OXIDATIVE STRESS AND PROTEIN PROFILING IN SWEET POTATO (*Ipomoeabatatas* (L.) Lam.) UNDER ABIOTIC STRESSES

by

AISWARYA N. S.

(2010-09-110)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE (INTEGRATED) IN BIOTECHNOLOGY

Faculty of Agriculture

Kerala Agricultural University, Thrissur

B.S.c.-M.S.c. (Integrated) Biotechnology

Department of Plant Biotechnology

COLLEGE OF AGRICULTURE

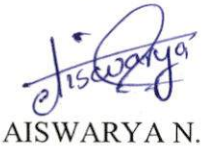
VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2017

DECLARATION

I hereby declare that this thesis entitled **"OXIDATIVE STRESS AND PROTEIN PROFILING IN SWEET POTATO (*Ipomoeabatatas* (L.) Lam.) UNDER ABIOTIC STRESSES"**is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.



Place: Vellayani

Date: 28/02/2017

AISWARYA N. S.

(2010-09-110)

भा कृ अनु प-केन्द्रीय कंद फसल अनुसंधान संस्थान
(भारतीय कृषि अनुसंधान परिषद, कृषि और किसान कल्याण मंत्रालय, भारत सरकार)
श्रीकार्यम, तिरुवनन्तपुरम - 695 017, केरल, भारत

ICAR-CENTRAL TUBER CROPS RESEARCH INSTITUTE
(Indian Council of Agricultural Research, Ministry of Agriculture and Farmers Welfare, Govt. of India)
Sreekariyam, Thiruvananthapuram-695 017, Kerala, India

ISO 9001:2008

CERTIFICATE

Certified that this thesis entitled "Oxidative stress and protein profiling in sweet potato (*Ipomoeabatatas* (L.) Lam.*)* under abiotic stresses" is a record of research work done independently by Ms. Aiswarya N. S. (2010-09-110) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place: Sreekariyam
Date: 21.02.2017

Dr. V. Ravi
Chairman, Advisory Committee

Dr. V. Ravi, Ph.D., A.R.S.
Head, Division of Crop Production
ICAR-Central Tuber Crops Research Institute
Sreekariyam, Thiruvananthapuram - 695 017

Phone : +91-471-2598551 to 2598554
Director (Per.) : +91-471-2598431
(Res.) : +91-471-2597211
Fax : +91-471-2590063
E-Mail : director.ctcri@icar.gov.in
ctcritvm@gmail.com

CERTIFICATE

We, the undersigned members of the advisory committee of Mrs. Aiswarya N. S., a candidate for the degree of Master of Science (Integrated) in Biotechnology, agree that the thesis entitled **"Oxidative stress and protein profiling in sweet potato (*Ipomoeabatatas* (L.) Lam.) under abiotic stresses"** may be submitted by Mrs. Aiswarya N. S., in partial fulfillment of the requirement for the degree

Dr. V. Ravi
(Chairman, advisory committee)
Principal scientist and Head
Division of Crop Production
Central Tuber Crops Research Institute
Sreekariyam, Thiruvananthapuram-695 017

Dr. Swapna Alex
(Member, advisory committee)
Professor and Head
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram- 695 522

Dr. Roy Stephen
(Member, advisory committee)
Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram- 695 522

Dr. R. V. Manju
(Member, advisory committee)
Professor and Head
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram- 695 522

Dr. C. Mohan
(Member, advisory committee)
Principal Scientist
Division of Crop Improvement
Central Tuber Crops Research Institute
Sreekariyam
Thiruvananthapuram-695 017

Dedicated to the God Almighty

ACKNOWLEDGEMENT

Foremost, it gives me an immense pleasure to express my sincere gratitude to my advisor Dr. V. Ravi for giving me an opportunity to work in Central Tuber Crop Research Institute, Sreekariyam. I am extremely grateful for his continuous support, patience, motivation, enthusiasm and immense knowledge which he shared during my research. His guidance helped me at the time of research and writing the thesis. The work presented in this thesis would not have been successfully accomplished without his encouragement, in rightful comments and critical analysis.

I express my heartful thanks to Dr. Sverup John, Dean, COA, Vellayani for all the facilities provided during my research.

I owe my gratitude and indebtedness to the advisory committee members Dr. B. R. Reghunath for his valuable suggestions and corrections, Dr. S. K. Chakrabarti, for granting the permission to work in CTCRI, Dr. K. Rajmohan, who has been the back bone of our course and project and Dr. C. mohan for being very co-operative all the time and for their kind hearted support, valuable suggestions and moral support throughout the study.

This thesis would be incomplete without expressing my deep sense of gratitude to Dr. Lekha Sreekhandan, Dr. Ummamaheswaran, Dr. Roy Stephen, Dr. Manju R.

V, Dr. Soni K. B,Dr. Swapna Alex and Dr. Deepa S. Nair for appreciating my research strengths and encouraging me to improve my research work areas.

I wish to express my deep gratitude to all teaching and non-teaching staff members of division of Crop Production CTCRI and College of Agriculture Vellayani for their timely help.

Dr. Saravanan Raju and Dr. Aju Ashok for their noteworthy advice and kindly help and my friend Sreekuttan K. S, for extending a helping hand throughout my study, I am also grateful to Vinoth Kumar(supporting staff, CTCRI) for him kind hearted support. My experience in lab was greatly enhanced due to these wonderful people who were with me as a team.

"There can be no friendship without confidence and no confidence without integrity".It is superfluous to thank them, But the memories of the times that we have shared shall be cherished always. I thank all my

beloved folks Dhanya, Rajalakshmi, Amritha and Jayalakshmi.With a grateful heart I thank the help, personal care and encouragement rendered by Dhanya, Aswathy and Sreekuttan.

I fall short of words to convey my deepest gratitude to my loveable family for their unceasing encouragement and blessing.

And finally I thank GOD for making all of this possible and given me the strength and confidence to withstand all the obstacles I faced during the period.

I acknowledge the favour of numerous person who, though not been individually mentioned here, have all directly or indirectly contributed to this thesis work.

AISWARYA N. S.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

EXPANSION OF ABBREVIATIONS AND SYMBOLS USED

^{o}c	Degree Celsius
$^{1}O_2$	Singlet Oxygen
µl	Micro Liter
µg	Micro Gram
%	Percentage
AP or APX; (EC 1.11.1.11)	Ascorbate Peroxidase
AOS	Active Oxygen Species
AR	Ascorbate Reductase
AsA	Acorbate
BSA	Bovine Serum Albumin
CAT; (E.C 1.11.1.6)	Catalase
CARs	Carotenoids
Cm	Centimeter
CO_2	Carbon Dioxide
CBB R-250	Coomassie Brilliant Blue R-250
DAP	Day after planting
DMSO	Dimethyl Sulfoxide
DCPIP	Dichlorophenol Indophenols
DTNB	5, 5'-Dithiobis Nitro Benzoic Acid
EDTA	Ethylenediaminetetraacetic Acid
EU	Enzyme Unit

GR; (EC 1.6.4.2)	Glutathione Reductase
GA	Giberilic Acid
GSSG	Glutathione Disulfide
H_2O_2	Hydrogen Peroxide
HSPs	Heat Shock Proteins
HTS	High Temperature Stress
IAA	Indol Acetic Acid
K_2HPO_4	Potassium Phosphate Dibasic
KH_2PO_4	Monopotassium Phosphate
LEA	Late Embryogenesis Abundant
Mm	Millimeter
Ml	Milliliter
Mg	Milligram
$MgCl_2$	Magnesium Chloride
Min	Minute
Nm	Nanometer
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NADH	Nicotinamide Adenine Dinucleotide
NaCl	Sodium Chloride
NR	Nitrate Reductase
O^{2-}	Superoxide
OH	Hydroxyl Radicals
PAGE	Poly Acrylamide Gel Electrophoresis
PMSF	Phenylmethylsulfonyl Fluoride

PH	Potential of Hydrogen
POX	Peroxidase
PSMs	Plant Secondary Metabolites
PVP	Polyvinylpyrrolidone
ROS	Reactive Oxygen Species
Rpm	Red Hat Package Manager
RWC	Relative Water Content
RGR	Relative Growth Rate
SOD; (EC 1.15.1.1)	Superoxide Dismutase
SDS-PAGE	Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis
TCA	Trichloroacetic Acid
TEMED	Tetramethylethylenediamine
Tris-HCL	Tris Hydrochloride
150 V	150 Volt
WDS	Water Deficit Stress
WP_L	Water Potential
UV	Ultra Violet Radiation
Sec	Second

INTRODUCTION

1. INTRODUCTION

Tropical tuber crops are those tuber crops cultivated in the tropical and subtropical regions of the world. In India cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas* (L.) Lam.), yams (*Dioscorea*) and aroids viz., taro (*Colocasia*), tannia (*Xanthosoma*), elephant foot yam (*Amorphophallus*) and arrow root (*Maranta*) are the primary tuber crops cultivated across our states. Tuber crops are important for several respects. They have a high biological efficiency as producers and it exhibits the highest rate of dry matter production per day per unit area, compared to other crops. The major mode of utilization of tuber crops is as human food in all most all countries where they are cultivated. Tuber is important for food industry and pharmaceuticals. Tuber crops are characterized by its high starch content that can be utilized for the synthesis of varieties of value added products.

Sweet potato belongs to the family convolvulaceae of Dicotyledonae. It is a hexaploid (2n = 90). It is a weak stemmed shrub which trails on the ground with simple, entire or palmately sect leaves and adventitious roots arising from each node. Some of the roots store food material and become tubers. Sweet potato tubers are specialized roots with 'eyes' or axillary buds distributed all over the surface which can sprout after harvest and produce plantlets.South America has been recognized as the centre of origin of sweet potato. It is grown more extensively in tropical and subtropical countries like china, Uganda, Nigeria, Indonesia, Vietnam, Japan, Tanzania and India. In India, it is grown around an area of about 1 lakh ha with a net production of 10 lakh tonnes. Though it is cultivated in all the states of India, the major area under this crop is confined to the states of Odisha, Bihar, Uttar Pradesh, West Bengal and Jharkhand.

Sweet potato tubers are used as subsidiary food after boiling, backing or frying. The tubers are also utilized as a raw material for the industrialized production of starch, alcohol, pectin and tuber vines form a best source of green fodder for milch animals and in certain countries like the Philippines, the fine

shoot tips are used as a leafy vegetable. The crop growth is best at above 24°C temperature.

Shading results in poor yield. It requires 500 mm rainfall during growing season. Heavy rainfall and water logging are antagonistic to the crop and it is susceptible to frost. Tuber yield is affected if drought occurs within the first six weeks after planting. Well drained, fertile, sandy loam soil is ideal for the crop. It is sensitive to saline and alkaline conditions. Sweet potato can be grown as a kitchen garden crop with round the year supply of tubers. The crop matures in 100-120 days but short duration varieties mature in 75-90 days. The tuber shape can be spherical, oval, fusiform or elliptic; tuber skin white, pink or purple and the tuber flesh white, cream, orange or deep orange in high carotene varieties and purple in anthocyanin rich varieties.

Sweet potato is grown as a rainfed crop during rainy (Kharif) or autumn (Rabi) seasons and as an irrigated crop during other periods. Apical vines of 20-30 cm length having 3 to 4 nodes are found to be ideal as planting material. It is also propagated through vine cuttings collected from sprouted tubers raised in nurseries. The vines with the leaves intact are stored under shades for two days in bundles, before planting in the field. The vine cuttings are planted on flat beds or on ridges at a spacing of 20 x 60 cms with the middle portion buried in soil and the ends kept exposed.

Free radicals are the bioactive compounds produced during normal metabolism in a living system. They possess unpaired electrons that can start off harmful chain reactions by targeting stable molecules, resulting in lipid peroxidation, damage of DNA and proteins. Antioxidants protect the cells from the threat posed by free radicals. Plants are easily susceptible by free radicals and even cause organallar destruction and respiratory chain break out. So they have developed numerous free radical quenching systems via antioxidant systems resulting in the production of numerous antioxidant enzymes and compounds and form a barrier in plants known as plantsecondary metabolites (PSMs). Antioxidants are also known to cordially work against several chronic diseases

posed by free radicals such as arthrosclerosis, cancer, diabetes, arthritis, inflammation and ageing related problems (Kaur and Kapoor, 2001).

During Development and growth, a plant has to undergo different internal and external stresses. Their ability to adapt to environmental and metabolic changes is essential for survival. The production of reactive oxygen species (ROS) such as superoxide $O_2^{\cdot-}$ hydrogen peroxide (H_2O_2) its toxic derivatives, singlet oxygen ($^1O_2$) and hydroxyl radicals ($OH^{\cdot}$) occurs every time during plant growth and development. This is increased when the plants are exposed to various other biotic and abiotic stresses (Elstner, 1982; Asada, 1994; Dat *et al.*, 2000). These toxic ROS oxidize unsaturated fatty acids, protiens and DNA, resulting in cellular damage and cell death.

To oxidative stresses, plants respond in different defense mechanisms. These include production of both non-enzymatic antioxidants as ascorbate and gluthathione and enzymatic antioxidants such as superoxide dismutase, catalase and ascorbate peroxidase. It will result in cell death when these defences fail to protect the plant from the ROS. Symptoms similar to those seen during leaf senescence before cell death are shown in many cases by the plants. For example, plants that are exposed to stresses such as ozone or UV-B irradiation, each resulting in increased levels of ROS. They responded by showing symptoms of premature senescence followed by necrotic cell death (Pell *et al.*, 1997; Jansen*et al.*, 1998). Also, infection by pathogens can induce premature senescence and localized cell death (Morel and Dangl, 1997).

Abiotic stresses such as drought, extreme temperatures and oxidative stresses are the primary causes of crop loss. Abiotic stresses lead to a series of morphological changes, physiological break downs, varying biochemical products and mutations that affect plant growth and productivity. Plants undergo oxidative cell burst under different abiotic stress conditions and generate free radicals which will oxidize lipids in the cellular membrane, damage the cell organelles and impair metabolic activities. Plants will also produce antioxidants to combat oxidative stress. Plants also synthesise certain special proteins known as Heat

Shock Proteins (HSPs). Abiotic stresses limits productivity to a extensive rate and also the expansion area of sweet potato. The present study aims for an understanding of metabolic response of sweet potato to abiotic stresses like heat and water deficit stresses and if it will help to combat the vagaries of climate change and improve sweet potato productivity.

REVIEW OF LITERATURE

2. Review of Literature

Sweet potato (*Ipomoea batatas* (L) Lam) holds the top sixth position for the most fundamental food crops of the world. By increasing the cultivation area production of sweetpotato may be increased. However, due to unfavourable soil properties or climatic conditions most of the lands available in the tropics are with limited productive capacity. Therefore, attention should be given while developing cultivars that are resistant to various stress conditions while increasing area under cultivation.

The accumulation of antioxidants although controlled genetically, is also largely influenced by various environmental and metabolic factors. Abiotic stresses have a key role in increased production of antioxidants. Abiotic stresses such as drought, extreme temperatures and oxidative stresses are posing major threat to today's agriculture scenario and are the principal causes for the drop in crop yield in major crops by more than 50% across the world.

Plants undergo oxidative stress under various abiotic stress conditions such as water deficit stress, high temperature (>33°C) stress and generate free radicals such as O^{-2}, H_2O_2,OH^-. The free radicals generated will oxidize lipids in the cellular membrane and damage the cell organelles and impair metabolic activities. This in turn will suppress plant growth and productivity. Plants will also produce antioxidants like ascorbate, glutathione and antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), ascorbate reductase, glutathione reductase (GR) to combat oxidative stress.

Plants also synthesis certain special proteins known as Heat Shock Proteins (HSP's) to combat abiotic stresses. Among 5 types of HSP's based on their molecular weights viz., HSP 90, HSP 60, HSP70, HSP 100, low molecular weight small HSPs (sum HSP) are found to be more prevalent in plants.

An understanding of its metabolic response to abiotic stresses to heat and WDS will help to combat vagaries of climate change and improve the sweet potato productivity.

Reactive O_2 species (ROS) are produced in both unstressed and stressed cells. Plants have well-developed defence system against ROS, involving both limiting the formation of ROS as well as instituting its removal. The formation and removal of O_2 balanced under unstressed conditions. However, the defence system can be overwhelmed, when presented with with increased ROS formation under stress conditions. Plants responds to a rise in ROS that the defence system is unable to remove with increased enzymatic or non – enzymatic antioxidant processes (Alscher and Hess, 1993), but the mechanisms underlying these processes is not well understood

Sweet potato is a perennial food crop of the morning- glory family, Convolvulaceae, but widely cultivated as an annual crop in tropical and warmer temperate climates (Huaman, 2002). The crop has played a key part in the food economy of nations as one of the world's most valued root crops with sweetish edible and vitamin-enriched roots. It is cultivated over a range of environment in more than one hundred countries, and between latitude 40^{o} N and S of the equator as well as between 2000-2300m above mean sea level (Hahn, 1977; Kay, 1987; Onwueme and Sinha, 1991; James, 1994). In some areas, sweet potato is the major source of nourishment for the people (NRCRI, 1983; Bourke, 1985; SPC, 1986). It is one among the seven leading world food crops and has marked an annual production of about 122 million Mt $year^{-1}$, and holding thirteenth rank globally in production value among agricultural goods (Collins, 1993; FAO, 2005). It ranks second in world root and tuber crop production after potato and second after cassava in importance in Africa (CIP, 1998). Sweet potato is a dependable and flexible crop with a great potential to feeding the hungry people in the tropical world, as it can be left in the ground and harvested piecemeal as needed or harvesting everything at once and preserved for food security (CIP, 1998). It has long been known as a calamity crop, because its suited for urgent situations, a well adaptable and major source of survival and therefore a strong base for agricultural renovation after disasters like typhoons, drought have occurred (CIP, 1998). The crop is easy to cultivate under different agro-ecologies

with little fertilizer input and produces a high volume of roots per hectare, matures fast has good keeping qualities and is affected by few pests and diseases. It tastes nice and is an excellent source of vitamin C (50%), vitamin A (10%), vitamin B1 (21%), vitamin B2 (8%), calcium (14%), iron (12%), niacin (12%), energy (13%), protein (13%) and some magnesium, potassium and zinc (raw or cooked). The young leaves and tips are always a good food source (whether in emergency or not), providing 167% vitamin C, 120% vitamin A, 8% vitamin B1, 23% vitamin B2, 56% calcium, 19% iron, 10% niacin, 17% protein and 2% energy (cooked) (Terry, 1987; SPC, 1986; CIP, 1998; Yang and Keding, 2009). In Ethiopia, for many years sweet potato is being cultivated which is important in their deit where the population growth is the highest, least land holdings and large scale starvation threat is present (Habtu 1995). Over 95% crop is produced in the Eastern, Southern and South West parts. For centuries it has remained as a major subsistence crop during drought period (Adhanom *et al.,* 1985).

The improved varieties of sweet potatoes are:

S1. No	Variety	Duration (days)	Yield t/ha	Special features
1	Sree Arun	90 – 100	20 - 28	Pink Skin, Spreading, Cream flesh, good cooking quality
2	Sree Kanaka	75 – 85	12 - 15	short duration hybrid which is rich in carotene (8.8-10mg/100g fresh weight
3	Kanjangad	110 – 120	15 - 20	Semi – spreding, reddish purple skin, light yellow flesh cooling quality good

2.1. Growth parameter

Plants are exposed to almost all environmental extremities. The unfavorable climatic conditions like water stress, salinity, extreme temperatures as well as irradiance have a definite impact on the various growth parameters like total vine length, number of leaves per plants and tuber yield per plant.

Growth parameters have been reported to influence crop growth. Tsubo *et al.*,(2003) showed that plant density affected crop productivity and resource use in intercropping and concluded that good crop growth resulted in high crop yield. Shamsuddin and Paul (1988) observed a positive correlation between relative growth rate (RGR) and NAR in sweet potato.

Crop physiology of sweet potato and the impact of high temperature stress on tuber yield have been reviewed (Ravi and Indira, 1999). The storage root formation and growth is influenced by air and soil temperature. Due to higher translocation of sugar from the shoot to roots during night time, the night air temperature is most crucial factor for storage root growth. Storage root formation and growth is promoted during night temperatures between 15 and 25°C. Maximum yield for cultivars of sweetpotato is in season with night air temperatures between 14 and 22°C (Negeve *et al.*, 1992). Storage root formation is suppressed due to higher night air temperature than 25°C, while promoting shoot growth (Nakatani, 1989). Air temperatures of more than 30°C, an increase in IAA oxidase activity leads to drop in storage root formation and growth increase because od the surge in gibberellic acid (GA) promoting shoot expansion (Chen, 1988). Soil temperatures between 20 and 30°C favor storage root formation and a temperature more than 30°C prop up shoot expansion at the cost of storage root expansion (Spence and Humphries, 1972).

The yield of sweet potato are majorly affected by water distribution time and its quantity. It decreases the under water deficit stress (WDS) if the present soli moisture declines to about 20% (Nair *et al.*,1996; Indira and Kabeerathumma, 1988; Chowdhury and Ravi, 1987). The root initiation time majorly depend on WDS because it directly affects storage root number (Indira and Kabeerathumma,

1988; Nair *et al.*, 1996; Ravi and Indira, 1996). Water deficiet stress at the peiod of storage root initiation promotes lignification of the storage root parts and reduces storage root development. Lignification along with the reduction in the storage root yield is higher in cultivars carrying a less sink capacity compared to those with greater sink capacity (Ravi and Indira, 1996).

The decline in storage root yield with WDS is in respect with physiological and biochemical difference developed in the leaves. In this WDS status, water potential (WP_L)or relativewater content (RWC) of sweet potato leaves decreases (Indira and Kabeerathumma, 1988; Ravi and Indira, 1995; Chowdhury and Naskar, 1993). Leaves permanently wilt when WP_L reduces to -1.3 MPa and at WP_L ranging from -1.6 to -2.0 MPa the leaves clearly senesce (Ravi and Indira, 1995). The reduction in WP_L hikes S_R to CO_2 exchange (Indira and Kabeerathumma, 1988; 1990) resulting a decrease in P_N rate (Ravi and Indira, 1996). Cultivars shows differences in their adaptance to WDS conditions (Chowdhury and Ravi, 1987; 1988; Ravi and Indira, 1996). Elevated S_R in tolerant varieties may be beneficial for preserving leaf water content at the expense of decrease in photosynthesis because of WDS.This aids tolerant varieties to have less desiccation rate in leaves compared to the susceptible varieties (Naskar and Chowdhury, 1995).

2.2. Physiological parameters

The changes in environment result in transient changes in various physiological features of the plant. Plants respond to the changes in the surrounding by changing its physiology there by adapting and acclimating to the new environment. Such physiological parameters that are influenced by abiotic stress are phytopigments, stomatal characteristics including stomatal frequency, stomatal conductance and stomatal index, specific leaf area as well as relative water content.

The study about the influence of light on growth in *Ageratum conyzoides* proved that under shaded conditions there is an increase in chlorophyll a, chlorophyll b and total chlorophyll (Sun *et al.*,2012). WDS plants exhibits a

considerable reduction in chlorophyll pigments viz., a, b and carotenoids than watered plants in lettuce (Agami, 2013).

In shade grown plants of *Andrographis paniculata*, plants had more chlorophyll per unit dry weight (Pratima, 1999). A significant reduction in chlorophyll b was observed due to WDS in *Grevellia robusta* (Nautiyal *et al.*, 1993) and a similar result was reported in tea (Rajasekar *etal.*, 1991).

