relations(Eds, EHLERINGER, J. R., HALL, A. E. AND FARQUHAR, G. D.) *Academic Press Inc* Pp. 435-450.

CONDON, A. G., FARQUHAR, G.D. AND RICHARDS, R. A., 1990, Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat leaf gas exchange and whole plant studies. *Aust. J. Plant. Physiol.*, **17:** 9-22.

COSIO, C., MARTINOIA, E. AND KELLER, C., 2005. Hyper accumulation of cadmium and zinc in *Thalspi caerulescens* and *Arabidopsis halleri* at the Leaf Cellular level. Plant physiol., **134**: 716-725.

CRAIG, H., 1953. The geochemistry of the stable carbon isotopes. *Geochem. Cosmochem. Acta.*, **3:** 53-92.

DAVIS, T., 1961, High root pressure in palms. *Nature,* **192**:277-278.

DRAPER AND HADLY, H., 1990, Estimation of Malondialdehyde content. *Methods Enzymology*, **105**: 121-126.

DURE, L., 1993, Structural motifs in LEA proteins. Current topics in plant physiology; An Americns Society of plant physiologists series. Vol 10 Plant responses to cellular dehydration during environmental stress. *American society of plant physiologists*, pp99-103.

EKANAYAKE, I. J., ORTIZ, R. AND VUYLSTEKE, D. R., 1994, Influence of leaf age, soil moisture, VPD and time of day on leaf conductance of various *Musa* genotypes in a humid forest-moist savanna transition site. *Ann. Bot.*, **74**:173-178.

ENG-CHONG, P., STEVENSON, S., SZU, W, L., PEI, L. AND JIAN-ZHANG, L., 2000, Expression of a UDP glucose pyrophosphate cDNA during fruit ripening of banana(*Musa acumunata*). Aust. J. Plant Physiol., **27**: 1151-1159.

FARQUHAR, G.D., 1983, On the nature of Carbon isotope discrimination in C_4 species. *Aust. J. Plant Physiol.*, **10**: 205-226.

FARQUHAR, G.D., EHLERINGER, J. R. AND HUBIC, K. T., 1989, Carbon isotope discrimination and photosynthesis, *Annu. Rev. Plant Physiol, Plant Mol Biol.*, **40**:503-537.

FARQUHAR, G.D., O' LEARY, M. H. AND BERRY, J. A., 1982, On the relationship between carbon isotope discrimination and the intercellular CO_2 concentration in leaves. *Aus. J. Plant Physio*l., **9:** 111-131.

FAZELI, F., GHORBANLI, M. AND NIKNAM, V., 2007, Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. *Biologia Plantarum,* **51 (1)**: 98-103.

FISCHER, R. A., 1980, Influence of water stress on crop yield in semi arid regions. In- Adaptation of plants to water and high temperature stress, eds. T. C. TURNER, P., KRAMER. Willey and Son, New York, pp. 323-340.

FOOD AND AGRICULTURAL ORGANIZATION (U. S. A) Statistical Department, 2007.

GRAAN, T. AND BOYER, J. S., 1996, Very high CO_2 partially restores photosynthesis in sunflower at low water potentials. *Planta***.,** **181**: 378-384.

GUTTRREZ, M. V. AND MEIZER, F. C., 1994, Carbon isotope discrimination and photosynthetic gas exchange in coffee hedge rows during canopy development. *Aust. J. Pl. Physiol.*, **21**: 207-209.

GUY, R. D. AND HOERING, T. D., 1987, Isotope fractionation during O_2 production and consumption by plants. Pp 597-600. In: J. BIGGINS (Eds) progress in photosynthetic research .III. Nizhoff, Dordrett.

HAGAR, H., UEDA, N. AND SHAL, S. V., 1996, Role of reactive oxygen metabolites in DNA damage and cell death in chemical hypoxic injury LLC-PK1 cells. *Amer. J. Physiol.*, **271**: 209-215.

HALL, A. E., ISMAIL, A. M. AND MENENDEZ, C., 1993, Implications in water use efficiency, carbon isotope discrimination and gas exchange. In.

EHLERINGER, J. J., HALL, A. E. AND FARQUHAR, G. D.) (Eds) stable isotope and plant carbon water relations. *Academic Press, San Deigo.*

HASEGAWA, P.M., BRESSEN, R.A., ZHU, J.K AND BOHNERT, H. J., 2000, *Annu Rev Plant Physiol Plant Mol Biol*, **51**:463-499.

HOFFMANN, H. P. AND TURNER, D. W., 1993, Soil water deficits reduce the elongation rate of emerging banana leaves but the night/day elongation ratio remains unchanged. *Sci. Hort.*, **54**:1-12.

HONG, Z., LAKKINENI, K., ZHANG, Z.AND VERMA, D. P. S., 2000, removal of feedback inhibition of Pyroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant physiol.*, **122:**1129-1136.

HUBIC, K. T. AND FARQUHAR, G.D., 1989, Carbon isotope discrimination and ratio of carbon gained to water lost in barley cultivars. *Plant Cell. Environ.*, **12:** 795-804.

HUBIC, K. T., FARQUHAR, G.D. AND SHORTER, R., 1998, Correlation between water use efficiency and carbo isotope discrimination in diverse peanut germplasm. *Aust. J. Plant Physiol.*, **13:** 803-814.

HUBIC, K. T., SHORTER, R. AND FARQUHAR, G.D. 1988, Heritability and Genotype x Environment interaction of Carbon isotope discrimination and transpiration efficiency in peanut. *Aust. J. Plant Physiol.*, **15**: 799-813.

INGRAM, J. AND BARTELS, D., 1996, The molecular basis of dehydration tolerance I plants. *Annu Rev Plant Physiol.,* **47**:377-403.

JACKSON, J. E., 2003, Biology of Apples and Pears. Cambridge University Press, Cambridge.

JACOBY, M., WEISSHAAR, B., DROGE-LASER, W., VICENTE-CARBAJOSA, J., TIEDEMANN, J., KROJ, T. AND PARCY, R., 2002, Bzip transcription factors in Arabidopsis. Trends in Plant Sci.,**1**: 106-111.

JAGTAP, V. AND BHARGAVA, S., 1995, Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of *Sorghum bicolor* (L.) Moench. exposed to high light, low water and high temperature stress. *J. Plant Physiol.*, **145**: 195-197.

JAKSON, R. B., SPERRY, J. S. AND DAWSON, T. E., 2000, Root water uptake and transport, using physiological processes in global predictions. *Trends Plant Sci.,* **5:** 482-488.

JIANG, M. AND ZHANG, J., 2001, Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant Cell Physiol.*, **42**: 1265-1273.

JOHNSON, D. A., ASSAY, K. H. AND READ, J. M., 1993, In stable isotopes and plant carbon/ water relations ((Eds. EHLERINGER, J. R., HALL, A. E. AND FARQUHAR, G.D.). *Academic Press New York*. Pp. 269-280. *Journal of Experimental Botany*, **56**: 417–423.

KALLARACKAL, J., MILBURN, J. A. AND BAKER, D. A., 1990, Water relations of the banana 3. Effects of controlled water stress on water potential, transpiration, photosynthesis and leaf growth. *Aust. J. Plant Physiol.*, **17**:79-90.

KANG, J., CHOI, H., IM, M. AND KIM, S. Y., 2002, *Arabidopsis* basic leucine zipper proteins that medicate stress-responsive abscisic acid signaling. *Plant Cell*, **14:** 343-357.

KEUTGEN, N., CHEN, K.AND LENZ, F., 1997, Responses of strawberry leaf photosynthesis, chlorophyll fluorescence and macronutrient contents to elevated CO2. – *J. Plant Physiol.* **150**: 395-400.

KICHEVA, M. I., TSONEV, T. D. AND POPOVA, L. P., 1994, Stomatal and non-stomatal limitations to photosynthesis in two wheat cultivars subjected to water stress. *Photosynthetica*, **30**: 107-116.