The net chlorophyll content in leaf tissues reduced in sweetpotato plants grown under WDS (Indira and Kabeerathumma, 1988; 1990; Chowdhury and Ravi 1987; 1988). In these WDS conditions, it is found out that nitrate reductase (NR) is the foremost enzyme in the nitrate assimilatory pathways which reduce NO_3^- to NO_2^- and this activity decreases in sweet potato leaf tissues (Chowdhury and Ravi, 1987; 1988; Indira and Kabeerathumma, 1990).

Carotenoids are lipophilic pigments seen in the chloroplast of all the photosynthetic and non-photosynthetic plants. They play a major number of functions in plant metabolism counting the role in oxidative stress tolerance and repairing UV degraded tissues. They are also known to be antenna molecules as carotenoids have capacity to absorb light in the wavelength of 450-570 nm from the visible spectra and surpass the confined energy on to the light haversting complex via Chl. In plastids, Carotenoids are the accessory pigments in complex light harvesting system; and also plays a more major role is their capability to quench various forms of free radicals. Carotenoids can either exist in its low energy state or in the excited states once it absorbs light from the corresponding wavelength. Carotemoids can exploit their antioxidant property in the following ways (1) by direct reaction with the free radicals products to cease the large devastating chain reactions, (2) by quenching $^1O_2$ and fluroscing the energy as heat, (3) by chemically interacting with 3Chl* or high energy chlorophyll (Chl*) molecules to thwart the production of $^1O_2$, or (4) by driving away the higher energy excitation energy via xanthophyll cycle. The major defending role of β-carotene in the photosynthetic machinery may be consummated via scavenging of 3Chl*, which thwarts $^1O_2$ production and hence slow down oxidative damage by

ROS (Collins, 2001). During scavenging of 3Chl*, excess energy is conveyed from Chlorophyl molecules to Carotenoids, which consequently fritter aways the excess devastating energy in a non-radiative heat form. Thus, carotenoids take action as a competitive inhibitor of $^1O_2$ production and this is supported by its close propinquity to Chl in the LHC. This way of safeguard is particularly important when light intensity peaks more than the saturating conditions (Collins, 2001). Another type of carotenoid, zeaxanthin produced from violaxanthin, has been concerned in the dissipation of heat energy, but the specific mechanism causing this dissipationis not yet understood. Zeaxanthin plays the important role in facilitating the photoconversion of 3Chl* to 1Chl* more proficiently than β-carotene (Mortensen *et al.,* 2001).

2.3. Biochemical Parameters

Biochemical pathways responsible for the synthesis of organic compounds in plants are highly influenced by various abiotic stress factors. The major biochemical compounds studied in the current experiment are total soluble proteins, proline.

The solute accumulation during the passage of stresses like compatible solutes viz proline, alpha betaine is found out to be important in stress escaping mechanism (Parthasarathy, 2004). Total soluble proteins and total sugars were found to be significantly lower under WDS in Lettuce plants (Agami, 2013).

The total soluble protein content significantly increased under WDS in *palmarose* (Fathima *et al.,* 2002). WDS significantly reduced soluble protein content whereas total leaf soluble protein increased 2.5 fold in Vinca major and nearly a 20 fold in pumpkin when shifted from shade to full sunlight (Logan *et al.,* 1998). The number of soluble protein, RUBISCO and chlorophyll per unit area of the leaf showed np change even when the cells are exposed to high CO_2 concentration in wheat (Delgado *et al.,* 1994).

In a comparative study of three species of Amaranthus namely, *A. hypocondriachus, A.tricolor* and *A. hybrids*; significant accumulation of proline

was reported depending on the onset of WDS (Slabbert and Kruger, 2014). Agami (2013) reported enhanced production of osmoprotectants like proline and soluble sugars under WDS conditions in lettuce.

Osmotic adjustments by proline production in amaranth and tomato plants during the stress periods were reported by (Umebese *et al.,* 2009). However a lower concentration of proline accumulation was reported in C4 plant, *Amaranthus cruentus* and sorghum when imposed with oxidative stress due to WDS (Brandt *et al.,* 2011). Exogenous application of proline under water deficit conditions was observed to decrease oxidative stress in cotton (Noreen *et al.,* 2013).

Drought tolerant sweet potato varities build up large quantities of proline in the leaf tissues and in fibrous roots compared to the plants free from WDS conditions (Chowdhury and Ravi, 1987; Indira and Kabeerathumma, 1988; Ravi and Indira, 1997). In tolerant and susceptible varieties, leaf tissues mount up large amount of the amino acid proline compared to the fibrous roots (Ravi and Indira, 1996). But its also reported that some susceptible varities are not under WDS also produce a appreciable quantity of proline in their leaves (Ravi and Indira, 1997).

2.4. ANTIOXIDANTS

In plants, reactive oxygen species (ROS) are continuously produced predominantly in chloroplasts, mitochondria and peroxisomes. Formation and elimination of ROS is firmly managed in the plant system. However the equilibrium between the formation and removal of ROS may be disturbed by a vast number of unfavourable abiotic factors viz as elevated light intensity, WDS, high temperature, low temperature and mechanical stress, which ultimately leads to oxidative stress (Elstner, 1991; Malan *et al.,* 1990; Prasad *et al.,* 1994; Tsugane, 1999).

Improvement of the plant antioxidant system protects them from oxidative stress harm is either by containment of ROS formation or by the quenching of ROS already formed in plants (Murgia *et al.,* 2004). ROS when present in excess

concentration results in lipid peroxidation of DNA damage and eventually apoptotic death of the cell (Sarowar *et al.,* 2005). Antioxidants defend a cell from oxidative stress imparement and thereby reduce the risk of cell damage (Smitha and Sudha, 2011).

Plants have developed a vast number of defence mechanisms with which they protect itself from oxidative stress. When these defences prove to be incompetant to defend the plant from against ROS, it results in cell death. In a number of cases the plant shows signs alike to those found at the time of leaf senescence prior to cell death. The plants which were under stresses viz UV-B irradiation, resulting in elevated levels of reactive oxygen species, for which plants underwent premature senescence and necrosis followed by apoptosis (Pell *et al.,* 1997; Jansen *et al.,* 1998). Infection from microbes and pathogens cause induction of premature senescence followed by cell death (Morel and Dangl, 1997).

Leaf senescence pertains to the complete degradation of chlorophyll, protein, nucleic acids and lipids and these results in significant metabolic changes. These autophagy processes, especially for lipids, results in elevated ROS formation (Thompson *et al.,* 1998) and the plant reacts by senescence mediated production of antioxidant enzymes for autophagy and ROS quenching (Buchanan-Wollaston, 1997; Jimenez *et al.,* 1998). Generally, the antioxidant prominence in leaf is reduced at the time of senescence; ROS levels are elevated, and many antioxidant enzymes such as SOD shows less activity and lipid peroxidation and autophagy increases (Del Rio *et al.,* 1998; Jimenez *et al.,* 1998; Thompson *et al.,* 1998; John *et al.,* 2001; Prochazkova *et al.,* 2001).

Different abiotic stress viz., drought and heat generate produce ROS such as hydroxyl ions, hydrogen peroxide, superoxide radicals (Zhu, 2001). Crops which have elevated amount of antioxidant enzymes are more resistant to various environmental stresses compared to those which have reduced enzyme activities. Production of AOS free radicals and initiation of antioxidant enzymes like, catalase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase,

ascorbate reductase under high temperature stress are studied in *Arabidopsis*, rice and tobacco (Wahid, 2007).

Heat shock proteins and late embryogenesis abundant (LEA) proteins are two major types of abiotic stress induced proteins. Heat shock proteins perform important physiological functions as molecular chaperones (Wang *et al.*, 2004). These functions of HSPs are deeply involved in resistance to temperature stresses (Wang *et al.*, 2004). Accumulation of HSPs under high temperature stress (38 – 40°C) has been reported in *Arabidopsis* (HSP 70) and carrot (HSP 17.7). Heat shock proteins (have been attributed for thermotolerance in *Arabidopsis* (Lee and Schoff, 1996) and in rice (Katiyar-Agarwal *et al.*, 2003). Similarly, LEA proteins have been reported to induce thermotolerance in rice (Babu *et al.*, 2004).

H_2O_2 is a type of ROS formed as a product of oxidative stress. H_2O_2 is produced by superoxide, more likely in an uncontrolled manner, when reaction continues to electron transport processes like photo phosphorylation and mitochondrial respiration for ATP synthesis. In response to abiotic stresses like intense light, cold and drought H_2O_2 production via ETC is elevated (Bartosz, 1997; Dat *et al.*, 2000). Plants acquire a series of antioxidant machineries viz., enzymatic or non-enzymatic, by the way ROS are quenched from the cell (Noctor and Foyer, 1998). Thus, an important equilibrium amidst the formation and metabolism of reactive oxygen species finalise the destiny of the cell.

Cellular reductants and antioxidant enzymes are copious in plants which are able to neutralize the AOS. These include the major antioxidant enzymes viz and peroxisomal and cytosolic catalase, superoxide dismutase, ascorbate and ascorbate peroxidase, and glutathione and glutathione peroxidase (Thompson *et al.*, 1987). Oxidative stress is the major reason behind the induction of antioxidant systems (Scandalios, 1993).

2.5. Enzymatic pathway for ROS-scavenging in plants

The important ROS-scavenging enzymes in plants comprises of ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione peroxidase (GPX),

catalase (CAT), peroxiredoxin (PrxR), glutathione and ascorbic acid (Noctor and Foyer, 1998), these enzymes make available cells with extremely proficient mechanism for ROS detoxification. The equilibrium among SODs and other H_2O_2 - quenching enzymes in plant cells is measured to be important in formating the balanced level of O_2^- and H_2O_2. This steady state, along with the compilation of ions by ferritin and metal-binding components, cease the production of the elevated amount of toxic hydroxyl radical by the Fenton reaction (Asada and Takahashi, 1987; Bowler, 1991). Fenton (Lara-Ortı´z, 2003; Keller, 1998) in the late nineteenth century described the oxidizing potential of hydrogen peroxide with ferrous salts. Forty years later, Haber Weiss reaction (Torres, 1998) recognized OH ion as the oxidant in the above reactions.

$$Fe_2^+ + H_2O_2 \longrightarrow Fe_3^+ + OH + OH^-$$

In plants, presence of limited ferrous ion will halt the reaction, but the same shall be recycled to ferrous ion by reductants like $O2^{\cdot-}$

$$Fe_3^+ + O_2 \longrightarrow {}^{\cdot}O_2 + Fe_2^+$$

Therefore, this reaction can be summarized as:

$$H_2O_2 + 2O^{\cdot} \xrightarrow{Fe_2^+,\ Fe_3^+} OH^{\cdot} + OH^- + O_2$$

Haber Weiss reaction

H_2O_2 is a strong oxidizing agentwhich can induce oxidative stress damage in leaf tissues priming to the hampering of metabolic reactions and failure of cellular maintance which results in senescence. The antioxidants accountable for ROS scavenging in the cell wall and apoplast not yet studied completely and the vacular ROS-quenching pathways are not reported.

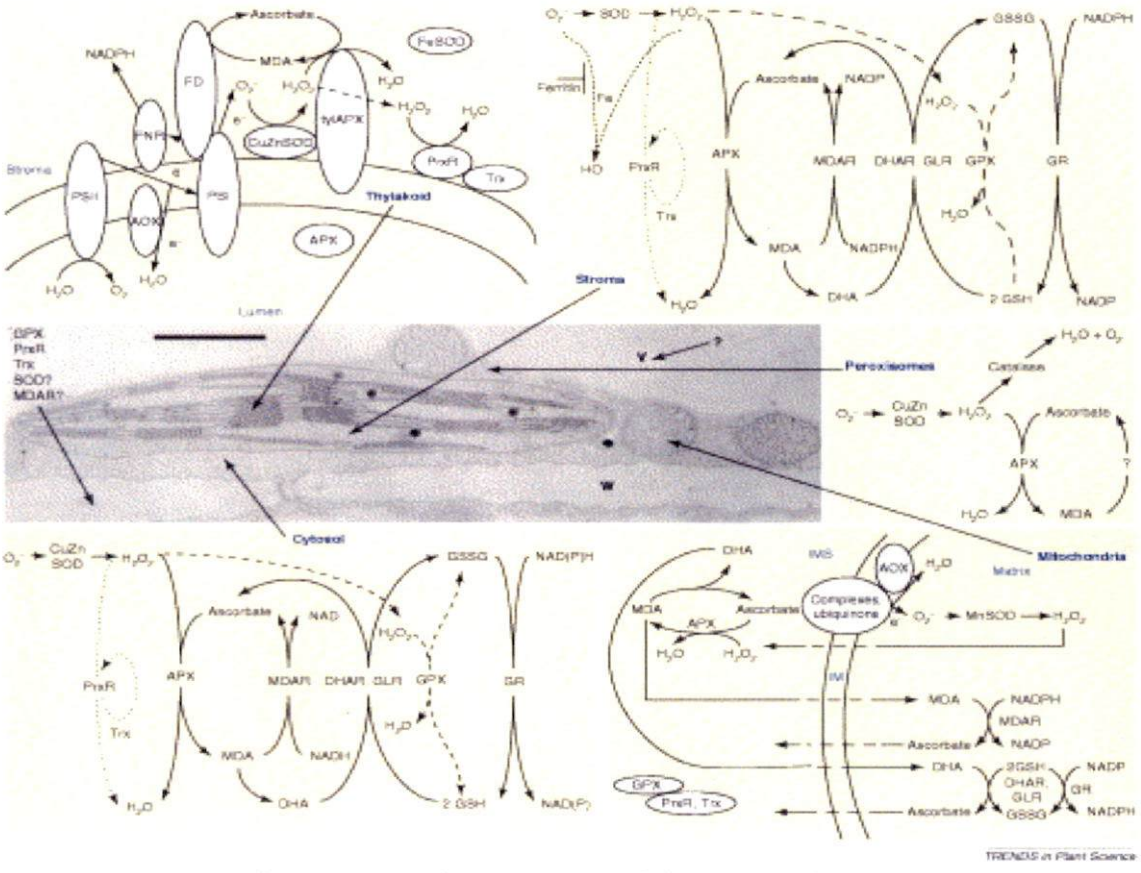


Fig.1. Enzymatic component of the ROS pathway

2.6. Enzymatic Antioxidants

The enzymatic antioxidants studied in the experiment include SOD, CAT, GR, APX and AR.

2.6.1. Superoxide Dismutase activity (EC.1.5.1.1.)

O^{2-} is frequently produced in the chain of electron transfer mechanism, and therefore O_2 reactions can occur in any organell of the cell (Elstner, 1991). SOD are a family of important antioxidants which catalyze the reduction of O^{-2} radicals into H_2O_2 and O_2, and envisages a key role in defending cell from the poisonous aftermath of superoxide ions formed in diverse organells. SODs are majorly found in chloroplasts, mitochondria, cell wall and cytosol,

There are totally three different typrs of enzymes distributed on the basis of their cofactors: Cu/Zn containing SOD, Mn cofactor SOD and the Fe mediated SOD isozymes. The Mn-SOD is seen in the mitochondria; Cu/Zn-SOD isozymes scattering the cytosol, and the other enyzymes in the chloroplasts of plants. The Fe-SODs are not frequently seen in plants, but in trace amounts Fe-SODs are usually coupled with the plastids.

Different forms of SODs are cloned from many varities. It exhibits SOD activities in cells in response to xenobiotic stresses like petroleum products and major pesticides and also environmental stresses like light, salinity and water logging. Evidently, SOD isozymes are autonomously regulated realting to the level of oxidative stress seen in the different compartments. Lipid peroxidation products from each organelle move out from the place of oxidative response to the nucleus where they elevate transcription of different SOD genes (Buchanan *et al.,* 2000).

The conserved sequence present in SOD is SRL/ARL which is absent in the prokaryotic Fe-SOD proteins which proves that the sequence is not important for carrying out its function (Van camp *et al.,* 1994). SOD expressing varities in *Oryza sativa* and maize are comprehensively studied, but the presence of Fe-SOD rice or maize is not yet reported.

Mn SOD have also been reported in the mitochondria of *Nicotiana tabacum;* (Bowler *et al.,* 1994), *Vigna mungo* (Reddy and Venkaiah, 1982). WDS had no influence on SOD activity in sorghum (Zhang and Kirkham, 1996). Sairam *et al.* (1998) reported that SOD activity was decreased with osmotic stress in upland rice. Elevated CO_2 treatment in soybean showed decreased activity of SOD (Booker and Fiscus, 2005).

A significant increase in antioxidant enzymes (SOD, POD, CAT, APX and GR) was reported in high light intensities and decreased in low light intensities under drought conditions in *Picea asperata* seedlings (Yang *et al.,* 2008). A fluctuating range of activities with the progress of stress were reported in a study of three genotypes of *Radixastragali* seedlings under WDS (Yong *et al.,* 2006).

Bartoli *et al.,* (1999) reported that in water stressed wheat plants, SOD activity remained unchanged. Gogorcena *et al.,* (1995) reported that SOD activity decreased in drought stressed pea nodules and a similar reduction in rice (Boo and Jung, 1999).

2.6.2 Ascorbate Peroxidase Activity (EC 1.11.1.11)

Ascorbate peroxidase (APX) is also an isoenzymes and envisages a key role in the metabolism of H_2O_2. Since ascorbate is a reducing agent of H_2O_2 in plastids (Foyer and Halliwell, 1977), the presence of APX (Groden and Beck, 1979; Nakano and Asada, 1981). The enzymatic number (EC 1.11.1.11) for APX appropriated because of the study conducted in Euglena enzyme and studied its various properties (Shigeoka *et al.,* 1980, b).

Takeda *et al.,*(1998, 2000), reported that ascorbate peroxides is classified under class I heme-peroxidases which is reported in higher plants and erthrophytes (Sano *et al.*, 2001), and the species of the protists (Shigeoka *et al.*, 1980b; Wilkinson *et al.*, 2002). The sequences of antioxidant enzymes from all wake of kingdoms are compiled in Peroxibase database (Oliva *et al.*, 2009), which also comprises a chain of bioinformatics tools helpful in analysing the sequences.

Passardi *et al.,* (2007) found that both the genomic and cDNA sequences obtained from the library are from large varieties of plant species widely distributed across the plant kingdom. These isozymes are determined by small gene families in the specific organisms. The different isozymes are analyzed and categorized according to its compartmentalization. Cytosolic, mitochondrial and plastid APX are soluble, isoforms seen in microbody, peroxisome and glyoxisome are membrane-bounded. The signal peptide sequences for different organelle-specific proteins and found in the N- and C-terminal regions determine its final destination. (Shigeoka *et al.*, 2002; Teixeira *et al.*, 2004, 2006).

Plant chlAPX isoenzyme specific genes are classified into two categories. The first category contains single genes which express two isozymes through alternative splicing which comprises plants such as spinach, tobacco, pumpkin

etc. The mechanism behind alternative splicing in chloroplast APX was reported in spinach (Kaur and Kapoor, 2001) and the results proved to be vital for stromal and thylakoid APX expression. This regulation is tissue-dependent based.In the second category, individual genes express individual isoenzymes which are normally spliced which comprises plants such as Arabidopsis, rice, and tomato.

The high temperatures changes the growth of plants adversely, yield and plant production quality shows a declining pattern (Mittler *et al.,* 2004; Scandalios, 2005). ROS production are seen to rise if plants are grown in stressful environment viz., extremely cold and hot temperatures. (Kawakami *et al.,* 2002) reported that in potato tubers, the brief accumulation of cAPX mRNA when exposed to cold conditions was greater in comparison with high-temperature exposure, exhibiting the APX expression in response to cold temperature, the rice cAPX genes for OsAPX1 and OsAPX2 were elevated after exposure in cold temperature. OsAPX3, OsAPX4, OsAPX6andOsAPX7 were considerably induced, at the same time OsAPX8 was supressed after exposure under cold temperature for 24hrs (unpublished data). (Park *et al.,* 2004) reported that exposure to high temperature in sweet potato, the cAPX gene of was greatly induced in leaf tissues .When exposed to high temperature, cAPX, sAPX and mAPX activities of cucumber plant elevated once it showed a decline in the initiation of the reaction. (Song *et al.*, 2005). When Arabidopsis was exposed to cold treatment, the expression of APX gene elevated slightly (Zhang *et al.*, 1997). APX activity was increased to 60% in resistant plants grown under control conditions in two cowpea resistant amd sensitive cultivars. A high elevation in transcripts of peroxisomal and cytosolic APX genes was observed in the susceptible drought stress variety (D'Arcy-Lameta *et al.*, 2006).

2.6.3. Ascorbate Reductase Activity (EC 1.6.5.4)

Under high temperature stress production of AOS free radicals and intitiation of antioxidant enzymes viz ascorbate reductase, catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidise are studied in rice, *Arabidopsis*, and tobacco (Wahid, 2007).

Bielski (1982) studied that AFR is comparatively steady and reacts with itself thus ceasing the free radical propogation reactions. It plays an important role in the cesation of oxidative damage by quenching superoxide, hydroxyl, and organic free radicals and, hence generates its own ROS, AFR (or monodehydroascorbate). AOS in plant cells viz O_2 and H_2O_2 is detoxified by catalase and other antioxidants including a-tocopherol, ascorbate, carotenoids flavonoids and glutathione (Elstner, 1982). Ascorbate peroxidase reduses H_2O_2 to form water by utilizing ascorbent as a reductant, generating AFR (Yamazaki and Piette, 1961; Hossain *et al.,* 1984). Hossain and Asada, (1985) reported that AFR reductase (monodehydroascorbate reductase) reduces AFR to ascorbic acid by utilizing NADH or NADPH as electron donor (Hossain and Asada, 1985). According to Hossain *et al.,*(1984), elevated levels of AFR reductase recreates incompletely oxidized ascorbate to reduced form, which are seen in plastids but its action is also reported in cytosol, mitochondria, and in microsome preparations and are seen to be extensively scattered among different species (Arrigoni *et al.,* 1981). AFR reductase enzyme was cloned from cucumber fruit by Sano and Asada, 1994 and pea plant by Murthy and Zilinskas, 1994, but the datas related to gene expression are not available. Since the generation of superoxide anion radicals (O_2-) is seen in chloroplast, Ascorbic acid are seen in elevated amounts because ROS production is high in ETC by photosynthesis and their conversion by superoxide dismutase to oxygen and H_2O_2 (Elstner,1982). (Tanaka *et al.,* 1985; Mehlhorn *et al.,* 1986; Luwe *et al.,* 1993) were studied that ascorbic acid are used in detoxification of ozone in plant cells and of respiratory-burst products H_2O_2 and at the process of seed germination (Cakmak *et al.,* 1993), in the aversion of damage caused by peroxide in N-fixing root nodules (Dalton *et al.,* 1993) against WSD (Smirnoff and Colombe, 1988) and low temperature treatment (Schoner and Krause, 1990; Kuroda *et al.,* 1991) wound, and in plant tolerance to nematode infection (Arrigoni *et al.,* 1979).

2.6.4. Glutathione Reductase Activity (EC 1.8.1.7)

Glutathione reductase, or GSR or GR (EC 1.8.1.7), comes under NADPH-dependent oxidoreductase and takes place in prokaryotic and eukaryotic organisms. GR is located in chloroplasts, cytosol, and mitochondria. 80% of its activity is seen in photosynthetic tissues and thir amounts vary according to according to differentially regulated stress. Abiotic stress resistanceshows an elevated amount of GR and GSH. GR plays an important role in cellular defense against ROS by economically preserving the cellular GSH in its reduced form through aiding GSSG to GSH reduction via the NADPH oxidation. The high amounts of GSH and GR is reported to participate in a major role in protecting plants against different forms of stress. Formation of a disulphide bond in glutathione (GSSG) GR catalyzed by NADPH-and is thereby crucial for maintaining GSH in reduced form. Thus, GSH and GR execute the quenching of free radicals and its metabolic products; hence offer resistance to plants in stressful condition.

GSH in reduced form can directly interact with ROS to quench them or pose as a reactant for ROS-qienching glutathione peroxidases and transferases (GPXs and GSTs; Ghezzi *et al.,* 2005). GSH is utilized to recreate ascorbate and thiol proteins in oxidized forms (Noctor *et al.,* 2002). Glutathione reductase regenertes oxidized glutathione (GSSG) by NADPH as a reductant (Wingsle and Karpinski, 1996). Glutathione is viewed as a key compound to determine redox status and is therefore is present only in millimolar concentrations (Mullineaux and Rausch, 2005).

The tripeptide GSH, seen mostly as thiol compound in most cells, reduces disulphide to protect the thiol groups of enzymes, regenerate ascorbate, and react with $^1O_2$ and $OH^{\cdot}$. GSH quenches herbicidal ROS by conjugation aided by the enzyme glutathione-*S*-transferase (GST), and controls gene expression in reaction to abiotic and abiotic stress. GSH regenerates ascorbate from DHA catalyzed by DHAR (Noctor and Foyer, 1998). GR mediates the generation of a disulphide bond in glutathione disulphide (GSSG) in the presence of NADPH and is thus

important for regulating the GSH in reduced form. the Halliwell-Asada pathway illustrates peroxide quenching by GSH and GR (Noctor and Foyer, 1998; Asada, 2000) in which GR catalysing step is the rate-limiting one. In pea umerous studies have been exhibited to prove that abiotic stress increases GR activity, cowpea, French bean. GR activity is elevated in plants with heavy metal stress. (Karuppanapandian *et al.,* 2006 a, b, c, 2009; Karuppanapandian and Manoharan, 2008, in wheat (Sairam and Srivastava, 2002). GR expression is expanded under stresses such as HL, mechanical wounding, temperature treatment, cold treatment and heavy metals exposure (Apel and Hirt, 2004; Karuppanapandian *et al.,* 2011).

2.6.5. Catalase Activity (EC. 1.11.1.6)

Catalase is the first enzyme to be purified in a highly transparency state. Isoforms of catalase have been reported in many plants. Plants with three isoforms of catalase viz., cat-1, cat-2 and cat-3 that are are differentially expressed and regulated in an independent manner. The cat-1 and cat-2 are found in peroxisomes and the cytosol respectively while cat-3 is mitochondrial origin. Catalase catalyses the dismutation of H_2O_2 into water and molecular oxygen. The enzyme is reported in eukaryotes and is crucial for the elimination of hydrogen peroxide produced in peroxisomes by oxidases engaged in β-oxidation of fatty acids and the glyoxylate cycle.

Catalase is very susceptible to light and has a high turnover rate, as a product of light absorption mediated by heme. Stress conditions, that reduce the protein turnover rateand stresses such as heat treatment, salinity, or cold treatment cause the reduction in the catalase activity. This may have consequence in the plant's capability to resist the oxidative products of the stresses.

Catalase activity was not affected by mild drought in sorghum (Zhang and Kirkham, 1996). Higher expression levels of catalase in transgenic rice plant resulted in augmented resistance for oxidative stress produced by chilling (Saruyama *et al.,* 2005). Catalase has been purified from germinating cotyledons of pumpkin seeds (Yamaguchi *et al.*, 1984), *Zantedeschia aethiopica* (Trindade *etal.*, 1998), Pea leaf peroxisomes (Corpas *et al.*, 1999) and in Van apple (Golden

delicious) (Yoruk *etal.*, 2005). Dill (*Anethum graveolensL*) (Gulnur 2001) and leaf in Sweet potato (Hsien-Jung *etal.*, 2011).

Yang *et al.*,(2008) have reported that lower CAT activity was observed in low light intensities under WDS condition in *Picea asperata*. Moran *et al.*, (1994) reported that catalase activity was decreased in pea plants under WDS. An intial rise in CAT levels were found in *Radix astragali*, with the onset of WDS, which decreased on subsequent days of stress treatment (Yong *et al.*, 2006).

2.7. Non-Enzymatic Antioxidants

2.7.1 Glutathione

Elicitor treated for biotic stress induction in alfalfa and bean cells raises the glutathione levels (Edwards et al., 1991). Regardless of such defenses, the oxidative burst drastically changes the AOS levels.

GSH or γ-glutamylcysteinglycine, occurrs in almost all organells such as mitochondria, plastids, vacuoles, ER, and cytosol hence mediating multiple functions (Noctor and Foyer, 1998). GSSG and GSH regulates redox equilibrium in the organelles. This allows for normalising cellular redox surroundings under ordinary conditions and, ahead the beginning of stress, and compounds for GSH stress signaling (Wang *et al.*, 2008). A cysteine residue in its active site is the reason for the high reducing capacity of GSH, whiche quenches cellular H_2O_2 and acts non-enzymatically with ROS, $^1O_2$, $O_2^{\cdot -}$, and $OH^{\cdot}$ (Noctor and Foyer, 1998; Wang *et al.*, 2008). The primary role of GSH in the plant protection is due to its capability to regenerate a dominant water-soluble antioxidant, such as phyochelatins via the AsA-GSH cycle (Noctor and Foyer, 1998; Halliwell, 2006). GSH is a pioneer of phytochealatins (PCs), that catalyses the sequestering of heavy metals. The quantity of GSH in cellular compartments has a serious outcome on its antioxidant function and it differs appreciably under various abiotic stresses. More than that, evidences has pointed towardsd rise in GSH concentration is concurrent with the capability of a plant to endure metal-derived oxidative burst. It has been reported that improved antioxidant activity in the leaf

tissues and plastids of *Phragmites sps* was correlated with a big amount of GSH that upshoted in defending the function of many photophosphorylation enzymes against the heavy metal burst of Cd (Pietrini *et al.,* 2003). Elevated concentration of GSH are found with the mounting concentration of heavy metal ions such as Cd, Cr, and Al in *Pisum sativum* green gram and black gram (Karuppanapandian and Manoharan, 2008). Lately, a rise in GR and GSH content under auxin 2,4-D has been studied to intiatevleaf senescence in mung bean leaves (Karuppanapandian *et al.,* 2011). Xiang et al. (2001) reported that Arabidopsis with decreased quantity of GSH were susceptible to even small amount of Cd ions because of reduced PC synthesis.

2.7.2 Ascorbic Acid

Ascorbic acid (Asc) is formed partially in the cytosol and partly in mitochondria, and is also seen in the apoplast, peroxisomes, vacuoles, and plastids. Ascorbic acid has a major role in photo protection as a cofactor in the xanthophyll cycle (Eskling *et al.,* 1997). Ascorbic acid mainly has four functions viz., an antioxidant, enzyme cofactor, precursor for tartrate and oxalate synthesis in plants. It take part in a range of processes, such as photophosphorylation, protection from photorespiration, the cell cycle, cell- wall growth via expansions production and cell development, synthesis of hormones ethylene, GA, hydroxyproline and anthocyanins, tolerance to abiotic stresses.

Kimball and Mitchell (1981), found no effect of CO_2 elevation on the similar species, though the excess concentration of CO_2 induced the synthesis of vitamin A. Low Asc in *vtc- 1* mutants of *Arabidopsis thaliana* increased the oxidative burst in plastids of water deficit stressed plants (Bosch and Alegre, 2002). In turfgrass, water deficit stress did not induce any increased of ascorbic acid concentration (Xunzhong and Schmidt, 2000). The ascorbate content increased in the seedling of *Picea asperata* under high light intensities (Yang *et al.,* 2008)

The ascorbate content in plants increased in response to excess level of Zn and high irradiance stress, indicating that ascorbate is one of the effective defense

mechanisms against stresses in plants (Michael and Krishnaswamy, 2011). The ascorbate poor *vtc-1* mutants of *Arabidopsis thalliana* exhibited more seusceptibility to drought stress than the wild type (Niu *et al.*, 2013). Cucumber seedlings when exposed to short term water deficit a decreased activity of ascorbate was reported (Fan *et al.*, 2014).

AA is another most important antioxidants available in almost all plant cells, organelles, and apoplast (Horemans *et al.*, 2000; Smirnoff, 2000). Normally AA survives mostly in the reduced form (90% of the ascorbate pool) in plastids (Smirnoff, 2000). The capability of AA to contribute electrons in an ample range of both enzymatic and non-enzymatic reactions which converts AA as the main ROS-scavenging compound in the aqueous solution. AA can quench $O2^{\cdot-}$, $OH^{\cdot}$, and $^1O_2$, and can reduce H_2O_2 to H_2O mediated by the APX reaction (Noctor and Foyer, 1998). In plastids, AA acts as a cofactor of the enzyme violoxanthin de-epoxidase, hence dissipating the excess fluroscence energy (Smirnoff, 2000). AA produces TOC from the tocoperoxyl radical (TOC), that gives membrane protection (Horemans *et al.*, 2000; Smirnoff, 2000). Thus, a rise in the levels of cellular AA in plants are essential to compensate oxidative stress along with regulating other metabolic process in plants (Smirnoff, 2000). AA oxidation occurs in two steps. First, MDHA is generated and, if the complex is not readily rereduced to ascorbate, it converts into AA and DHA. The reproduction of AA inside plastids gives an accepted mechanism for the maintanance of ETC. In accounting to be a effective antioxidant, AA is associated in the pH-mediated intonation of PSII activity, and its reduction in expression is related with the type of carotenoid known as zeaxanthin. This is an important apparatus for inhibiting photo-oxidation.

MATERIALS AND METHOD

3. MATERIALS AND METHODS

The present study entitled "Oxidative stress and protein profiling in sweet potato (*Ipomoeabatatas* (L.) Lam.) under abiotic stresses" was carried out in the Division of Crop Production, Central Tuber Crops Research Institute (ICAR-CTCRI), Sreekariyam, Thiruvananthapuram during the period October 2014-June 2015. The details of experimental materials and methodology adopted for the study are presented in this chapter.

3.1. Plant materials

Five varieties/ genotype of sweet potato *viz.*, Sree Arun,Sree Kanaka,Kanjangad, 'varieties' S-1464 (pale green)and S-1466 (purple), were planted under field conditions with three replications, with each replication twenty-five plants each (Plate 1). The crop was planted on 25 January 2015 plants were grown under irrigated, field conditions in Block I of Central Tuber Crop Research Institute during 2014 (Plate 2).Water deficit stress (WDS) was imposed during secondand third month after planting by withholding irrigation for 4 days. High temperature stress was imposed by enclosing the plants in light transparent poly chambers. Plants were exposed to high temperature stress at 40°C during the day time. HTS will be imposed for a period of seven days during second and third month after planting. Activity of antioxidative enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate reductase and ascorbate peroxidase (AP), were assayed in the leaves and tuber of five varieties of sweet potato using spectrophotometer EVOLUTION 201 UV-visible spectrophotometer (Thermo scientific)adopting standard procedures. Total protein was extracted from the leaves and tubers of sweet potato plants and 1 D protein profiling was done using PAGE (Poly Acrylamide Gel Electrophoresis).Growth parameters viz., leaf production, leaf retention, plant height were recorded monthly whereas yield components such as tuber yield, tuber number and mean tuber weight were recorded at the time of harvest.

Plate 1. Five varieties/ genotypes of sweet potato (*Ipomoeabatatas* (L.) Lam.)

Plate 2. Field view of sweet potato field in Block 1 of Central Tuber Crop Research Institute.

3.2. Growth parameter

3.2.1. Vein length

Vein length was calculated from the base to the apex of the plant using meter scale for were recorded for 120 days with an interval of 30 days.

3.2.2. Number of leaves per plant

Leaf number was counted from the base of the plant to the apex of the plantwas recorded for 120 days with an interval of 30 days.

3.2.3. Leaf retention percentage

Leaf Retention percentagewere recorded in 2nd and 3rd monthaccording to the formula.

$$\text{Leaf Retention Percentage} = \frac{Number\ of\ leaves\ present}{Total\ no.\ of\ leaves}\ X\ 100$$

3.3. Yield parameter were recorded after 3 month after planting

3.3.1. Number of tuber per plant

At the time of harvest, tubers were collected from the base of each plant and were counted.

3.3.2. Mean tuber weight

The average weight of the tubers was documented with the aid of weighing balance (Ultra-electronics).

3.4. Quantitative estimation of protein

Protein content of sweet potato leaf and tuber forming root sample was estimated using Bradford (1976) method. The assay is chanelled on the report that the absorbance maximum of an acidic solution of Coomassie Brilliant Blue G-250 changes from 465 nm to 595 nm whilst interacting with a protein. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, resulting in a visible color change. The assay is constructive because the extinction coefficient of a dye-albumin complex solution is stable over a 10-fold concentration range.

3.4.1. Preparation of Acetone Powder

2 g of plant tissue was taken from leaves, roots and tuber forming roots respectively and was cut into small pieces (1-2 cm length). Homogenate the samples with chilled acetone using homogenizer (IKA®T18 basic ULTRA - TURRAX®). The homogenate was then filtered through Buchner funnel with Whatmann No.1 filter paper under suction. The residue was washed with diethyl ether. The powder on the funnel was dried with suction and air dried by spreading on Whatmann No.1 filter paper for about 1 hour.

3.4.2. Total protein extraction for protein quantification and enzyme assay

Sweet potato leaves and tuber were washed with distilled water and grated into a pre- chilled pestle and mortar containing liquid nitrogen and ground to a fine paste with the help of ice cold phosphate buffer (Appendix I) (pH 7.0, 2 ml per gram of fresh tissue). The homogenate was then transferred into a 20 ml centrifuge tube. The sample was spun for 2-3 minutes at 5000 rpm. The clear supernatant was transferred to a clean sterile tube and stored at 20°C till further analyzed. This clear extract was used as enzyme source for the assay by suitable method and to determine amount of protein in the sample.100 µl of protein extract was pipetted out into a test tube. To this 3ml Bradford reagent (Appendix I) was added. The contents of the tube was mixed thoroughly and kept for 10 minutes in normal room temperature. The absorbance was taken after 10 minutes at 595 nm. Three aliquots from each sample were used for the assay. The protein content was estimated using standard curve.

3.4.3. Protein profiling using SDS PAGE

The leaves of the control and test plants (under stress) were gathered and the protein fractions were kept ready for use followed by protein profiling of these fractions was analyzed by SDS-PAGE (Laemmli, 1970).

3.4.3.A. Gel casting

The electrophoresis glass plates, comb and spacers were cleanesed with acetone to eliminate any grease. The spacers were positioned amidst the two plates. The plates were then perfectly fixed on to the gel casting apparatus. The resolving gel (Appendix II) was poured using a Pasteur pipette between the glass plates making sure that there is no leakage. The resolving gel was placed on top of ethanol to cease oxygen from entering into the gel and hampering polymerization. After the polymerization finishes, the over lie was poured off and the top was washed repeatedly with deionized water to remove any unpolymerized acrylamide. Enough care was given to drain the rest of the fluid from the top of the gel and then the remnants were removed with the edge of paper towel. On the top of the polymerized resolving gel, the stacking gel (Appendix II) was applied. Immediately after pouring the stacking gel, the comb (Teflon comb) was placed on the gel to form well. After the polymerization was completed, the comb was slowly and carefully removed and taken in consideration not to tore the wells and the wells were immediately washed with deionized water to remove the traces of unpolymerized acrylamide.

3.4.3.B. Preparation of the sample

The protein extracted from the leaves and tubers, adopting the procedure stated above, were used for protein profiling. The protein concentration of the supernatant was adjusted such that an aliquot containing 200 µg/ml was taken in a centifuge tube and equal volume of the SDS sample bufferwas added (Appendix II). The tubes were then placed on a boiling water bath for 3 minutes and are cooled. Sample volumes containing 100 µg of the protein were used for a electrophoretic run. PageRuler ^{TM} unstained protein ladder (Fermentas life sciences, Thermo scientific) was also loaded and run along the samples each time, for comparing the molecular weight of the protein bands separated after electrophoretic study.

3.4.3.C. Electrophoretic run

The protein samples and ladder were loaded on to the corresponding wells. The reservoir buffer (Appendix II) was filled in the electrode chambers and the electrodes were then connected to the power pack. The apparatus were set initially at 150 V for 50minute until the dye front reached the resolving gel and later at 100 V till the dye front reached the end. After running the gel was removed and subsequent wash in distilled water and stained with the Coomassie blue solution (Appendix II).

3.4.3.D. Staining with coomassie blue

The gel was deep in at least 100ml of staining solution and kept overnight at normal room temperature. The gel was then taken for destaining by soaking it in the destaining solution 3-4 times (Appendix II). After destaining, the gels were saved in 20% glycerol in a sealed plastic bag. The molecular weight of the bands seen on the gel was analyzed by the comparison with the position of the molecular weight of marker proteins.

3.5. Physiological Parameters

3.5.1. Estimation of total chlorophyll and carotenoids

The total chlorophyll content of the leaves was estimated according Sadasivam and Manickam (1992). A weighed quantity of fresh leaf sample (0.5g) was taken and cut into small bits. These bits were put in test tubes and incubated overnight at room temperature with 10 ml DMSO: 80 % acetone mixture (1:1V/V). The coloured solution was decanted into a measuring cylinder and made up to 25 ml with the DMSO – acetone mixture. The absorbance was calculated at 663,645,470 and 510 nm using a spectrophotometer. The chlorophyll content was measured by substituting the absorbance values in the given formula:

$$\text{mg of chlorophyll a/g tissue} = 12.7\,(A663) - 2.69(A645)X\frac{V}{1000\ X\ W}$$

$$\text{mg of chlorophyll b/g tissue} = 22.9(A645) - 4.68(A663)X\frac{V}{1000\ X\ W}$$

$$\text{mg of total chlorophyll (per g tissue)} = 20.2\ (A645) + 8.02\ (A663) \times \frac{V}{1000XW}$$

$$\text{Total carotenoid} = \frac{(1000 \times A470) - (1.82 \times \text{chl a}) - (85.02 \times \text{chl b})}{198} X \frac{V}{1000 X W}$$

Where, A = Absorbance at specific wave lengths,

V = Net volume of chlorophyll extract in 80% acetone

And W = Fresh weight of the tissue extracted

3.5.2. Relative water content (RWC)

RWC was measured by calculating the fresh weight, dry weight and turgid weight of known number of leaf disc from the treatment plants. After measuring the fresh weight of the sample, it was submerged in the distilled water for 3 hours and then turgid weight was taken. The dry weight of the sample was measured after keeping the sample in the oven at 80°C for three consecutive days. The RWC of the treatment was calculated using the following formula.

$$RWC = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} X\ 100$$

3.6. Biochemical parameters

3.6.1. Estimation of Proline

Proline content was estimated according to Bates et al., (1973).The midribs of a leaf were taken and 500 mg of the fresh leaf tissue was weighed. It was homogenised with 10 ml of 3% sulphosalicylic acid (Appendix III) in a mortar and pestle. The homogenate was subsequently filtered through a Whatmann No.2 filter paper. This procedure was repeated again with the residue and the filtrates were pooled.

3.6.1.A. Estimation

2.0 ml of the filtrate was taken and 2.0 ml of acid ninhydrin (Appendix III) and 2.0 ml of glacial acetic acid were added. The tubes were incubated for 1 hour

at 100°C on a water bath. The tubes were transfered to an ice bath to cease the reaction. 4.0 ml of toluene was then added and mixed vigorously for 15 to 20 seconds. Thechromophore with toluene was aspirated from the aqueous phase. It was then allowed to reach room temperature and the absorbance was measured at 575 nm. A reagent blank was maintained. A standard curve was obtained using a known concentration of proline.

$$\mu\text{moles of proline/g tissue} = \frac{\mu\text{g proline/ml x ml toluene}}{115.5} \text{ x } \frac{5}{\text{g sample}}$$

Where, 115.5 is the molecular weight of proline.

3.7. Antioxidantenzyme assays

3.7.1. Glutathione reductase assay (GR, EC 1.6.4.2)

Glutathione reductase activity was found out by utilizing the method of Foyer and Halliwell (1976).The leaf tissue was homogenized in a pre-cooled mortar and pestle with the Glutathione reductase extraction mixture (Appendix IV), the homogenate was centrifuged at 15000 rpm for 20 minute and the supernatant was used for the enzyme assay.

3.7.1.A. Enzyme assay

2ml of Glutathione reductase assay mixture (Appendix IV) was taken in a test tube. The reaction was initiated by the addition of NADPH at 25°C and the absorbance was recorded at 310 nm. For 2-3 min with an interval of 15 seconds. One unit of GR is expressed as NADPH oxidized/ minute/gram. The GR activity was calculated by the formula.

$$\text{U /ml} = \frac{(\Delta A340nm/\text{ min})\ X\ 3\ X\ \text{wt of sample}}{6.22\ X\ \text{volume of enzyme}}$$

Where, Δ = change in absorbance

3 = volume of reaction mixture

6.22 = millimeter extinction co-efficient

3.7.2. Superoxide Dismutase assay (SOD, EC 1.15.1.1.)

The activity of superoxide dismutase was studied by the method of Beauchamp and Fridovich (1971). The leaf material was homogenized in a mortar and pestle with 5 volumes of the SOD extraction buffer (Appendix V) and the homogenate was centrifuged at 6000 rpm for 10 sec. The pellet was then washed with extraction buffer and centrifuged again. The supernatants were pooled and used for the enzymatic assy.

3.7.2.A. Enzyme assay

To the SOD assay mixture (2ml) (Appendix V), riboflavin was added as the last component and the reaction was started by placing the tubes under two 15-V fluorescent lamps. The reaction was ceased after 10 min by removing the reaction tubes from the light source. Both nonilluminated and illuminated reactions without supernatant served as calibration standards and the absorbance was measured at 560 nm. SOD is calculated by the formula,

$$Protein\ EU/mg = \frac{(\Delta A560nm/\ \text{min})\text{X}2.5\ X\ \text{wt of sample}}{0.0436\ X\ \text{volume of enzyme}}$$

Where, 0.0436 mμ = Extinction co-efficient

2.5 = volume of reaction mixture

EU = Enzyme Unit

3.7.3. Catalase assay(CAT, EC 1.1 1.1.6)

The activity of catalase was assayed by a modified method by Luck, (1974). The leaf material was homogenized in a pre-cooled mortar and pestle with 5volumes catalase extraction mixture (Appendix VI) and the homogenate was centrifuged at 8000rpm for 10 min at 4°C. The pellet was combined with cold phosphate buffer, allowed to stand with occasional stirring. The combined supernatants were used for the assay.