KIZIS, D. AND PAGES, M., 2002, Maize DRE-binding proteins DBF1 and DBF2 are involved in rab17 regulation through the drought-responsive element in an ABA-dependent pathway. *Plant J.,* **6:** 262-267.

KNIGHT, H. AND KNIGHT, M. R., 2001, Abiotic stress signaling pathways: specificity and cross talk. *Trend Plant Sci.*, **6**: 262-267.

KOBAYASHI, Y., YAMAMOTO, S., MINAMI, H., KAGAYA, Y. AND HATTORI, T., 2004, Differential activation of the rice sucrose nonfermentingl-related protein kinase2 family by hyperosmotic stress and Abscisic acid. *Plant Cell,* **16**: 4085-4090.

KRAMER, I., BECKETT, R, P., WORNIK, S., ZORN, M. AND PFEIFHOFER, H. W., 2002, Revival of ressurection plant correlates with its antioxidant status. *Plant J.*, **31:** 13-24.

KRIEDEMANN, P.E. AND BARRS, H.D., 1981, Citrus orchards. In-Water deficit and plant growth, ed. T. T. Kozlowski. Academic Press, New York, pp. 325-417.

LARCHER, W., 1980, *Physiological plant ecology*. Springer-Verlag, Berlin.

LARCHER, W., 2000, Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosystems,* **134**: 279-295.

LEE, G., POKALA, N. AND VIERLING, E., 1995, Structure and in vitro molecular chaperon activity of cytosolic small heat shock proteins from pea. *J Biol Chem***, 270:** 10432-10438.

LEE, Y.H. AND CHUN, J.Y., 1998, A new homeodomain-leucine zipper gene from *Arabidopsis thaliana* indiced by water stress and abscisic acid. *Plant Mol Biol,* ***37****: 377-384.*

LEUNG, J., BOUVIER-DURAND, M., MORRIS, P. C., GUERRIER, D., CHEDFOR, E. AND GIRAUDAT, J., 1994, *Arabidopsis thaliana,* ABA-responsive gene ABII: Features of a calcium modulated protein phosphatase. *Science*, **264**: 1448-1452.

LIU, J. J., GOH, C. J., LOH, C. S., LIU, P. AND PUA, E. C., 1998, A method for isolation of total RNA from fruit tissues of banana. *Plant molecular biology reporter*, **16**:87.

LIU, Q., KASUGA, M., SAKUMA, Y., H., MIURA, S., YAMAGUCHI-SHINOZAKI, K. AND SHINOZAKI, K., 1998, Two trascription factors, DREB1 and DREB2, with an EREBP/ AP2 DNA binding domain separate two cellular signal transduction pathway in drought and low-temperature responsive gene expression, respectively in *Arabidopsis. Plant Cell,* **10:** 1391-1406.

LU, P., WOO, K., AND LIU Z. T., 2002, Estimation of whole-plant transpiration of bananas using sap flow measurements. *J. Exp. Bot.*, **53**:1771-1779.

MANSFIELD, T. A., DAVIES, W., 1981, Stomata and stomatal mechanisms. – In: PALEG, L.G., ASPINALL, D. (ed.). The Physiology and Biochemistry of Drought Resistance in Plants. Pp. 315- 346. Academic Press, Sydney – New York – London – Toronto – San Francisco 1981.

MEINZER, F. C., RUNDEL, P. W. AND SCHULTZ, L. M., 1990, Effects of leaf resin on stomatal behavior and gas exchange of carrea tridenta (DC). *Cov. Funct. Ecol.*, **4**: 579-584.

MICHEL, D., EURINI, A., SALAMINI, E. AND BARTELS, D., 1994, Structure and regulation of an ABA and desiccation responsive gene from the resurrection plant *Craterostigma plantagineum. Plant Mol Biol.*, **24:** 549-560.

MILBURN. J. A., 1992, Advances in studying water relations in banana and other plants. *Transaction Malaysian society plant physiology,* **3**: 35-42.

MITTLER, R., 2002, Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.,* **7:** 405-410.