3.7.3.A. Enzyme assay

H_2O_2-phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the increased addition of 40µl of enzyme extract and is mixed thoroughly. The time procured for a reduction in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer. The enzyme solution with H_2O_2-free phosphate buffer was served control. One enzyme unit was measured as the amount of enzyme required to reduce the absorbance at 240nm by0.05 units. Catalase is calculated by the formula:

$$Protein\ EU/mg = \frac{(\Delta A240/\text{min}) \text{ X } 3.04 \text{ X wt of sample}}{0.036X \text{ volume of enzyme}}$$

Where, 3.04 = volume of reaction mixture

0.036= extinction co-efficient

3.7.4. Ascorbate peroxidase assay (APX, EC 1.1 1.1.1 1)

The enzyme activity of ascorbate peroxidase was assayed by the method of Nakano and Asada (1987). The leaves were homogenized in a pre-chilled pestle and mortar with the ascorbate peroxidise extraction buffer (Appendix VII) the homogenate was filtered through four layers of cheese cloth and then centrifuged at 20,000 rpm for30 min. The supernatants were used for the enzyme assay.

3.7.4.A. Enzyme assay

In the ascorbate peroxidise assay mixture (Appendix VII), the hydrogen peroxide-dependent oxidation of the ascorbate was trailed by a reduction in the absorbance at 290 nm. The enzyme activity was calculated using the equation,

$$\text{Protein EU/mg} = \frac{(\Delta A290/\text{min}) \text{ X } 2 \text{ X wt of sample}}{2.8\ X \text{ volume of enzyme}}$$

Where, 2.8 = Extinction co-efficient

2 = volume of reaction mixture

3.7.5. Assay for Ascorbate Free Radical Reductase (EC 1.6.5.4)

The activity of ascorbate free radical reductase was assayed by the method of (Hossain et al., 1984). The frozen leaf tissue of cassava of 1 *g* f was powdered in liquid N, using a pestle and mortar and then homogenized at 0°C for 4 min in ascorbate free radical reductase assay buffer 10 ml (Appendix VIII)using a Tekmar homogenizer. Extracts were then centrifuged down at full speed in a bench-top centrifuge for 30 min at 0°C, and the supernatants were squeezed in through Mira cloth.

3.7.5.A. Enzyme assay

15-pLaliquots were assayed in a final volume of 1mL. AFR reductase activity was procured spetrophtometrically by trailing the reduction in A340due to NADH oxidation.

3.8. Non enzymatic Anti-oxidant Assay

3.8.1. Estimation of Ascorbic Acid

Ascorbic acid content was estimated volumetrically by the method of Sadasivam and Manickam (2008).

3.8.1.A. Extraction of Ascorbic acid

Fresh leaf was homogenized with 5 ml of 4% oxalic acid in arepeatedly pestle and mortar. The homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was saved and the pellet was re extracted repeatedly with the same volume of the solvent. The supernatants were pooled and used for estimation of net content of ascorbic acid.

3.8.1.B. Estimation of Ascorbic acid

Working standard solution (5 ml) was pipetted out into a 100 ml conical flask. 4% oxalic acid was added to it and titrated against the dye (V_1 ml). End point was noted on the first appearance of pink colour which continued for a few minutes. The sample (0.5 – 5g) was weighed and ground in a mortar with pestle using 15 ml 4% oxalic acid. The homogenate was filtered through a double

layered cheese cloth. The filtrate was made up to a known volume and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and made up to 25 ml using oxalic acid. 5.0 ml aliquot was pipetted into a conical flask to which 10 ml of 4% oxalic acid was added. This was titrated against Dichlorophenol indophenols (DCPIP) solution, until the appearance of pink colour (V_2 ml.)

$$\text{Amount of ascorbic acid } \left(\frac{\text{mg}}{100\text{g}}\text{sample}\right) = \frac{0.5}{V1ml} X \frac{V2}{5ml} X \frac{100}{wt.of\ sample} X100$$

Whereas, V_1 = volume of standard

V_2 = volume of sample

3.8.2. Estimation of reduced glutathione

Reduced glutathione was estimated by the method of Moron et al. (1979). Reduced glutathione on reaction with DTNB (5, 5'-dithiobis nitro benzoic acid) produces a yellow coloured product that absorbs at 412nm. The homogenate was prepared with 0.5g of the plant sample with 2.5ml of 5% TCA. The precipitated protein was centrifuged at 1000rpm for 10 minutes. The supernatant (0.1ml) was used for the estimation of GSH. The supernatant (0.1ml) was made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 M were also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer at 412nm after 10 minutes. The values were expressed as moles GSH/g sample.

RESULTS

4. RESULTS

The present study entitled the "Oxidative stress and protein profiling in sweet potato (*Ipomoeabatatas* (L.) Lam.) Under abiotic stresses" was conducted during the period 2014-2015 at the, CTCRI, Division of Crop Production and the Department of Plant Biotechnology, CoA, Vellayani, Tvm. The results obtained from the study are summarised below.

4.1. GROWTH PARAMETER

4.1.1. Changes in the vein length of five sweet potato varieties/ genotypes as affected by WDS and hightemperature stress conditions

Under control condition the vein length of five varieties/ genotypes of sweet potato was measured for 120 days at interval of 30 days and the results are given in (Fig. 1). At 30 days after planting (DAP), the average vein length of five varieties recorded was viz., Sree Arun (74 ± 4.39 cm), S-1466 (30 ± 8.26 cm), Sree Kanaka (21 ± 5.29 cm), Kanjangad (56.83 ± 28.26 cm) and S-1464 (31.33 ± 5.03 cm). The maximum vein length was observed in the variety Sree Arun (74 ± 4.39 cm) and the variety Sree Kanaka had the minimum vein length (21 ± 5.29 cm). After 60 DAP, the average vein length of five varieties of sweet potato increased: Sree Arun (117.67 ± 12.42 cm), S-1466(79.67 ± 8.02 cm), Sree Kanaka (87.33 ± 30.99cm), Kanjangad (118.67 ± 15.50 cm) and S-1464 (93.67 ± 4.50 cm). After 60 DAP, the vein length of the variety Kanjangad showed increased to (118.67 ± 15.50 cm) and minimum change in vein length was observed in the variety S-1466 (79.67 ± 8.02 cm). At 90 DAP, the average vein length of varieties increased significantly viz., Sree Arun (157.64 ± 34.42 cm), S-1466(121.54 ± 4.25 cm), Sree Kanaka (116.22 ± 32.01 cm), Kanjangad (135.46 ± 8.57 cm) and S-1464 (117.33 ± 7.63 cm). From the study it was concluded that the variety Sree Arun had the maximum average vein length (157.64 ± 34.42 cm) and the variety Sree Kanaka had the minimum average vein length (116.22 ± 32.01 cm). Under 4 days after WDS during 2nd and 3rd month (Fig. 2) and high

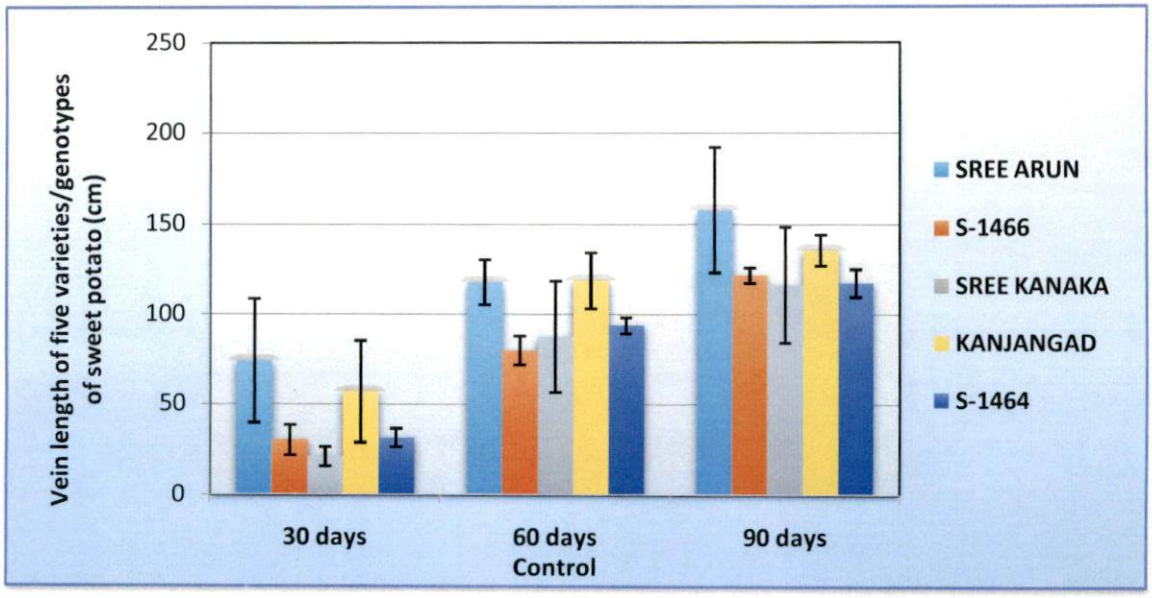


Fig. 1. Changes in the vein length of five varieties/ genotypes of sweet potato under irrigated condition

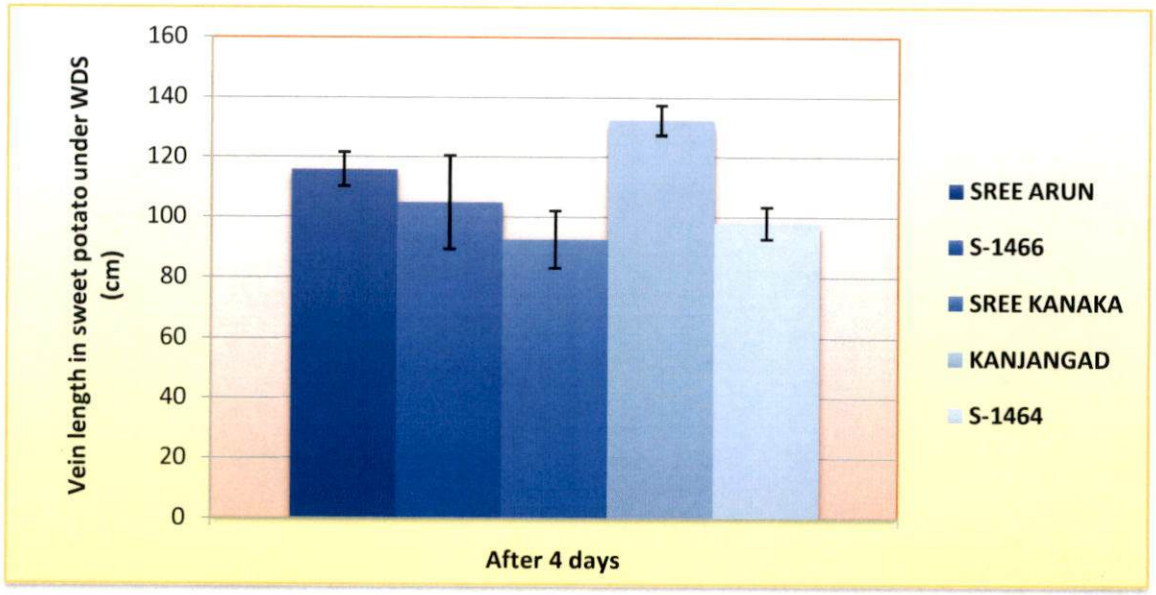


Fig. 2. Changes in the vein length of five varieties/ genotypes of sweet potato after WDS condition

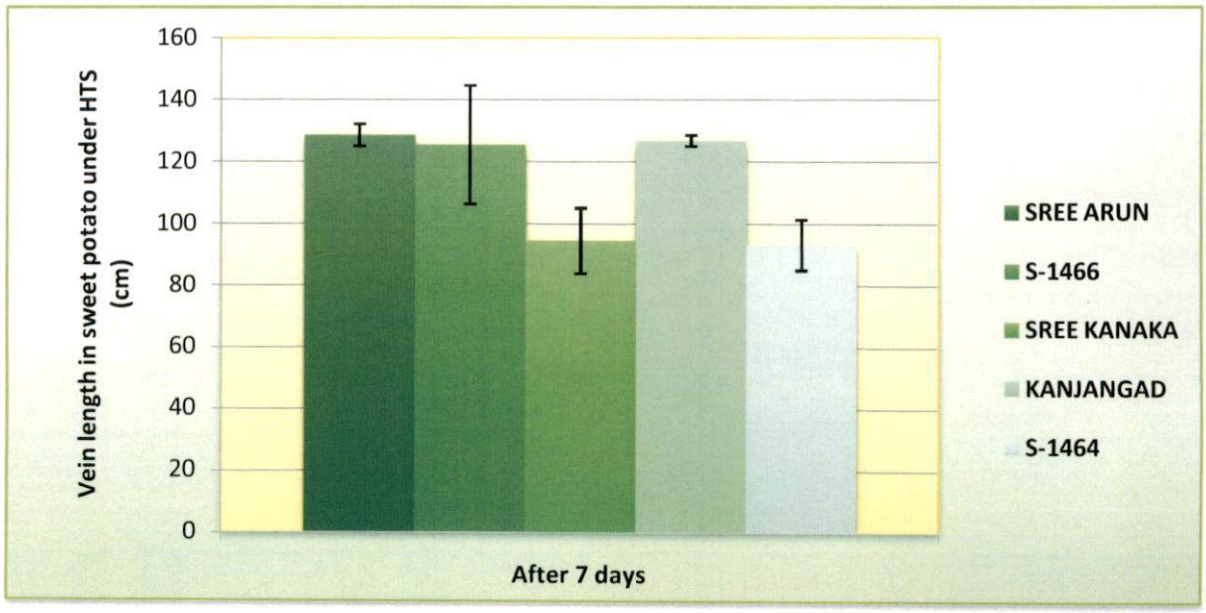


Fig. 3. Changes in the vein length of five varieties/ genotypes of sweet potato after heat stress condition

temperature stress condition during 2nd and 3rd month (Fig. 3) the five varieties/ genotypes did not show much variation in vein length.

4.1.2. Changes in the leaf production of five sweet potato varieties as affected by WDS andhigh temperature stress conditions

Under control condition plant leaf production was recorded between 30 and 120 days and depicted in (Fig. 4). It was observed that at 30 DAP, the average leaf production per plant was viz., variety Sree Arun (66.33 ± 8.73), S-1466 (59.66 ± 14.74), Sree Kanaka (55.33± 26.50), Kanjangad (51.66 ± 10.59) and S-1464 (50.33 ± 7.37). The average leaf production per plant of the five sweet potato varieties varied between (66.33± 8.73) and (50.33 ± 7.37). At 30 DAP, the maximum leaf production was observed in the variety Sree Arun, and minimum leaf production was observed by the variety Pale green. At 60 DAP, the maximum average leaf production was observed in the variety Sree Arun (136 ± 29) and minimum leaf production was observed in the variety Sree Kanaka (66.66 ± 14.04). At 90 DAP,the leaf production increased when compared to other two months. Under 4 days of WDS applied during (2nd and 3rd month) there was a reduction in the leaf production in all the varieties viz., Sree Arun (72.5 ± 10.60), S-1464 (91 ± 11.31), S-1466 (97.5 ± 6.36), Sree Kanaka (64.5 ± 12.02) and Kanjangad (98 ± 28.28) compared to the 90 DAP. Similar pattern was recorded in the case of high temperature stress condition during (2nd and 3rd month) for 7 days and the leaf production was reduced to Sree Arun (83.5 ± 14.84), S-1464 (81 ± 18.38), S-1466 (71.5 ± 19.09), Sree Kanaka (79.5 ± 7.77) and Kanjangad (92 ± 4.24). From the study it is concluded that the average leaf production was reduced under both stress conditions WDS (Fig. 5) and high Temperature Stress (Fig. 6) compared to stress free conditions.

4.1.3. Changes in leaf retention percentage of five sweet potatovarieties/ genotypesas affected by WDS and high temperature stress conditions

Leaf retention percentage of five varieties/genotypes of sweet potato*viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangad was estimated for 90

days with an interval of 30 days. At 30 DAP the variety Sree Kanaka had the maximum leaf retention (46.47 ± 11.03%) and the minimum leaf retention was observed in the variety Sree Arun (23.05 ± 11.03%) (Fig. 7). At 60 DAP, there was a reduction in leaf retention in the variety Sree Kanaka (32.33 ± 10.59%) whereas there was a elevation in leaf retention in the variety Sree Arun (35.33 ± 12.50%). The maximum leaf retention was observed in the variety Sree Arun at 60 DAP (35.33 ± 12.50%) and minimum leaf retention was observed in Kanjangad (21.33 ± 8.62%). After 90 DAP there is increase in leaf retention in the variety Kanjangad (41.04 ± 12.50%) and minimum leaf retention was observed in the variety S-1466 (17.39 ± 4.46%). At 4 days after WDS for 2^{nd} and 3^{rd} month it was observed that the variety Kanjangad (84.49 ± 6.93%) had the maximum leaf retention percentage and minimum leaf retention percentage was observed in variety Sree Kanaka (57.02 ± 0.64%) (Fig. 8). In plants under HTS for 7 days applied during 2^{nd} and 3^{rd} month it was found that the variety Kanjangad (66.16 ±6.32%) and minimum leaf retention was observed in the S-1466 (65.45 ± 4.37%) (Fig. 9). From the study it was concluded that the variety Sree Kanaka had the maximum leaf retention percentage under stress conditions.

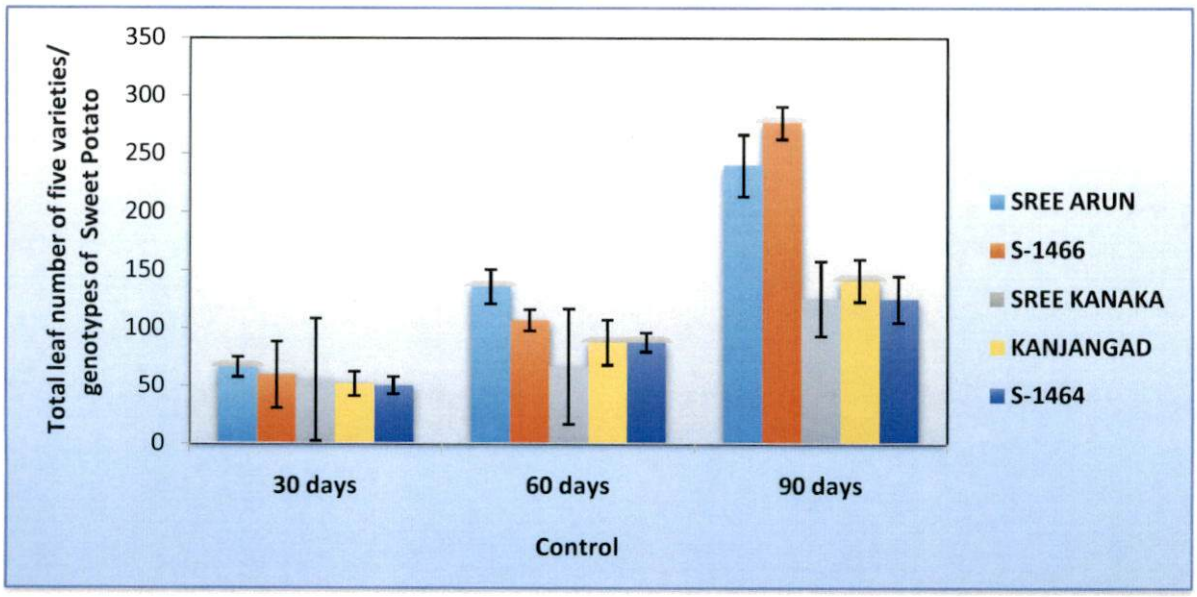


Fig. 4. Changes in the leaf production of five varieties of sweet potato under control condition

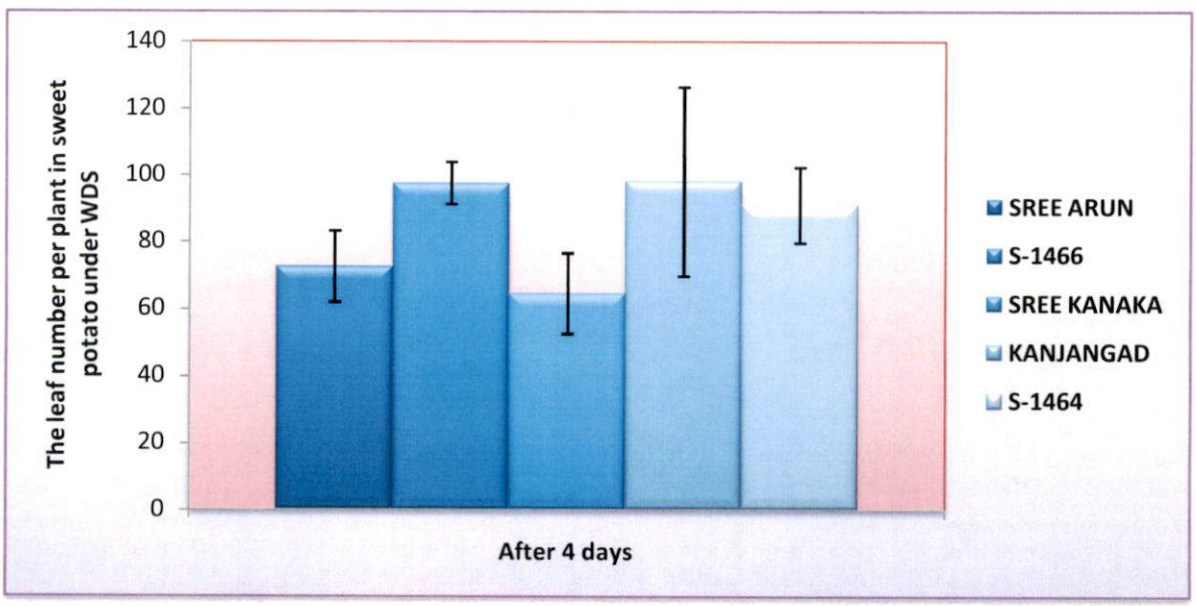


Fig. 5. Changes in the leaf production of five varieties of sweet potato under WDS condition

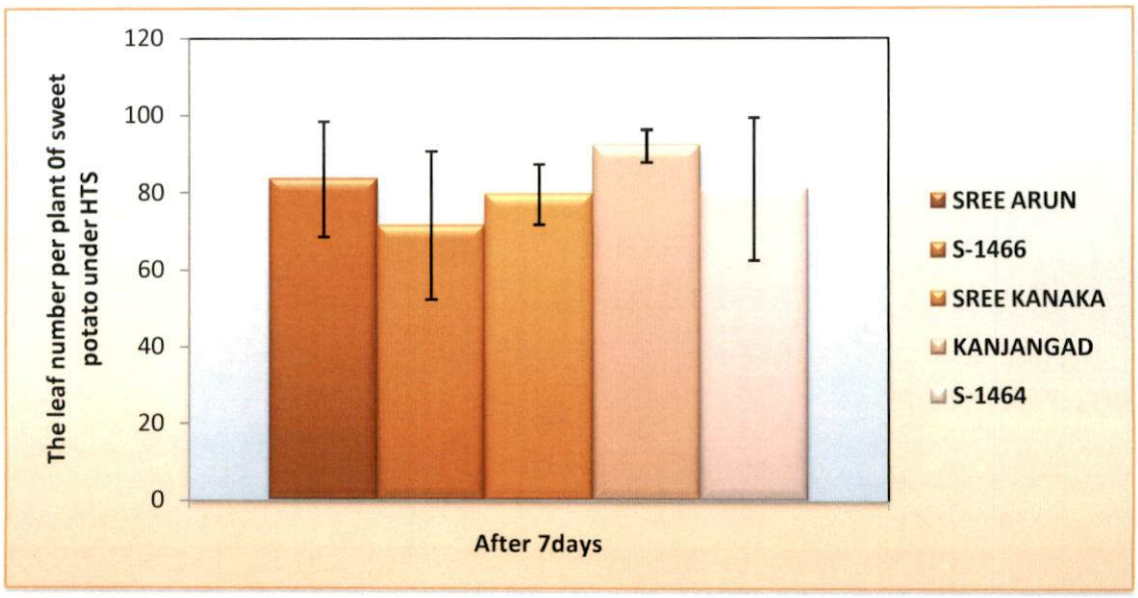


Fig. 6. Changes in the leaf production of five varieties of sweet potato after HTS condition