MOHMOOD, R.I., MOHMOOD, K. Y., MOHMOOD, H. A., MARZIAH, M. AND ZULKIFILI, S., 2000,Growth and physiological processes of banana (*Musa spp.*) plants to soil water deficit. Proceedings of the first national banana seminar at Avana Genting and country resort. UPM: Serdang (MYS), 2000. 343 p.

MUNDREE, S. G., BAKER, B., MOWLA, S., PETERS, S., WILLIGEN, C. V., GOVENDER, K., MAREDZA, A., MUYANGA, S., FARRANT, J. M. AND THOMSON, J. A., 2002, Physiological and molecular insights into drought tolerance. *African J Biotech.,* **1(2)**: 28-38.

NATIONAL HORTICULTURAL BOARD (NHB) DATA BASE, 2006.

NEILL, S. J., DESIKAN, R., CLARKE, A., HURST, R.D. AND HANCOCK, J. T., 2002 Hydrogen peroxide and nitric oxide as signalling molecules in plants. – *J. exp. Bot.*, **53**: 1237-1247.

NOORWOOD, M., TOLDI, O., RICHTER, A. AND SCOTT, P., 2003, Investigation into the ability of roots of the poikilohydric plant *Craterostigma plantagineum* to survive dehydration stress. *J Expt Bot.*, **54(391**): 2313-2321.

O' LEARY, M. H., 1984, Measurement of the isotope fractionation associated with diffusion of CO_2 in aqueous solution. *J. Phys. Chem.*, **88:** 823-825.

O' LEARY, M. H., 1988, Carbon isotope discrimination in photosynthesis. *Bio. Sci.*, 38-336.

O' LEARY, M. H., 1993, biochemical basis of carbon isotope discrimination. In: Stable isotopes and plant carbon water relations (Eds. EHLERINGER, J. R., HALL, A. E. AND FARQUHAR, G. D.). *Acdemic pr*ess. Pp. 19-20, C. K., SIMMONDS, L. P. AND MATHEWS, R. B., 1987, Responses to saturation deficit in genotypes of groundnut, growth and development. *Annu. Bot.*, **59**:121-128.

OONO, Y., SEKI, M. AND TOKIHIKO, 2003, Monitoring expression profiles of *Arabidopsis* gene expression during rehydration process after dehydration using Ca. 7000 full-length cDNA microarray. *Plant J.*, **34**: 868-887.

PEI, Z. M., MURATA, Y., BENNING, G., THOMINE, S., KLU¨SENER, B., ALLEN G. J., GRILL, E. AND SCHROEDER, J. I., 2000, Calcium channels activated by hydrogen peroxide mediated abscisic acid signalling in guard cells. *Nature*, **406**:731–734.

PHILIPS, J. R., OLIVER, M. J. AND BARTELS, D., 2002, Molecular genetics of desiccation and tolerant systems. In CAB *International Desiccation and Survival* in plants: Drying without dying, M. BLACK AND H. PITCHARD. (E ds), pp. 319-341.

POPENOE, W., 1941, Banana culture around the Caribbean. *Tropical Agriculture*, **18**:8-12 and 33-38.

PROCTOR, J. T. A, WATSON, R. L. AND LANDSBERG, J. J., 1976, The carbon budget of a young apple tree. *J. Am. Soc. Hort. Sc*i., **101**:579-582.

RAMANJULU AND BARTELS, D., 2002, Drought and Desiccation induced modulation of gene expression in plants. *Plant Cell En*viron, **25:** 141-151.

RAO, V. N. M 1984, Banana. A monograph. ICAR Publication.

RATNAYAKA, H. H., MOLIN, W.T. AND STERLING, T. M., 2003, Physiological and antioxidant responses of cotton and spurred anoda under interference and mild drought. *J. exp. Bot.*, **54**: 2293- 2305.

READ, J. J., JOHNSON, D. A. AND TIEZEN, C., 1991, Carbon isotope discrimination, gas exchange and WUE in crested wheat grass clones. *Crop. Sci.*, **31**:1203-1208.

RICHARDS, C. J. AND TESZIEN, L. L., 1993, Carbon isotope discrimination, water relations and gas exchange in temperate grass species and accession. In: stable isotopes and plant carbon water relations (Eds. EHLERINGER, J. R., HALL, A. E. AND FARQUHAR, G.D.). *Acdemic Press Inc*. Pp 281-296.

RIECHMANN, J., HEARD, G., MARTIN, L., REUBER, C.Z., JIANG, J., KEDDIE, L., ADAM, O., PINEDA, O.J., RATCLIFFE, R.R., SAMAHA, R., CREELMAN, M., PILIGRIM, P., BROUN, J. Z., ZHANG, D., GHANDEHARI, B.K., SHERMAN, G. AND YU L., 2000, *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science*, **290**: 2105-2110.

ROBINSON, J. C., 1995, System of cultivation and management. In: GOWEN, S. (Ed.) Bananas and plantains. London: Chapman & Hall, chapter 2, p.15-65.

ROBINSON, J. C., 1996, Bananas and Plantains. CAB International, Wallingford.

SAKUMA, Y., LIU, Q., DUBOUZET, J. G., ABE, H., SHINOZAKI, K. AND YAMAGUCHI-SHINOZAKI, K., 2002, DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration and cold-inducible gene expression. *Biochem Biophys Res Commun.*, **290**(3):998–1009.

SCEBBA, F., SEBASTIANI, L. AND VITAGLIANO, C., 1998, Changes in activity of antioxidative enzymes in wheat (*Triticum aestivum*) seedling under cold acclimation. - *Physiol. Plant.*, **104**: 747-752.

SEKI, M., NARASUKA, M. AND KAMIYA, A., 2002, Functional annotation of a full length Arabidopsis cDNA collection. *Science*, **296**: 141-145.

SESTAK, Z. AND SIFFEL, P., 1997, Leaf-age related differences in chlorophyll fluorescence. – *Photosynthetica,* **33**: 347-369.

SGHERRI, C. L. M. AND NAVARI-IZZO, F., 1996, Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defence mechanisms. *Physiol. Plant.*, **93**: 25-30.

SHINOZAKI, K. AND YAMAGUCHI-SHINOZAKI, K., 2000, Molecular response to dehudration and low temperature: Differences and cross talk between two stress signalong pathways. *Curr Opin Plant Biol.*, **3:** 217-223.

SHINOZAKI, K., YAMAGUCHI-SHINOZAKI, K. AND SEKI, M., 2003. Regulatory network of gene expression in the drought and cold stress responses. *Curr Opin Plant Biol.*, **6**: 410-417.

SHMUELI, E., 1953, Irrigation studies in the Jordan Valley. 1. Physiological activity of the banana in relation to soil moisture. *Bull. Res. Counc. Isr.* **3**:228-247.

SIMMONDS, N. W., 1962, The classification and nomenclature of bananas some implications. *Proc Linn. Sol Ion.,* **173**: 111-113.

SIMMONDS, N.W., 1959, Bananas, Longmans, Green and Co. Ltd., London.

SREENIVASULU, N., RAMANJULU, S., RMACHANDRA-KINI, K., PRAKASH, H. S., SHEKAR-SHETTY, H., SAVITHRI, H. S., SUDHAKAR,C., 1999, Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fox-tail millet with differential salt tolerance. *Plant Sci.*, **141**: 1-9.

SRIKUL, S. AND TURNER, D.W.,1995, High N supply and soil water deficits change the rate of fruit growth of banana(Cv.Williumd) and promote tendency to ripen. *Scientia Horticulture*, **62:** 165-174.

STOVER, R. H. AND SIMMONDS, N. W., 1987, *Bananas*. 3rd ed. Longman, London. 468 pp.

STREETER, J.G., LOHNES, D.G. AND FIORITTO, R.J., 2001, Pattern of pinitol accumulation in soybean plants and relationship to drought tolerance. *Plant Cell Environ.*, **24**: 429-438.

TANNY, J. AND HAIJUN, L., COHEN, S., 2006, Airflow characteristics, energy balance and eddy covariance measurements in a banana screenhouse. *Agric. For. Meteorol.*. **139**:105-118.