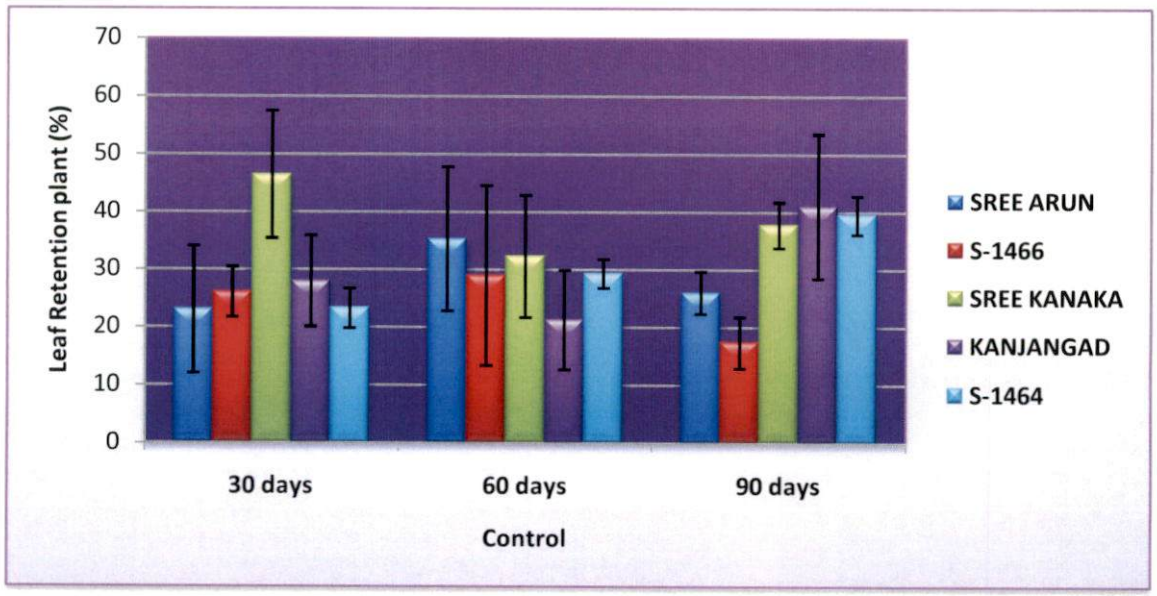


Fig. 7. Changes in the leaf retention in five varieties /genotypes of sweet potato under control condition

4.2. YIELD PARAMETER

4.2.1. Tuber Number

The total number of tubers per plant was recorded under control condition, WDS and high temperature stress conditions (Table 1, Plate 3). In control condition the variety Sree Arun (5.66 ± 0.57) and Sree Kanaka (4.33± 1.52) recorded the maximum number of tuber per plant and S- 1464 had minimum number of tuber per plant (3.33 ± 1.15). In the plants under WDS conditions day 2nd and 3rd month, the variety Sree Kanaka and S- 1466had the maximum tuber number (5.00 ± 1.00), (5.00 ± 1.00) and Kanjangad had the minimum tuber number (4.00 ± 1.73). In the plants under high temperature stress treatment after 2th and 3ih month, it was observed that the Sree Arun had the maximum number of tubers (5.00 ± 1.00) and minimum number of tubers were observed in Sree Kanaka(4.33 ± 1.52), Kanjangad (4.33 ± 0.577) and S- 1464 (4.33 ± 0.57). From the present study it is concluded that plants under controlcondition will produce almost equal number of tubers compared to WDS and high temperature stress conditions.

4.2.2. Mean Tuber Weight

The mean tuber weight per plant was recorded under control condition, WDS and high temperature stress conditions (Table 2). Maximum tuber weight was recorded for the variety Kanjangad under control condition (5.19 ± 0.22) and minimum tuber weight was observed in the variety S- 1464 (1.98 ± 0.44). For the plants under WDS condition during 2nd and 3rdmonth, it was observed that the variety Kanjangad had the maximum tuber weight (4.75 ± 0.39) and S-1464 had the minimum tuberweight (1.78 ± 0.53). Similar results were found inplantsunder high temperature stress during 2nd and 3rdmonth. Kanjangad had the maximum mean tuber weight (5.09 ± 0.19) and minimum mean tuber weight was found in S-1464 (1.97 ± 0.42). From this study it was found that there is almost no or negligible difference in the mean tuber weight in both treatment condition (WDS and high temperature stress) and control conditions.

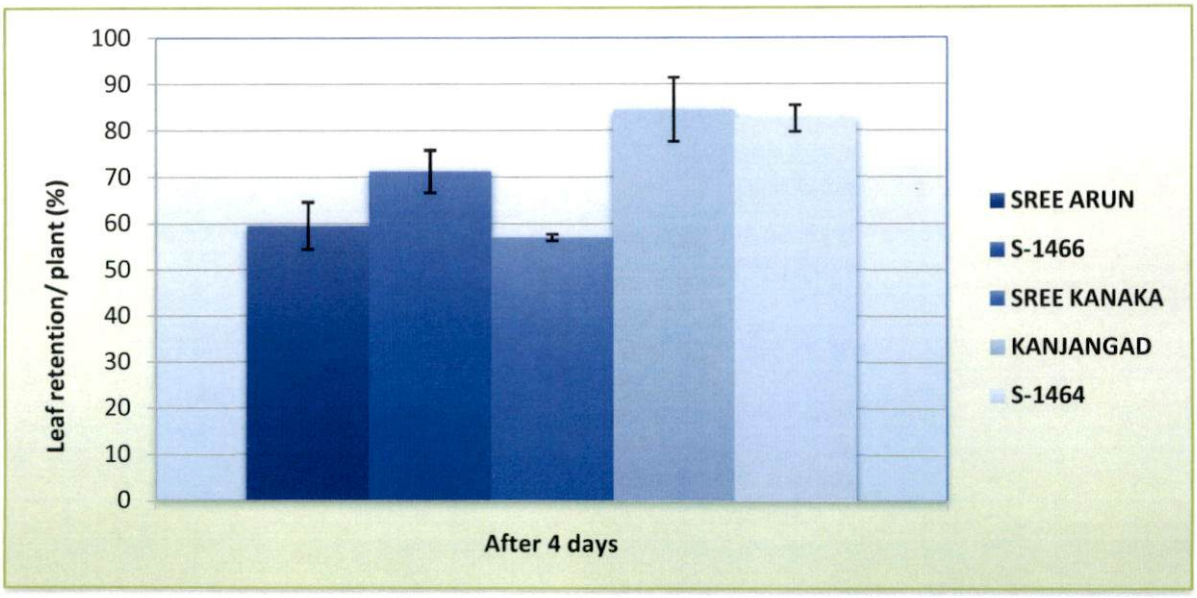


Fig.8. Changes in the leaf retention in five varieties/genotypes of sweet potato after WDScondition

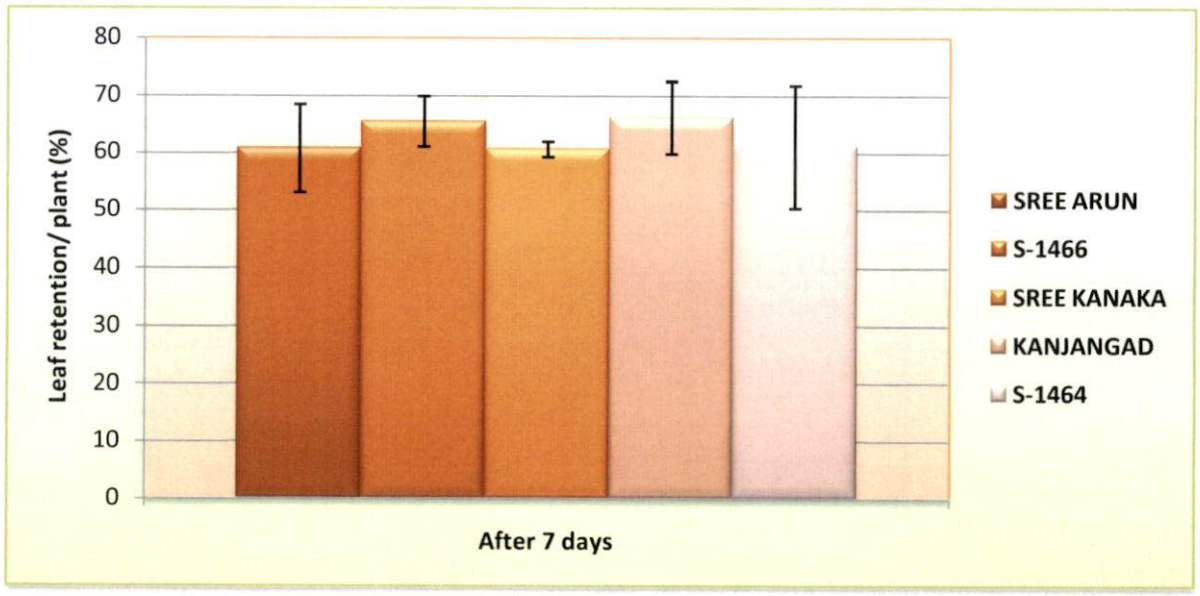


Fig. 9. Changes in the leaf retention in five varieties/genotypes of sweet potato under HTS condition

Table. 1. Changes in number of tuber under different treatments and control condition

Variety/genotype	Number of tubers per plant		
	CONTROL	HTS	WDS
Sree Arun	5.66 ± 0.57	5.00 ± 1.00	4.66 ± 0.57
Purple	4.00 ± 1.00	4.66 ± 0.57	5.00 ± 1.00
Sree Kanaka	4.33 ± 1.52	4.33 ± 1.52	5.00 ± 1.00
Kanjangad	4.00 ± 1.00	4.33 ± 0.57	4.00 ± 1.73
Pale Green	3.33 ± 1.155	4.33 ± 0.57	4.33 ± 1.15

Table. 2. Changes in mean tuber weight under different treatments and control condition

Variety/genotype	Mean Tuber Weight (Kg/plant)		
	CONTROL	HTS	WDS
Sree Arun	3.12 ± 0.82	2.97 ± 0.60	2.89 ± 0.58
Purple	2.52 ± 0.48	2.4 ± 0.48	2.36 ± 0.42
Sree Kanaka	3.02 ± 0.49	2.98 ± 0.43	2.96 ± 0.47
Kanjangad	5.19 ± 0.22	5.09 ± 0.19	4.75 ± 0.39
Pale Green	1.98 ± 0.44	1.97 ± 0.42	1.78 ± 0.53

Plate 3. Tubers of five varieties / genotype of sweet potato

4.3 ESTIMATION OF PROTEIN

4.3.1 In the leaves total content of protein of five sweet potato varieties as affected by water deficit stress (WDS) and high temperature stress condition

Total protein was extracted from the leaves, tubers and tuber forming roots of five sweet potato genotypes *viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and kanjangad grown under irrigated conditions, stress condition, water deficit stress (WDS) and high temperature stress, and was quantified (Fig. 10). In stress free condition it was observed that Kanjangad had the maximum total leaf protein content (31.14 ± 0.07mg/g leaf) compared to other four varieties Sree Arun (24.74 ± 0.02 mg/g leaf), Sree Kanaka (26.77 ± 0.01 mg/g leaf), S-1466 (23.12 ± 0.01 mg/g leaf) and S-1464 (24.52 ± 0.01 mg/g leaf). Under WDS applied during 2nd and 3rd month, it was observed the there was reduction in total protein content of all five varietiessweet potato viz., Sree Arun (14.37 ± 0.02 mg/g leaf), S-1466 (18.31 ± 0.11 mg/g leaf), Sree Kanaka (15.87 ± 0.01 mg/g leaf), Kanjangad (17.11 ± 0.01 mg/g leaf) and S-1464 (12.89 ± 0.01 mg/g leaf) compared to stress free condition. Plants under high temperature stress condition it was found that the two varieties S-1466 (29.86 ± 0.08 mg/g leaf) and S-1464 (28.74 ± 0.03 mg/g leaf) had high protein content compared to stress free condition. From the study it was concluded that total leaf protein content varied in control and treatment condition. The result of total protein content was statistically analyzed using ANOVA and it was found that significantly difference between ($P > 0.001$) treatments. Where compared using Tukey contrast and it was clear that protein content in plants varied significantly ($P > 0.001$) in WDS condition compare to control and HTS condition.

4.3.2. Total protein content in tubers of five sweet potato varieties as affected by water deficit stress (WDS) and high temperature stress condition

The total protein was extracted from the tubers of five sweet potato varieties /genotypes *viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangad grown under irrigated and stressed conditions (WDS and high temperature stress)

(Fig. 11). In control it was observed that the variety Sree Kanaka (14.57 ± 0.31 mg/g tuber) had maximum tuber protein content compared to other four varieties Sree Arun (5.28 ± 0.34 mg/g tuber), S-1466 (2.30 ± 0.04 mg/g tuber), Kanjangad (7.17 ± 0.05mg/g tuber) and S-1464 (4.59 ± 0.25 mg/g tuber). Similar result were observed in the case of plants subjected to WDS conditions during 2nd and 3rd month it was observed that the variety Sree Kanaka had the maximum protein content (14.78 ± 0.68 mg/g tuber) match up to other four varieties and smallest amount protein content was experimental in the variety S-1466 (2.50 ± 0.11 mg/g tuber). Under high temperature stress during 2nd and 3rd month it was observed that the variety Sree Kanaka had the maximum protein content (15.20 ± 1.17 mg/g tuber) and minimum protein content were observed in the variety S-1466 (2.26 ± 0.16 mg/g tuber). S-1464 under high temperature stress condition had more tuber protein content (5.56 ± 0.87 mg/g tuber) compared to the plant in WDS condition (4.63 ± 0.76 mg/g tuber). From the study it is concluded that total tuber protein content varied negligibly between the stress and stress free condition.The result of total tuber protein content was statistically analyzed using ANOVA and it was found that there was no in significant difference between the treatments.

4.3.3. Total protein content in tuber forming roots of five sweet potato varieties as affected by water deficit stress (WDS) and high temperature stress condition

Total protein was extracted from the tuber forming roots of five sweet potato varieties/ genotypes *viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangad grown under irrigated and stress conditions (WDS and high temperature stress) (Fig. 12). It was observed that under control condition the variety Sree Kanaka (16.55 ± 0.13 mg/g protein) had the maximum protein content and minimum protein content was observed in the variety S-1464 (6.61 ± 0.78 mg/g protein). In plants subjected to WDS condition during 2nd and 3rd month it was observed that the the variety Sree Kanaka (16.844 ± 0.901 mg/g protein) showed maximum protein content and minimum protein content were observed in the variety S-1464 (6.85 ± 0.57 mg/g protein). In plants under high

temperature stress during 2nd and 3rd month it was observed that the variety Sree Kanaka (15.79 ± 0.87mg/g protein) had the maximum protein content and minimum protein was observed in S-1464 (6.64 ± 0.91 mg/g protein). From the study it is concluded that the varieties had maximum protein content under WDS condition compared to high temperature stress and stress free conditions. The result of total protein content in tuber forming roots of sweet potato was analyzed using ANOVA and the protein content significantly differed between($P > 0.001$) the treatments but there was no significant difference between the varieties.

4.4 PROTEIN PROFILING

4.4.1. Changes in the profile of leaf proteins as affected by WDS and high temperature stress conditions

Protein extracted from the leaves of five varieties of sweet potato under control and stress (WDS and high temperature stress) conditions. The protein profiling was done by PAGE. Protein extracted was loaded keen on the wells of SDS gel along with the marker protein. After the electrophoretic run for 50 minutes at 150V the gel was removed, stained in staining solution and kept overnight for destaining. After destaining, it was observed that there were no protein bands observed in both treatment condition (WDS and high temperature stress) and stress free condition. From the study it is concluded that presence of mucilage in sweet potato appears to trap the protein and this prevented clear band formation in the gel.

4.4.2. Changes in the profile of tuber proteins as affected by WDS and high temperature stress conditions

Total Protein was extracted from the tubers of five varieties of sweet potato under control and stress (WDS and high temperature stress) conditions. The protein profiling was done by PAGE. Proteins extracted were loaded into the wells of SDS gel along with the marker protein. After the electrophoretic run for 50 minutes at 150V the gel was removed, stained in staining solution and kept overnight for destaining. After destaining, it was observed that plants under stress

free condition had significant protein bands indicating high protein content in stress free condition (Plate 4). The variety Sree Kanaka showed protein band in the region between50 KDaand the variety Sree Arun and Kanjangad showed band at the region near to 40 KDa and the variety S-1466, Sree Kanaka, Kanjangad and S-1464 had the band at the region near to 60KDa and the variety Kanjangad showed clear band at the region near to 70 KDa. At 4 days after WDS applied during 2nd and 3rd month protein bands were observed that significant bands were observed in the varieties Sree Kanaka, Kanjangad and S-1464 for which protein band were obtained at the region between 60 KDa and the variety S-1466 showed band at the region near to 30KDa and the variety Sree Arun and S-1466 showed very thin band near to 10 KDa (Plate 5). Bands obtained are not much clear compared to the plants under stress free condition. Under high temperature stress condition during 2nd and 3rd it was observed that there were no bands were obtained for all the five varieties of sweet potato indicating low protein content due to denaturation of protein overnight for destaining. After destaining, it was observed that plants under stress free condition obtain significant protein bands on the gel and it showed high protein content in tuber forming root. The variety of Sree Arun, S-1466, Sree Kanaka, Kanjangad and S-1464 showed clear bands near to the 60KDa (Plate 6). The WDS and HTS condition do not obtain significant protein bands on the gel because of low protein content in tuber forming root.

4.5. PHYSIOLOGICAL PARAMETER

4.5.1. Change in the relative water content of five varieties of sweet potato as affected byWDS and high temperature stress conditions

Under control condition relative water content of five varieties of sweet potato was estimated during first, 2nd and 3rd month. First month it was observed that the varieties Kanjangad and Sree Kanaka had the higher RWC 70.98% and 68.70% and it was found during 3rd month andminimum RWC showed by the varieties Sree Arun (52.89 %) and S-1464 (59.45 %) respectively (Fig. 13). At WDS condition applied during2nd and 3rdmonth it was observed that RWC reduced drastically. The variety Kanjangad had the maximum RWC (59.10%) and the

variety Sree Arun had the minimum RWC (43.30%) (Fig.14).Under hightemperature stress applied during 2nd and 3rdmonth it was observed that the RWC is high compared to WDS treatment. The high temperature stress maximum RWC showed by the variety S-1464 (63.53%) and minimum RWC showed by the variety Sree Arun (52.82%) (Fig.15).From the study it was concluded that RWC reduced at WDS and high temperature stress compared to control condition.

4.5.2. **Changes in total chlorophyll and carotenoid content in the leaves of five varieties of sweet potato as affected by WDS and high temperature stress conditions**

The total chlorophyll (Chl) content of the leaves of five varieties of sweet potato was estimated in control and under stress conditions (Fig. 16). In control plants the total Chl content varied between (0.21 ± 0.004mg/g) and (0.01 ± 0.002mg/g). Maximum Chl content was observed in the variety Sree Arun (0.21 ± 0.004 mg/g) and the minimum Chl content was observed in the variety S-1464 (0.01 ± 0.002mg/g). Under water deficit stress (WDS) during 2nd and 3rdmonth the variety Sree Kanaka had the maximum Chl content (0.16 ± 0.09 mg/g) and S-1464 had the minimum Chl content (0.05 ± 0.004 mg/g). Compared to control the Chl content in the variety Sree Arun was reduced drastically under WDS conditions. When plants were subjected to high temperature stress conditions during 2nd and 3rdmonth the maximum Chl content was observed in the variety Sree Arun (0.09 ± 0.004 mg/g) and minimum Chl was in the variety S-1464 (0.02 ± 0.005 mg/g). From the results it isclear that under high temperaturestress plants had more chlorophyll degradation than water deficit stress in all the varieties. The total chlorophyll content in leaves of sweet potato plant varieties significantly decreased under control and high temperature stress conditions compared to plant under WDS conditions.