TAYLOR, S. E. AND SEXTON O. J., 1972, Some implications of leaf tearing in Musaceae. *Ecology*, **53:**143-149.

THOMAS, D. S. AND TURNER, D. W., 2001, Banana (*Musa* sp.) leaf gas exchange and chlorophyll fluorescence in response to soil drought, shading and lamina folding. *Sci. Hort.*, **90**:93-108.

THOMAS, D. S., 1995, The influence of the atmospheric environment and soil drought on the leaf gas exchange of banana (*Musa* spp.). The University of Western Australia. PhD thesis.

THOMAS, D.S. TURNER, D. W. AND EAMUS, D., 1998, Independent effects of the environment on the leaf gas exchange of three banana (Musa sp.) cultivars of different genomic constitution. *Sci. Hort.*, **75**:41-57.

TUNNACLIFFE, A., GOYAL, K. AND WALTON, L. J., 2005, LEA proteins prevent protein aggregation due to water stress. *Biochemical J.*, **388**: 151-157.

TURNER, D. W. AND THOMAS, D. S., 1998, Measurements of plant and soil water status and their association with leaf gas exchange in banana (*Musa* spp.): a laticiferous plant. *Sci. Hort.* **77**:177-193.

TURNER, D. W., 1995, The response of banana plant to the environment. In: *Bananas and Plantains.* Gowen, S. (Ed), pp. 206-229. Chapman and Hall, London.

UDAYA KUMAR, M. AND PRASAD, T. G., 1994, ^{13}C isotope discrimination in plants- A potential technique to determined WUE. In: selection for WUE in grain legumes(RAO, R.C.N. AND WRIGHT, G. C. Eds), A report of workshop held at ICRISAT Centre. Andhra Pradesh, India. Pp 42-45.

UPADHYAYA, H. AND PANDA, S. K., 2004 Responses of *Camellia sinensis* to drought and rehydration. *Biol. Plant.*, **48**: 597-600.

VILLALOBOS, M. A., BARTELS, D. AND ITURRIGA, G. 2004, Stress tolerance and glucose insensitive phenotypes in Arabidopsis overexpressing the CpMYB10 transcription factor gene. *Plant Physiol.*, **135**: 309-324.

VINICOV, I. AND BASTOLA, D. R. 1999, Transgenic over expression of the transcription factor A1ftn 1 enhances expression of the endogenous

MSPRP2 gene in alfalfa and improves salinity tolerance of the plants. *Plant Physiol.*, **120**: 473-480.

WANG, Z. AND HUANG, B., 2004, Physiological recovery of Kentucky bluegrass from simultaneous Drought and Heat Stress. *Turfgrass Sci.,* **12**: 113-118.

WHEMEYER, N. AND VIERLING, E., 2000, The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in dessication tolerance. *Plant Physiologic.*, **122**: 1099-1108.

WHITE, T. W. 1993, Implications of carbon isotope discrimination studies for breeding common bean under water deficits. In stable isotopes and plant carbon/ water relations (Eds. EHLERINGER, J. R., HALL, A. E. AND FARQUHAR, G.D.). *Acdemic Press Inc.* Pp 389-396.

YAMAGUCHI-SHINOZAKI, K. AND SHINOZAKI, K., 1993, The palnt hormone abscisic acid mediadtes the drought induced expression but not the seed-specific expression of rd22, a gene responsive to dehydration stress in *Arabidopsis thaliana. Mol Gen genet.,* **238**: 17-25.

YANCEY, P. H., CLARK, M. E., HAND, S. C., BOWLUS, R. D. AND SOMERO, G. N., 1982, Living with water stress: evolution of osmolyte systems. *Science*, **217**: 1214-1222.

ZHANYUAN AND WILLIUM, J., 1994, Superoxide Dismutase activities in senescing apple fruit. *J. of Food Science.*, **186**: 421-424.

ZHU, J. K., 2002, Water stress signaling. *Annu Rev Plant Physiol.,* **62**: 247-279.