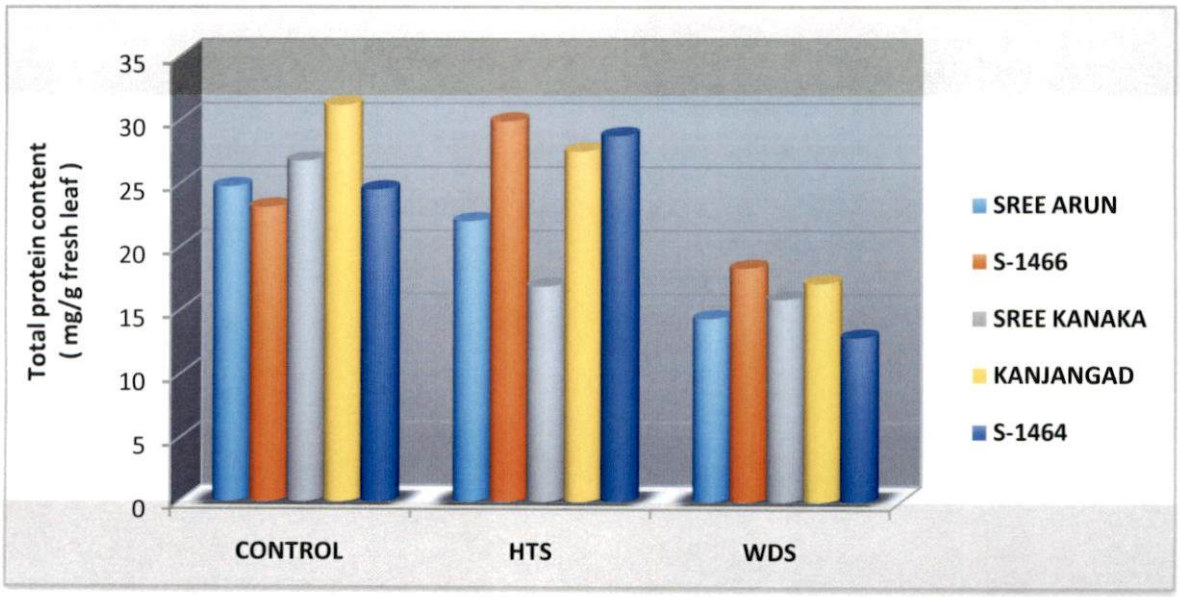


Fig. 10. Changes in leaf protein content of five sweet potato varieties/genotypes as affected by WDS and high temperature stress condition

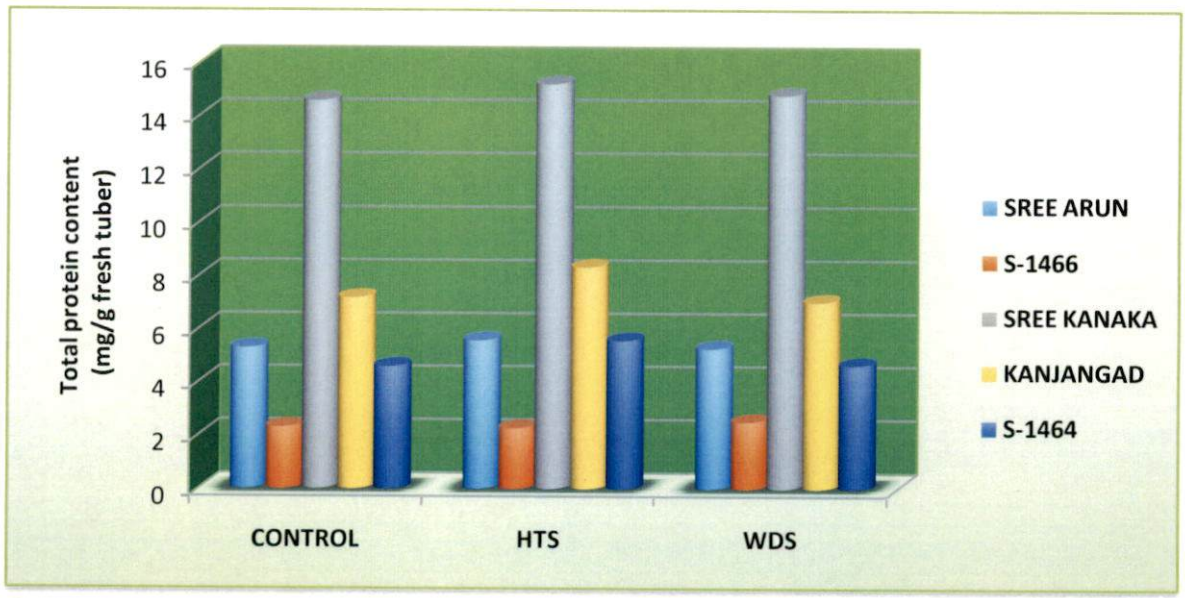


Fig.11. Changes in tuber protein content of five sweet potato varieties/genotypes as affected by WDS and high temperature stress conditions

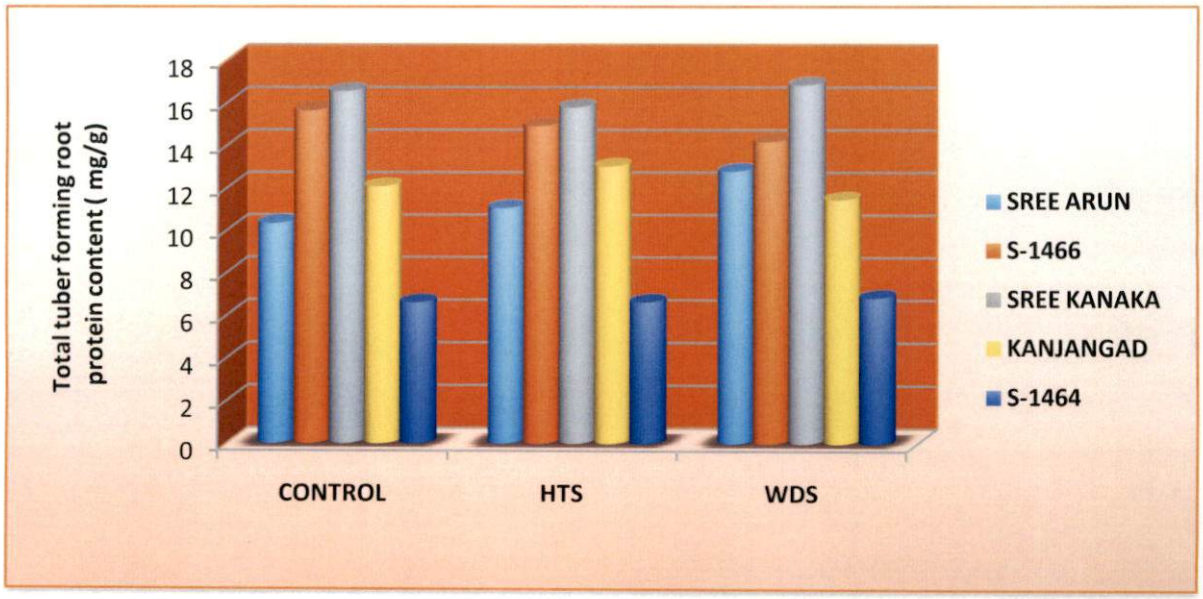


Fig. 12. Changes in tuber forming roots protein content of five sweet potato varieties/ genotypes as affected by WDS and high temperature stress condition

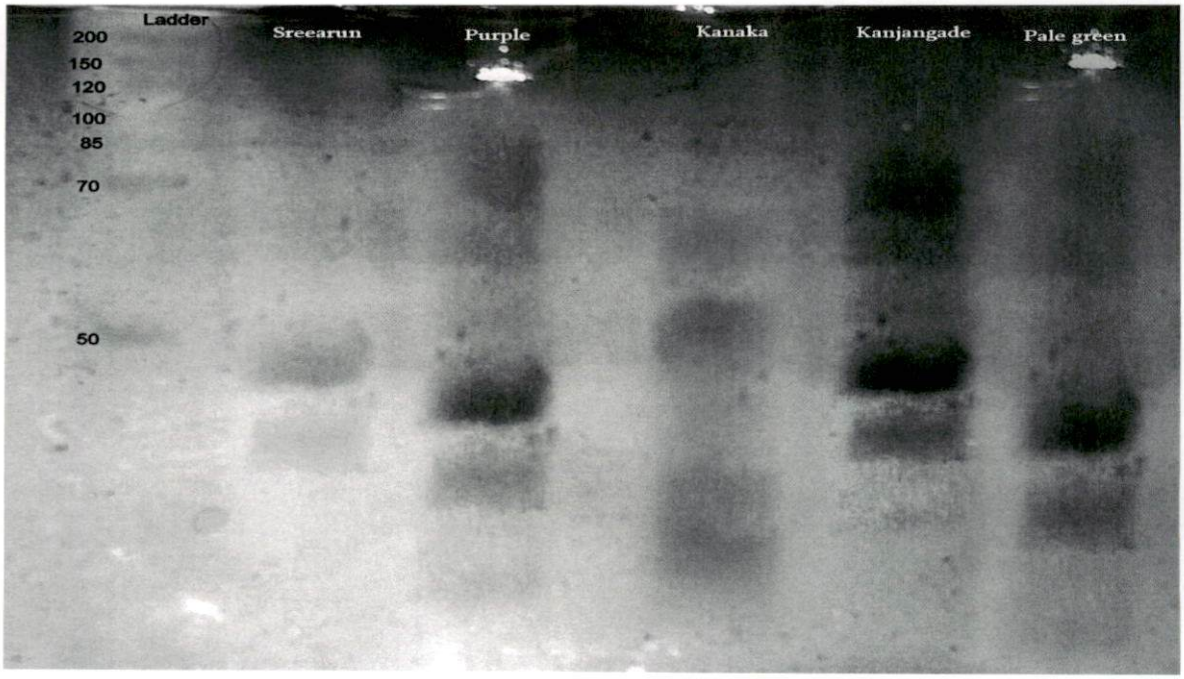


Plate 4. Changes in the total tuber protein profiling pattern in five varieties/ genotypes of sweet potato under control condition

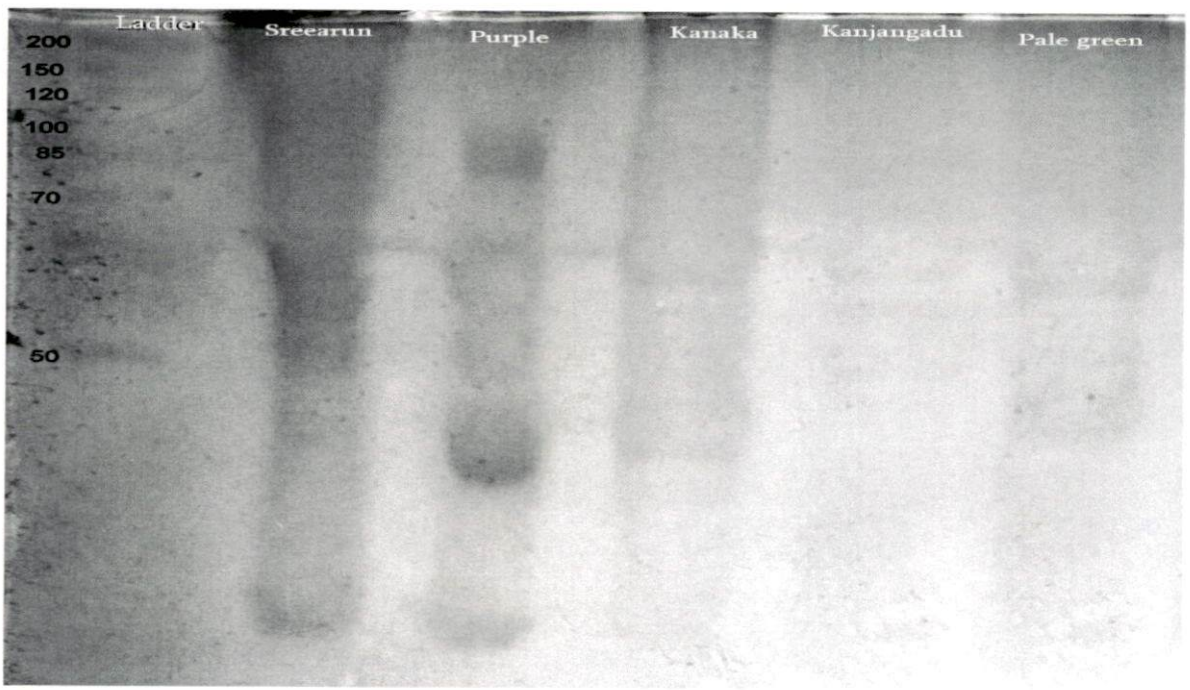


Plate 5. Changes in the total tuber protein profiling pattern in five varieties/ genotypes of sweet potato under WDS condition

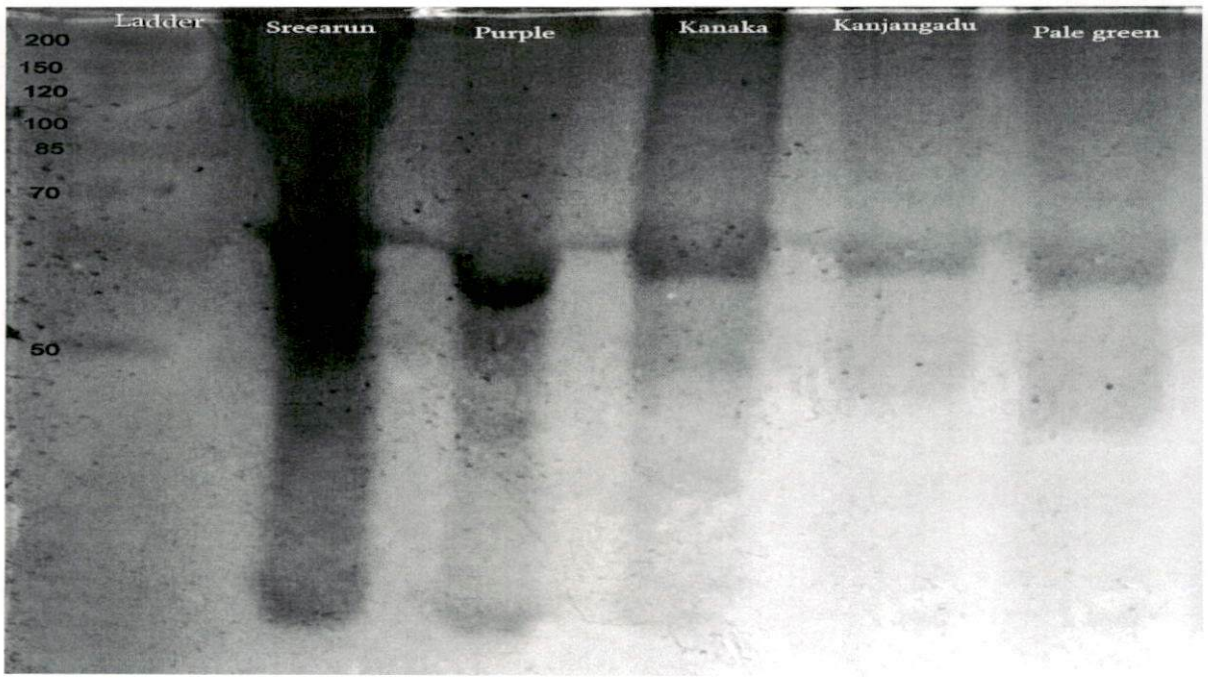


Plate 6. Changes in the total tuber forming root protein profiling pattern in five varieties/ genotypes of sweet potato under stress free condition

Maximum carotenoid content was observed in the variety S-1466 (0.06 ± 0.002mg/g) undercontrol conditions. Again heat stress led to more degradation of carotenoid than water deficit stress in all varieties (Fig.17). The result of carotenoid content was analysed using ANOVA.There was significant difference ($P > 0.01$) in carotenoid content in plants between treatment Comparing the means of different treatments using Tukey contrast it was clear that there was significant decrease in carotenoid content of heat stressed plants from that of control and theresult of chlorophyll contentwas analysed using ANOVA and it was observedthat there wassignificantdifference ($P > 0.01$) in chlorophyll content in plants under different treatment. On comparing the means using Tukey contrast it was found that chlorophyll content of plants under high temperature stress differed significantly from that of control ($P > 0.01$) and WDS ($P > 0.05$) plants. The chlorophyll content in the variety S-1464 significantly high than that of the varieties Sree Arun and S-1466 ($P > 0.001$), Sree Kanaka ($P > 0.05$).

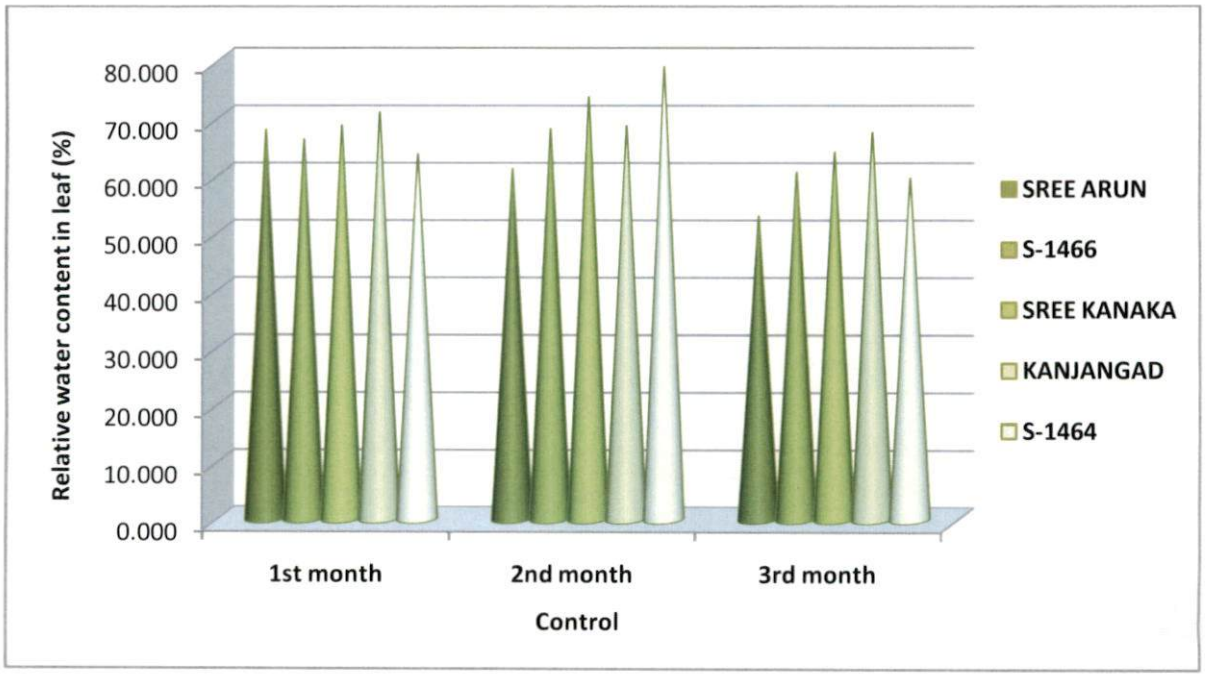


Fig. 13. Changes in the relative water content of five varieties/ genotypes of sweet potato under control Condition

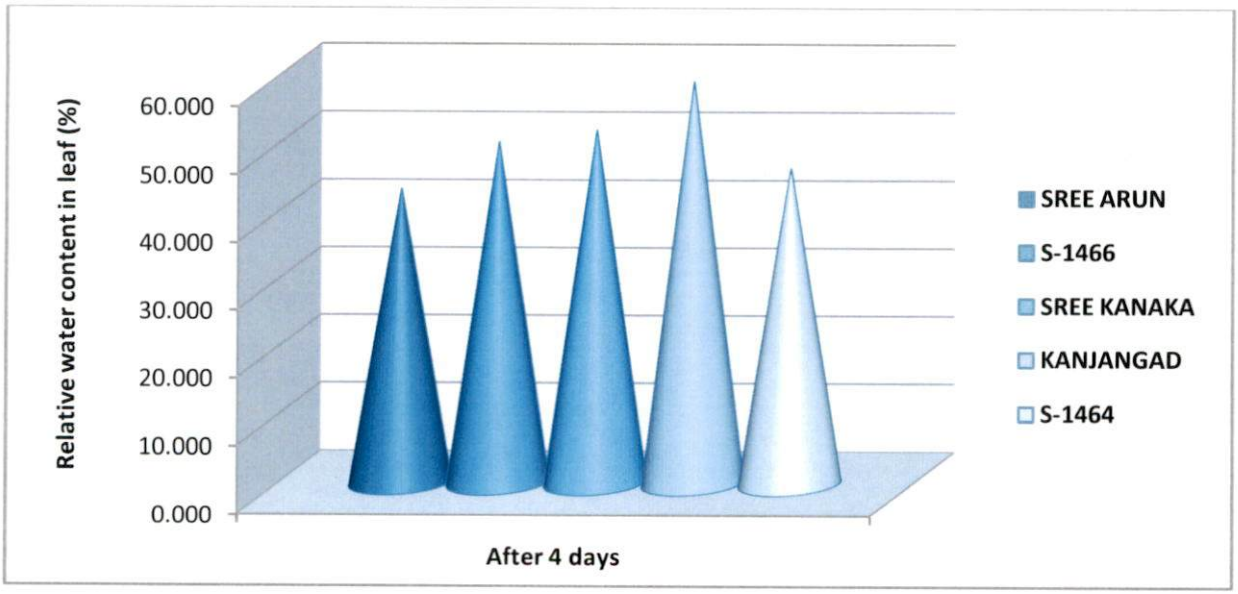


Fig. 14. Changes in the relative water content of five varieties/ genotypes of sweet potato under WDS Condition

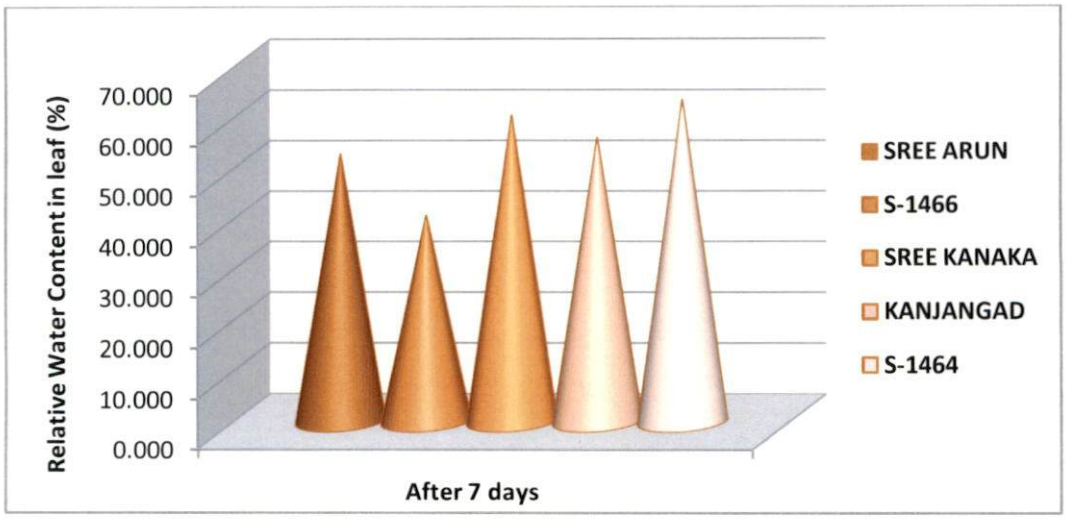


Fig. 15. Changes in the relative water content of five varieties/ genotypes of sweet potato under HTS Condition

4.6. BIOCHEMICAL PARAMETER

4.6.1. Changes in proline content in leaves of five sweet potato varieties as affected by WDS and high temperature stress conditions

Proline was extracted from the leaves of all the five varieties /genotypes *viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangadgrown under control, WDS condition and high temperature stress during 2ndand 3rdmonth (Fig. 18). Since proline is anstress indicator amino acid its level increases during the stress conditions. Under control condition the variety S-1464 had the maximum proline content (24.21 ± 0.6 µ moles/g) compared to other four varieties viz., Sree Arun (12.87 ± 0.82 µ moles/g), S-1466 (15.83 ± 0.43 µ moles/g), Sree Kanaka (19.94 ± 0.45 µ moles/g) and Kanjangad (23.67 ± 0.12 µ moles/g) . In plants subjected to WDS conditions it was observed that the genotypeS-1464 had the maximum proline content (29.56 ± 0.81 µ moles/g), whereas the variety S-1466 had the minimum proline content (22.29 ± 0.75 µ moles/g). In plants under high temperature stress Kanjangad showed maximum proline content (25.95 ± 0.51 µ moles/g) and S-1466 showed minimum proline content (21.1 ± 0.44 µ moles/g). From the results it was concluded that proline content increased under both stress conditions compared to stress free conditions. Proline content was analyzed in using ANOVA and it was found that here be a significant difference ($P > 0.001$) linking the treatments. Means were analyzed using Tukey contrast and it was found that proline content in heat stress plant was significantly greater ($P > 0.01$) as compared to stress free plants.

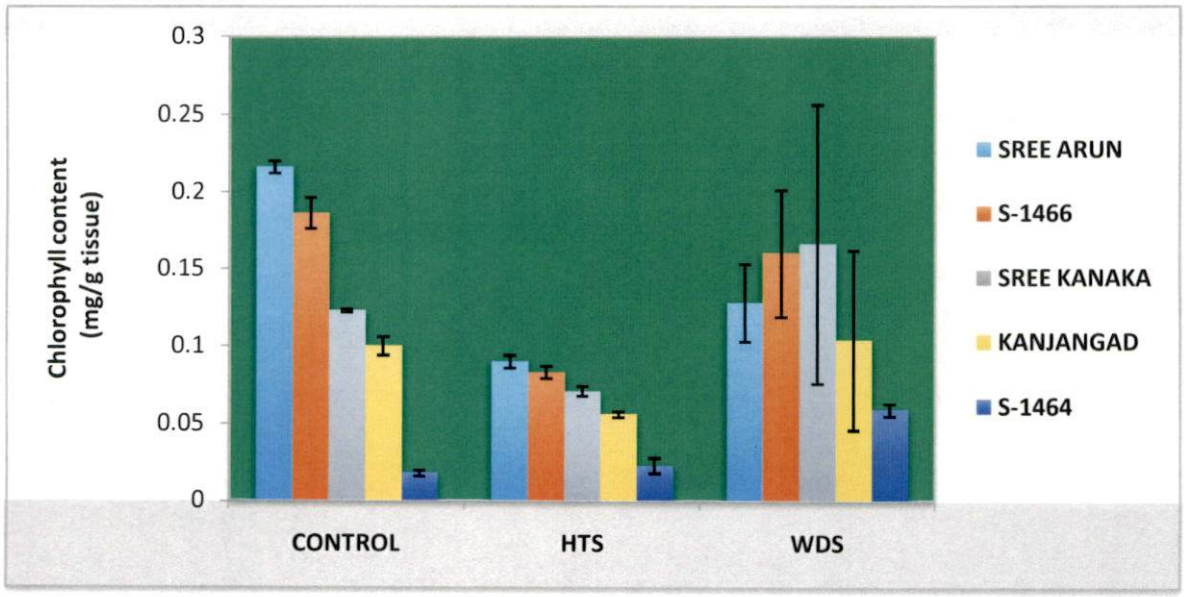


Fig. 16. Changes in total chlorophyll content in the leaves of five varieties/ genotypes of sweet potato as affected by WDS and high temperature stress conditions

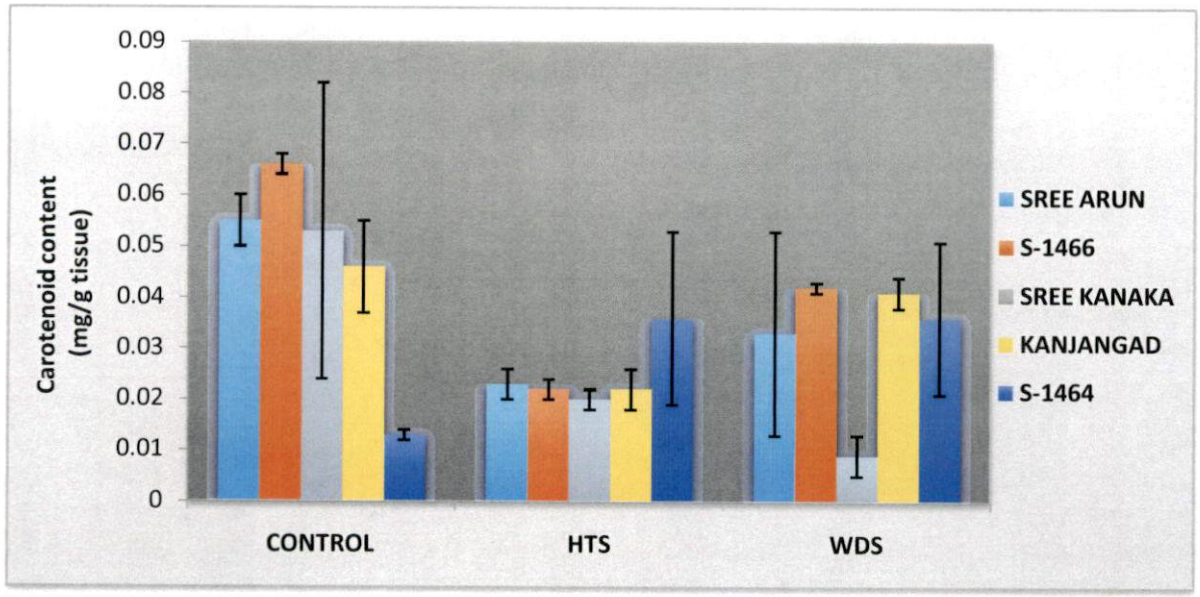


Fig. 17. Changes in carotenoid content in the leaves of five varieties/ genotypesof sweet potato as affected by WDS and high temperature stress conditions

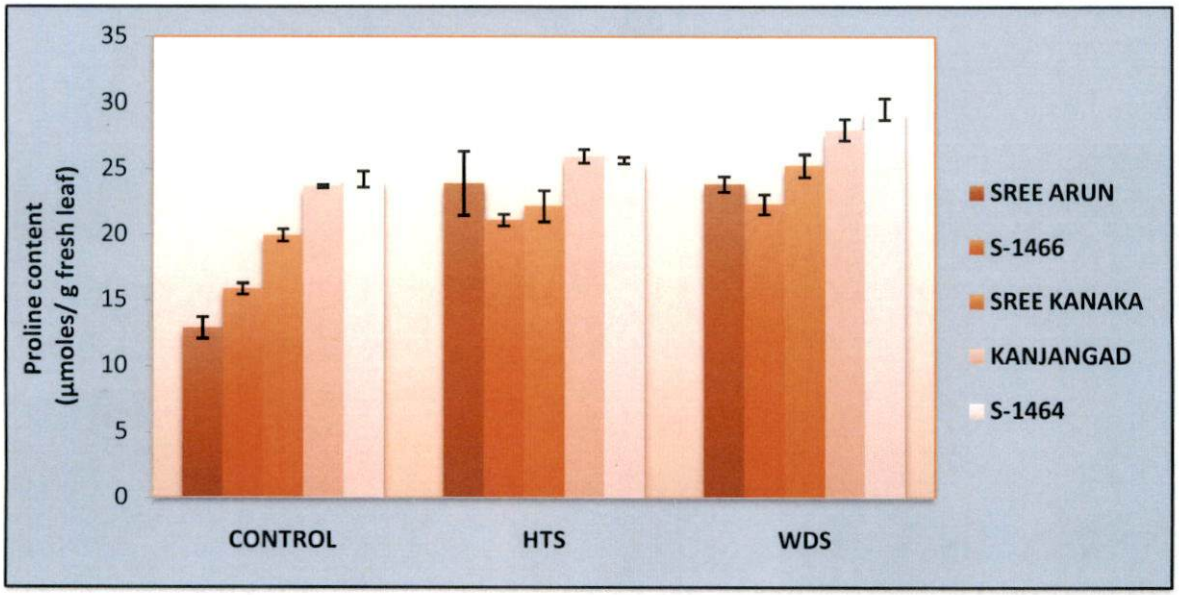


Fig. 18. Changes in proline content of leaves of five sweet potato varieties/ genotypes as affected by WDS and high temperature stress conditions

4.7. ENZYMATIC ANTIOXIDANTS

4.7.1. Changes in APX (Ascorbate peroxidase), (EC 1.1 1.1.1 1) leaves activities in the five sweet potato varieties as affected by WDS and high temperature stress conditions

The Ascorbate Peroxidase activity (APX) was assayed in the leaves of control, WDS and high temperature stress conditions (Fig. 19.). Among the five varieties of sweet potato *viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangad. Under control condition APX activity varied between (3.27 ± 0.81EU/mg protein)and (6.69 ± 0.29 EU/mg protein). The maximum enzyme activity was observed in the variety S-1464 (6.69 ± 0.29 EU/mg protein) and the variety purple had the minimum enzyme activity (3.27 ± 0.81 EU/mg protein). Under WDS condition the variety S-1464 (7.94 ± 0.60 EU/mg protein) had themaximum APX activity and the variety Sree Arun had the minimum enzyme activity (4.64 ± 0.08 EU/mg protein). All the varieties/ genptypeshad maximum protein content in WDS compared to stress free condition. Under high temperature stress conditions for 2^{nd} and 3^{rd} month Kanjangad had maximum APX activity (5.70 ± 0.52 EU/mg protein) and the variety S-1466had the minimum enzyme activity (4.48 ± 0.04 EU/mg protein). In high temperature stress S-1464 showed maximum APX activity compared to the plant in WDS condition. From the present study it was concluded that APX enzyme activity is more under WDS conditions compared to high temperature stress and control conditions. The APX enzyme activity statistically analysed using ANOVA it was found that significantly difference between ($P > 0.001$) treatments. On comparing the means using Tukey Contrasts it was found that WDS differ significantly from control ($P > 0.001$) and heat stress ($P > 0.05$) on comparing the APX activity different varieties it was clear that S-1464 significant difference in ascorbate activity from Sree Arun ($P > 0.01$) and S-1466 ($P > 0.05$).

4.7.2. Changes in CAT (catalase), (EC 1.1 1.1.6) activity in leaves of five sweetpotatovarieties as affected by WDS and high temperature stress conditions

The results of (CAT) activities in the leaves of five varieties of sweet potato are given in (Fig. 20). Under control conditions the (CAT) activity varied between (0.76 ± 0.002 Eu/mg protein) and (0.21 ± 0.07 Eu/mg protein). The maximum catalase activity was observed in thevariety S-1466 (0.76 ± 0.002 Eu/mg protein) and minimum activity was observed in the variety Kanjangad (0.21 ± 0.07 Eu/mg protein). The CAT activity increased when plants weresubjected to WDS during 2nd and 3rdmonth and high temperature stress conditions during 2nd and 3rdmonth. Under WDS conditions CAT activity varied between 0.59 ±0.002 Eu/mg protein (S-1464)and 0.41 ± 0.005Eu/mg protein (Sree Kanaka). The CAT activity of WDS condition the five varieties of sweet potato did not differ significantly. Under high temperature stress plants showed had significantly different CAT activity and it varied between 0.40 ± 0.01Eu/mg protein (Sree Arun) and 0.27 ± 0.09 Eu/mg protein (Kanjangad). From the present study it was concluded that CAT activity increased during stress (both WDS and high temperature stress) conditions as compared to control condition. The result of catalase activity was statistically analyzed using ANOVA and the results showed that there was no significant difference in catalase activity of sweet potato plants between the treatments and the varieties.

4.7.3. Changes in SOD (superoxide dismutase), (EC 1.15.1.1) activity in theleaves of five sweet potato varieties as affected by WDS and high temperature stress conditions

The superoxide dismutase (SOD) activity was assayed from the leaves of five sweet potato varieties grown under control, WDS and high temperature stress during 2nd and 3rd month and the results are presented in (Fig. 21). Under control conditions variety S-1466had the maximum SOD activity (8.64 ± 0.95 Eu/mg

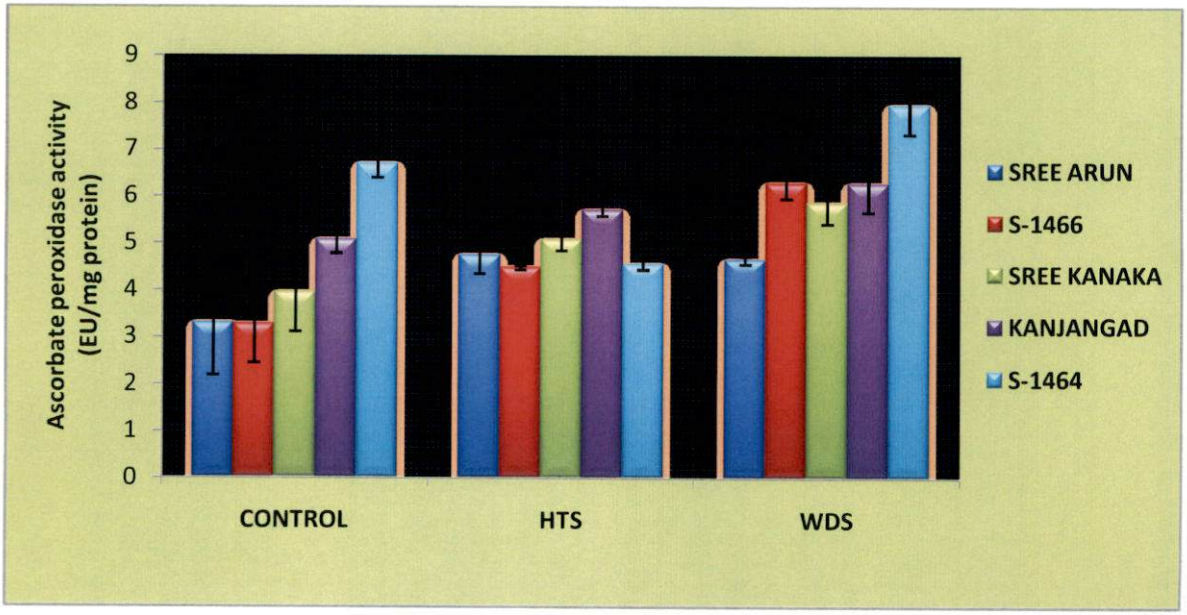


Fig. 19. Changes in ascorbate peroxidase activity in the leaves of five varieties/ genotypes of sweet potato as affected by WDS and high temperature stress condition

protein) whereas minimum SOD activity was observed in the variety Kanjangad (3.45 ± 0.74 Eu/mg protein). The SOD activity significantly varied among the five genotypes of sweet potato under control condition. Under WDS conditions thevarietiesS-1466 had maximum enzyme activity (25.92 ± 2.24 Eu/mg protein), whereas S-1464 had the minimum enzyme activity (12.09 ± 1.97 Eu/mg protein). Under WDS conditions SOD activity significantly varied among the five varieties /genotypes of sweet potato Sree Arun (20.30 ± 2.55 Eu/mg protein), S-1466 (25.92 ± 2.24 Eu/mg protein), Sree Kanaka (14.25 ± 1.12 Eu/mg protein), Kanjangad (17.28 ± 1.45 Eu/mg protein) and S-1464 (12.09 ± 1.97 Eu/mg protein). In plants under high temperature stress, the variety Sree Arun had the highest SOD activity (31.10 ± 2.07 Eu/mg protein) and the variety Sree Kanaka had minimum SOD activity (17.71 ±1.74 Eu/mg protein). The result of SOD was statistically analyzed using ANOVA and it was clear that there was significant difference (P >0.001)betweentreatments mean of the result were compared using Tukey contrastand it was clearthat SOD activity in plants varied significantly (P > 0.001) in stress condition compare to thecontrol condition and it also observedthat SOD activity varied significantly (P >0.05) in high temperature stress and WDS condition. There was no significantly difference between the SOD varieties.

4.7.4. Changes in GR (glutathione reductase), (EC 1.6.4.2) activity as affected by WDS andhigh temperature stress conditions

Glutathione reductase enzyme was extracted from the leaves of five sweet potato varieties/ genotypes viz., Sree Arun, S-1464, S-1466, Sree Kanaka and kanjangad under control, WDS and high temperature stressed condition. GR activity varied between 2.99 ± 0.67 Eu/mg protein (Sree Arun) and 2.02 ± 0.33 Eu/mg protein (Kanjangad) in the leaves of control plants. GR activity varied between (1.93 ± 0.28Eu/mg protein) and (1.15 ± 0.71 Eu/mg protein) in theleaves of sweet potato plant under WDS conditions applied during 2ndand3rd month. S-1464 (1.93 ± 0.28Eu/mg protein) showed maximum GR activitywhereas Sree Kanaka had the minimum GR activity (1.15 ± 0.71 Eu/mg proteins). GR activity varied between (3.92 ± 0.03 Eu/mg protein) and (0.86± 0.31 Eu/mg protein)

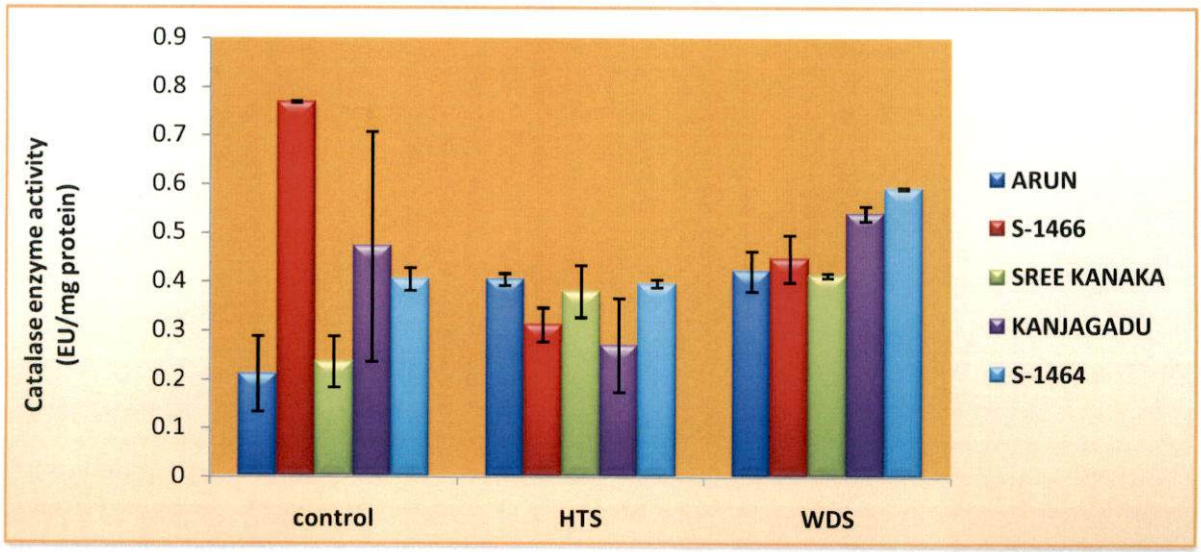


Fig. 20. Catalase enzyme activity under stress and control conditions of five Varieties/ genotypes of sweet potato

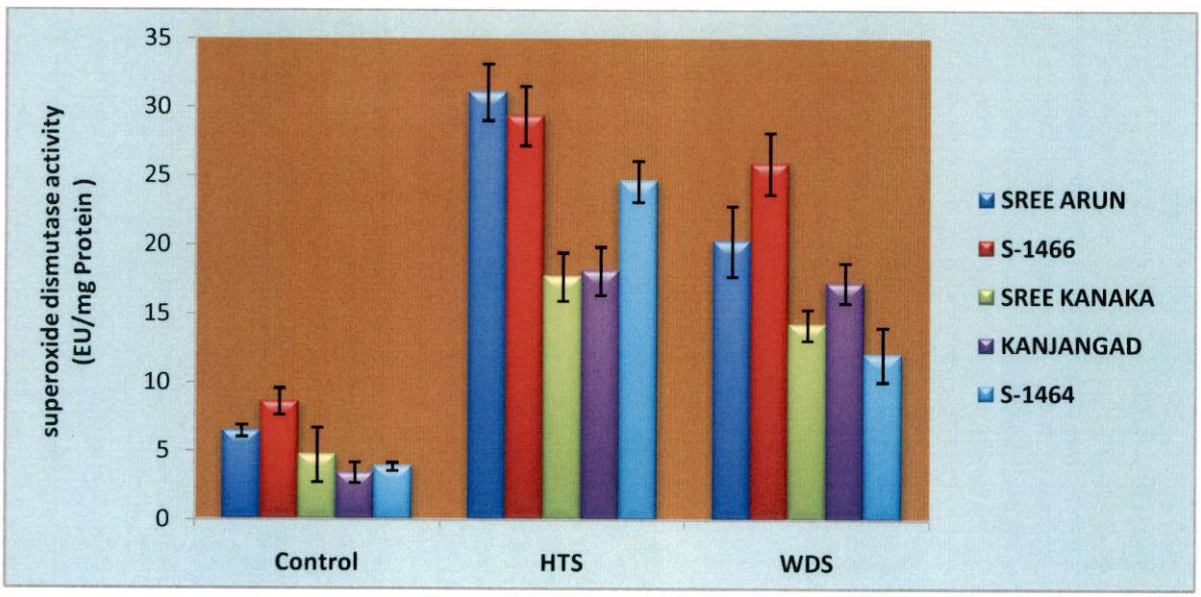


Fig. 21. Changes in superoxide dismutase activity in the leaves of five varieties/genotypes ofsweet potato as affected by WDS and high temperature stress conditions

in the leaves of sweet potato plants under high temperature stress condition applied during 2nd and 3rd month. The variety Sree Kanaka had the maximum GR activity (3.92 ± 0.03 Eu/mg protein) whereas minimum GR activity was observed in Sree Arun (0.86 ± 0.31 Eu/mg proteins). It was found that during stress conditions, GR activity decrease marginally than the control conditions. On comparing the GR activity between stress conditions it was observed that S-1464 showed maximum GR activity in WDS condition (1.93 ± 0.28Eu/mg protein) than in high temperature stress. Similar observation was made in Kanjangad and Sree Arun, whereas the variety S-1466 had maximum GR activity (3.56 ± 0.01 Eu/mg protein) in high temperature stress condition compared to WDS (Fig.22). The result of GR was statistically analysed using ANOVA it was found that there was no significant difference between treatment and five varieties of sweet potato used for the study.

4.7.5. Measurement of ascorbate reductase (AFR), EC 1.6. 5. 4) activity in the leaves of five sweet potato varieties as affected by WDS and HTS conditions

The activity of ascorbate free radical reductase was assayed by the method of (Hossain *et al.*, 1984). The leaf tissue of cassava of 1 g was crushed in liquid Nitrogen, using a mortar and pestle then homogenized at 0 °C for 4 min in ascorbate free radical reductase assay buffer 10 ml. Centrifuge the extracts for 30 min at 0°C at full speed in a bench-top, the supernatants were filtered through using Mira cloth, volume of 1mL 15-pLaliquots were assayed in a final reaction. To determine the AFR reductase activity spetrophtometrically the decrease in A340 due to NADH oxidation was monitored. However, there was no change in absorbance and therefore, the enzyme activity could not be measured.

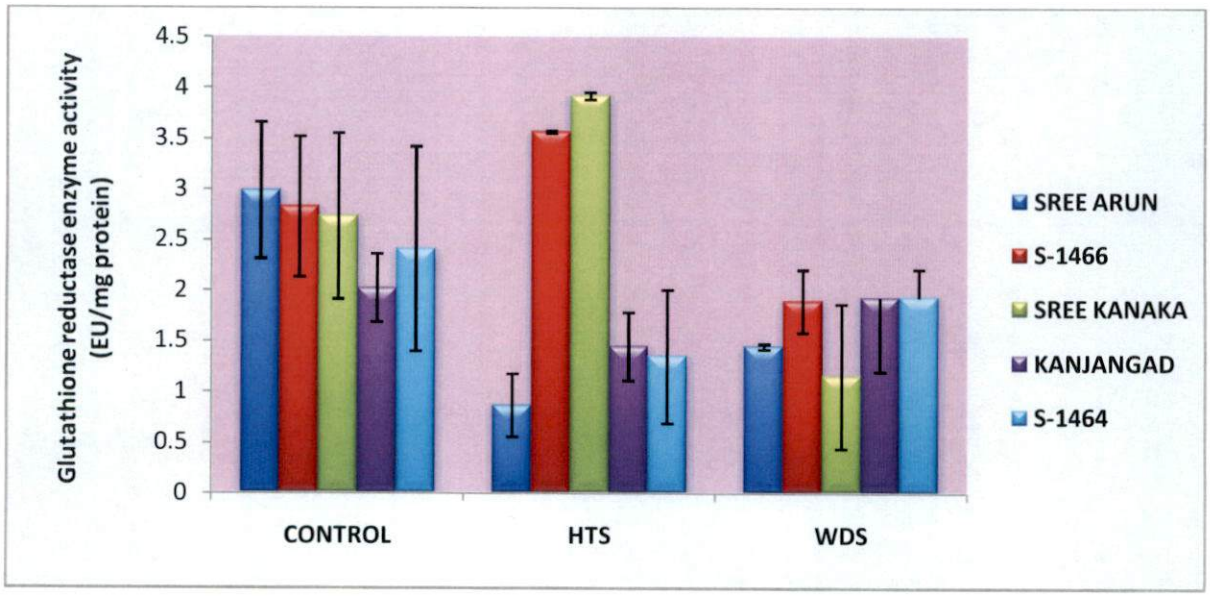


Fig. 22. Changes in glutathione reductase activity in leaves of five sweet potato varieties/ genotypes as affected by WDS and high temperature stress conditions

4.8. NON ENZYMATIC ANTIOXIDANTS

4.8.1. Ascorbic acid content in leaves as affected by WDS and high temperature stress conditions

Ascorbic acid (AA) content was estimated by volumetric analysis. In the presence of AA the dye (Dichlorophenol indophenol) will develop a pink colour. From the study, it was observed that extract of all the five genotypes/varieties of sweet potato *viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangad leaves developed faint pink colour in control and both stress condition (Fig. 23.). Under control condition the variety Sree Arun had the maximum ascorbic acid content (700mg/100g) and the variety S-1464 had the minimum ascorbic acid content (300mg/100g). In plants under WDS it was observed that the variety Kanjangad (4300mg/100g) had the maximum ascorbic acid content and minimum ascorbic acid were showed by the variety Sree Arun(1300 mg/100g). Under high temperature stress condition Sree Arun (300 mg/100g) and Kanjangad (300 mg/100g) had the maximum ascorbic acid content and minimum ascorbic acid content were found Sree Kanaka (200 mg/100g) and S-1464 (200 mg/100g). The variety S-1466 had no ascorbic acid content in both stress treatment and stress free condition. From the study it is concluded that plants under WDS showed maximum ascorbic acid content compared to high temperature and stress free condition. From the observations it is clear that the AA content in sweet potato leaves under WDS is moresignificantly different fromcontrol andhightemperature stress. The AA content in control and high temperature stress ($P > 0.001$) was almost same comparing to WDS the means using Tukey contrast. On comparing the varieties it was found that purple and Kanjangad ($P > 0.05$) is more significant compare to other varieties.

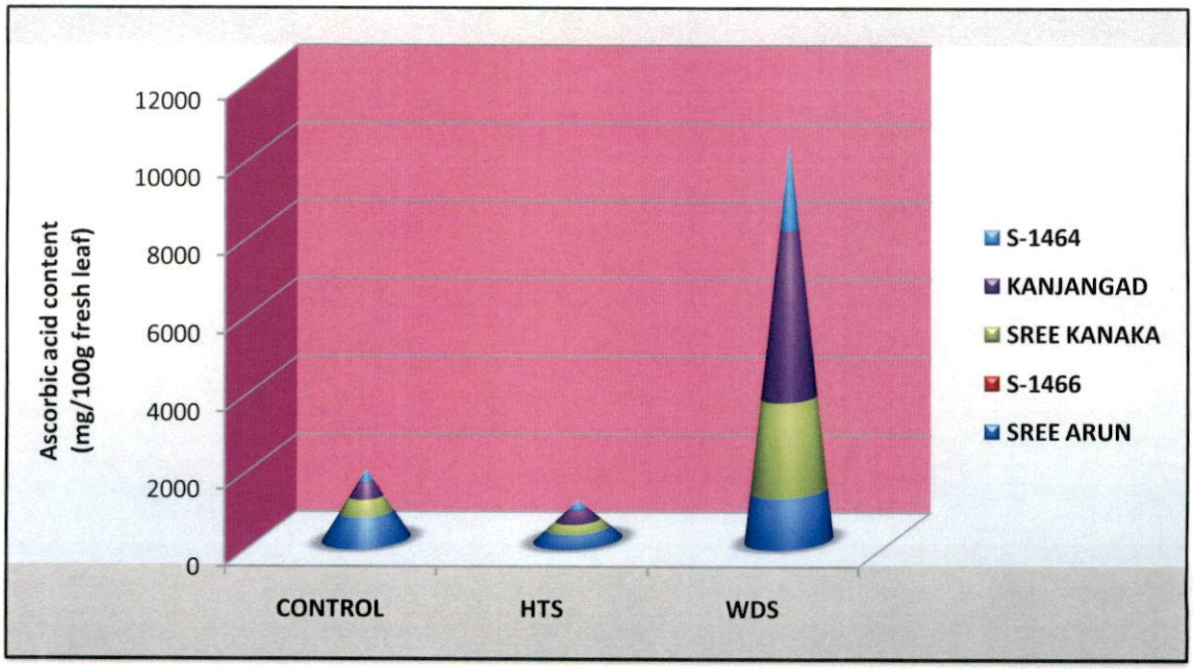


Fig. 23. Changes in ascorbic acid content in leaves of five sweet potato varieties/ genotypes as affected by WDS and high temperature stress conditions

4.8.2. Glutathione content in leaves of cassava plant as affected by WDS and high temperature stress conditions

Determination of Reduced glutathione was done by the method of Moron et al. (1979). DTNB Reduced glutathione and produces reaction (5, 5'-dithiobis nitro benzoic acid) on a yellow colour that was absorbs at 412nm. The 0.5g homogenate was prepared the sample with 2.5ml of 5% TCA. 1000rpm for 10 minutes the precipitated protein was centrifuged. The (0.1ml) supernatant was used for the estimation of GSH. The (0.1ml) supernatant was made up to 1.0 ml with 0.2 M sodium phosphate buffer (pH 8.0). Standard GSH ranging between 2 and 10 M were also prepared corresponding to concentrations. Freshly prepared 2 ml of DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer at 412 nm after 10 minutes. There was no change in absorbance the glutathione content could not be determined. Other method tried also didn't give results.

DISCUSSION

5. DISCUSSION

Abiotic stresses viz., salinity, drought, chemical pollutants and temperature, are concurrently acting on the plants resulting in cell injury, wounds and generating secondary stresses viz., osmotic and oxidative (Wang et al., 2003). Plants are not able to change their places to surpass such stresses, but have conspired many ways in adaptating morphologically to resist these stresses. Defence at the gene level are vital for the exitence and growth of plants. Plants exhibit a set of molecular responses and signal transduction to these responses. This consequence of thermal stress echoes the temperature dependence of Michaelis–Menton constant (Km) of each enzyme contributing in the process (Mitra and Bhatia, 2008). Plants must adjust thermal stress for continued existence, so they materialized varying mechanisms including the regulation of cell membrane integrity, quenching the reactive oxygen species (ROS), production of antioxidants, sequestration and osmoregulation of osmoticum, initiation of some kinases that responding to stress, Ca-dependent protein kinase pathways, and increasing the transcription chaperones like BIPs(Wahid et al., 2007).

Plant response studies to oxidative stress and protein profiling provide information on the physiological basis of mechanism by which they adapt to the ever changing environment. Due to the changing environmental condition plants have to face and overcome various types of stress. Heat stress, water stress and salinity are the most important environmental factors at regulate plant growth and development and contribute to an average yield loss of more than 50% for most major crop plants (Bray et al., 2000). Plant can respond and adapt to these stresses to certain limit by altering their cellular metabolism and evoking various defence mechanisms. Therefore, understanding the mechanism underlying plant abiotic stress response and generation of stress tolerant plants has received much attention in recent years.

Sweet potato being a tropical tuber crop which is highly prone to heat stress, water stress and other oxidative stress assessing these oxidative stress and

changes in the protein profile in sweet potato under abiotic stress in contrasting genotypes (short and long duration varieties) of sweet potato gain importance.The main objective of this study was to find out the antioxidative enzyme activity and protein profiling of different varieties and genotypes of sweet potato (Sree Arun, S-1464, S-1466, Sree Kanaka and kanjangad) under different stress treatments (heat and water deficit) and identify physiological parameter as a marker for identifying / developing genotypes / varieties for environmental stress tolerance such as WSD and high temperatures (>33°C). During the study proper procedures for finding out the antioxidative enzymes activity were determined which will be useful to the similar studies on other crops.Identification of marker physiological parameters can help to identify / develop varieties for tolerance to abiotic stress condition.

5.1 EFFECT OF ABIOTIC STRESS ON GROWTH PARAMETER

Abiotic stress viz. WDS and heat stress HTS were imposed on five different varieties of sweet potato plant at 2nd and 3rd month and 2nd and 3rd month after planting respectively for a period of 4 days. Observations on vein length, leaf number and leaf retention were made for 120 days at an interval of 30 days. The influence of WDS and HTS on use parameters discussed in the following section.

Sweet potato cultivated as an annual crop. Measuring the growing conditions and varieties, crop yielding period varies between 12 and 35 weeks (Chen and Xu 1982; Hahn and Hozyo 1984) whereby a long period of 25-50 weeks were also been reported for some varieties (Huett 1976; Huett and O'Neill 1976).

In the present study it was found that vein length of five different sweet potato varieties were not differentially affected by heat and water deficit stress. However, an increasing trend in vein length were observed under both stress and control conditions.

The leaf production of five different sweet potato varieties/ genotypes were not affected by both heat and water stress, and the same is maximum in the

case of WDS than HTS. In the case of leaf production among five varieties Sree Arun had higher tolerance to both stress condition. In a similar study in sweet potato (Ravi and Indira, 1995) reported that leaf tissues permanently wilt when WP_L reduces to -1.3 MPa and at WP_L amidst -1.6 to -2.0 MPa and the leaves senesce. The leaf retention of four varieties/ genotypes were differentially affected by both heat and water stress and leaf retention is greather under WDS than HTS. A decreasing trend in leaf retention was observed in stress condition as compared to control plants.

5.2 .EFFECT OF ABIOTIC STRESS FACTOR ON YIELD PARAMETER

Sweet potato grows the best when cultivated at 25% available soil moisture content and there is no rise in storage yield by regulating soil moisture >50% (Hernandez and Barry 1966; Hammett *et al.*1982). Under physiological production conditions, the crop procures 500 mm water for a 16-20 week growth time (King 1985; Kay 1987; Onyekwere and Nwinyi 1989; Chukwu 1995). But the storage root yields are affected by amount, timing, and distribution, of water. Storage root yield reduces under WDS, specially when the present soil moisture reduces below 20% (Chowdhury and Ravi 1987, 1988; Indira and Kabeerathumma 1988; Nair *et al.* 1996). Observations on storage root number and mean tuber weight (Kg/plant) were made. The influence of WDS and HTS on use parameters is discussed in the following section. There is only negligible difference in the yield parameters between stress and control condition. However there is significant difference in yield parameters among the varieties. Among the five different sweet potato varieties/ genotypes under study SreeArunhad higher yield parameters except storage root width under stress condition. The little difference in yield parameter between the control and stress conditions may be due to the increased oxidative stress which might have reduced the assimilatory activities in plant resulting in reduced yield character. Findings of the present study is in agreement with Ramanujam, (1990); Ravi and George, (2003). The storage root induction period is the most susceptible to WSD due to its consequence on storage root number (Indira and Kabeerathumma 1988; Nair *et al.*

1996; Ravi and Indira 1996). WSD at the root initiation time induces lignification of storage roots and reduces storage root growth. Lignification and subsequent decrease in the storage root yield is higher in varieties with less sink capability compared to those with greater sink capacity (Ravi and Indira 1996). It was also reported that the initiation and production of heat-shock proteins because of high temperature stress are common occurrence in all living organisms from microbes to human beings (Parsell andLindquist, 1993; Vierling, 1991; Gupta et al., 2010). It seems that the generation of these proteins is highly expensive energy-wise that is casted on the yield of the cropsMohamed (2010).

5.3. EFFECT OF ABIOTIC STRESS ON TOTAL PROTEIN CONTENT AND PROTEIN PROFILING

Total soluble protein of leaf and tuber was found to be lower in plants exposed to stress conditions. This is in favour of earlier reports by Parthasarathi (2004) and Agami (2013). The decrease in protein and amino acid content was due to impaired protein metabolism and synthesis. The protein fractions were converted into compatible cytoplasmic osmotic agents (Walter 1995). Total soluble protein in the tuber forming roots showed an increasing trend under HTS condition. This might be due to the low water content experienced by the plants. This was in agreement with the finding of Kosakivska*et al.*,(2008). Under the HTS conditions of short-term heat stress condition, observed a 2-fold increase of protein content in seedlings of*Rumexpatienta L. x R. tianshanicus* was observed.

In corn (*Zea mays* L.) and barley (*Hordeum vulgare* L.) plants, NR activity reduced because of a decline in protein synthesis but not because of the NO_3^- in stressed tissue (Arriaga et al. 1972; Huffaker et al. 1970). If the reduction in NR activity in stress exposed sweet potato leaf tissues, it is due to a decline in the enzyme activity itself due to a reduced protein synthesis or because of decrease inNO_3^- uptake or both. In sweet potato, because of WSD, the interface among reduced N, WPL, ABA, and stomatal closure is not understood. Because the integration of inorganic N into plants is majorly regulated by NR activity,

decrease in NR activity under WDS may decrease growth, protein synthesis and development. Sweet potato varieties resistant to WDS have higher NR activity compared to senzitive ones (Naskar and Chowdhury 1995).

Thermal stress and other abiotic stresses can set off some machineries of defense such as the ultimate gene expression that are normally not expressed under ''usual'' conditions (Morimoto, 1993). The rapid modification in protein expression results in an increase in the production of specific protein groups. called ''heat-shock proteins' (Hsps), ''Stress-induced proteins'' (Lindquist and Crig, 1988; Gupta et al., 2010). Most of the stresses initiate gene expression and production of heat-shock proteins in cells that are exposed to stress (Feder, 2006). Single dimensional protein profile was done in the present study for leaves and tubers. It was found that brighter bands appeared for protein from control condition whereas protein samples of plants from WDS and HTS condition failed to show such a distinct band pattern. In the case of tuber forming root brighter bands appeared in the control condition compared to HTS and WDS condition and in the case of storage root protein dark bands appeared in the WDS condition. So one dimensional protein profiling could not reveal the HSP in sweet potato. Presence of mucilage in sweet potato also appears to trap the protein which prevented clear band formation in the gel.

5.4. EFFECT OF ABIOTIC STRESS ON PHYSIOLOGICAL PARAMETER

The effect of abiotic stress on various physiological parameter like relative water content, chlorophyll and carotenoid content was studied. Among the given treatments with increase in WDS and HTS, the chlorophyll and carotenoid content were reduced in all the five varieties/ genotypes. Comparing HTS and WDS, chlorophyll and carotenoid content was found to be lower in plants exposed to HTS. The results are in congruent with the finding of Gargi (2014) and Parthasarathi (2004). According to Walter (1995), the reduction of the chlorophyll might be due to the destruction of thylakoids and the chloroplast swelling and

breaking down under stress condition. The generation of ROS induced by WDS also have resulted in the damage of membrane and cause photo oxidation. This can be the reason for reduced chlorophyll under stress conditions (Lambers et al., 1998). It was also reported that a significant reduction in chlorophyll b was observed due to WDS in *Grevellia robusta* (Nautiyal*et al.,* 1993) and a similar result was reported in tea (Rajasekar*etal.,* 1991). The net chlorophyll number in leaf tissues reduced in sweet potato and plants exposed to WDS (Indira and Kabeerathumma, 1988; 1990; Chowdhury and Ravi 1987; 1988).

Relative water content of plant content varied under both WDS and HTS condition. A Higher RWC was observed in plants exposed to HTS condition compared to WDS condition Rahimi *et al.* (2010). In the case of Cassava being a C3 plant needs efficient strategies for efficient water management under stress condition. The plant might able to tolerate well with available moisture with the deeper root system and increased dry matter production.Reported that RWC is more sensitive to drought stress than heat stress which is on line with our finding. It was also reported that under conditions of WDS, water potential (WP_L)or relativewater content (RWC) of sweet potato leaf tissues reduces (Indira and Kabeerathumma, 1988; Ravi and Indira, 1995; Chowdhury and Naskar, 1993).

5.5. EFFECT OF ABIOTIC STRESS ONBIOCHEMICAL PARAMETER

Drought tolerant sweet potato varieties mounts up to a higher quantity of proline in the leaf root tissues compared to the plants under WDS free conditions (Chowdhury andRavi 1987; Indira and Kabeerathumma, 1988; Ravi and Indira, 1997). In both resistant and sensitive varietiess, leaf tissues accumulate a high amount of proline than the non-storage roots (Ravi and Indira, 1996). But some sensitive cultivars that do not produces yield but exists under WDS also accrue a good quantity of proline in their leaves (Ravi and Indira, 1997). Since most of the proline accumulation happens after growth has stopped, proline have no influence on sweet potato plant development during WDS. But, greater the accumulation the more amount of proline in the leaf tissues during WDS has been revealed in others to aid the plant to exists and maintain the leaves through osmotic

adjustment (Ford and Wilson 1981; Hanson and Hitz 1982). Although sensitive varieties accrue a good quantity of proline under WDS, their exceptionally week sink potential may describe their low crop yield. If sweet potato plants sequesters other organic solutes because of WDS it may contribute toward tolerating water deficiet. Proline status of plants under stress condition followed an increasing trend suggesting that proline can be considered as an indicator of stress for cassava. This was in line with the earlier findings Agami (2013) and Slabbert and Kruger (2014). The plant might have experienced severe abiotic stress can also be the reason for the increasing trend of proline content in the plant.In the present study Proline content data was analyzed in using ANOVA and it was found that there was significant difference between the treatments and it was found that proline content in heat stressed plant increased significantly compared to stress free plants.

5.6. EFFECT OF ABIOTIC STRESS ON ENZYMATIC ANTIOXIDANTS

The effect abiotic stress factors on various enzymatic antioxidants such as glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), ascorbate reductase (AFR) and ascorbate peroxidase (APX) were studied.

Among the five different sweet potato varieties/genotypes studied Sree Kanakahad maximum GR activity under HTS condition. Between the treatments it was found that GR activity showed an increasing trend in plants from stress free condition compared to HTS and WDS conditions. The plant tries to maintain the equilibrium of ROS generation to defend the oxidative damage during stress condition experienced by the plant. Similar results were also reported byMay and Leaver (1993) and Madamanchi et al. (1994) stating that upon the incidence of oxidative stress, the living pool of reduced glutathione (GSH) is changed to oxidized glutathione (GSSG) and the biosynthesis of glutathione roused.

Catalase activity of five varieties of sweet potato plant varied among different stress condition (WDS and HTS) compared to control. The catalase activity was lower under control condition. Enhanced production of ROS might

not have been occurred in the plant to induce an intensified CAT activity. The CAT activity increased under WDS. This is in agreement with the earlier finding of Hertwig et al. (1992). In general, a rise in catalase activity was found with an increased stress level withthe purpose of hunting the free radical produced during the stress.

Abiotic stress particularly HTS and WDS induces oxidative injury and alter the activity of the SOD, CAT and GR in many plant species (Foyer et al. 1994; Gong et al. 1997). In the present study, SOD activity showed an increasing trend under stress conditions. This finding is in agreement with Tsang et al. (1991).There was an increase in SOD in tobacco leaves during heat shock due to the accumulation of CU/Zn SOD mRNA.

The Ascorbate Peroxidase (APX) activity was found to have significant variation with the given treatment (WDS and HTS). Plants under stress treatment might have experienced considerable level of oxidative stress. This might be the reason why the ascorbate peroxidase activity increased under stress conditions. Findings of the present study is in agreement with the finding of Park et al., (2004) and he reported that the sweet potato cAPX gene was majorly produced in leaf tissues after subjected to thermal stress. The activities of cAPX, sAPX and mAPX elevated after the cucumber plants were exposed to heat stress after an initial slight decrease. The expression of sAPX also shows a similar pattern (Song et al., 2005).

5.7. CONCLUSION

It is obvious from the results that an appreciable amount of labour has been done over the one year and this study has aided to widen our information of physiological parameters, biological parameters, yield parameters, growth parameters, protein profiling and enzymatic and non-enzymatic antioxidant enzyme activity in sweet potato. Sweet potato formation may be elevated by rise in yield per unit area or mounting area under irrigation. Yield elevation per unit area can be attained by a breeding and followed by a selection process. However,

almost all land present in the tropics is restricted in its productive capability by unflavorable soil properties and climatic conditions. Hence for rising area under irrigation, concentration must be pointed to develop varieties that are tolerant to different stress situations. Under HTS condition SOD activity increased in Sree Arun and S-1466 alone whereas all the five varieties had increased SOD activity under WDS. The variety Kanjangad had maximum APX activity in HTS whereas S-1464 had higher APX activity in WDS. From the present study, it is inferred that SOD activity can be used as a biochemical marker for developing HTS tolerant varieties as it had consistent changes under both WDS and HTS. APX activity can be used as marker for identifying WDS tolerant varieties. Detailed molecular biochemical and physiological information will be necessary using different varieties for getting appropriate activities as the marker to screen abiotic stress tolerant varieties.

SUMMARY

SUMMARY

A study on "Oxidative Stress and Protein Profiling in sweet potato (*Ipomoeabatatas* (L.) Lam.) Under Abiotic Stress" (WDS and HTS stress) was conducted at CTCRI, Thiruvananthapuram-14 and Department of Biotechnology College of Agriculture Vellayani during 2014- 2015.

The present study was focused on five varieties/genotypes of sweet potato *viz.*, Sree Arun, S-1464 (pale green), S-1466 (purple), Sree Kanaka and kanjangad with three replications, each replication having five plants. There were three treatments viz., control, (water deficit stress (WDS) condition, and high temperature stress conditions. In WDS free conditions, plants were well irrigated and WDS for 4 days was imposed during 2^{nd} and 3^{rd}month after planting by withholding irrigation. For high temperature stress conditions, plants were incubated under the light transparent poly chambers for 7 days during 2^{nd} and 3^{rd} month. Plants were exposed to high temperature stress at 40°C during day time for six hours between 9.0 AM to 4.0 PM daily for a period of 7 days.

Growth parameter such as leaf production, leaf retention, vein length was recorded for 120 days with an interval of 30 day and yield components such as tuber number, mean tuber weight were recorded.

Total protein extracted from the leaves, tubers and tuber forming of five varieties of sweet potato plants was estimated by Bradford method and protein profiling was done by SDS-PAGE. Chlorophyll was estimated from five varieties/genotypes by using DMSO method. Proline is a stress indicator amino acid and its level increased under stress conditions. Proline content was estimated by spetrophtometrically using standard procedure from the literature. Activity of enzymatic antioxidants like superoxide dismutase, catalase, ascorbate peroxidise, glutathione reductase and ascorbate reductase were assayed from the leaves of five varieties/genotypes of sweet potato by using standard procedures from the literature. Non enzymatic antioxidant like ascorbate, glutathione was estimated using standard procedure.

Growth parameter such as plant height, leaf number and leaf retention showed gradual increase in each interval of time. There is no change in the plant height in stress condition compared to control. Reduction in leaf number and the leaf retention in stress treatment condition compared to stress free condition. Yield parameter such as tuber number showed negligible variation in stress treatment condition compared to control condition. The variety Sree Arun had the maximum tuber number in control and HTS condition and control condition and minimum tuber number was observed in the variety S-1464. The maximum mean tuber weight was observed in Kanjangad under control and treatment conditions and minimum mean tuber weight was observed in S-1464 under control and treatment conditions.

All the five varieties/genotype of sweet potato *viz.*, Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangad had variation in their total protein content in leaf when plants were subjected to stress conditions. All four varieties expect S-1466 and S-1464 had the maximum protein content in the HTS condition whereas Purple had its maximum protein content in WDS condition and in stress free condition it was observed that Kanjangad had the maximum total leaf protein content. In the case of total tuber protein content, Sree Kanaka showed maximum protein content and Purple showed minimum protein content in the control, WDS and high temperature stress condition. Total protein content in storage root forming root content Sree Kanaka shows same as the treatment difference in high temperature stress, control and WDS condition and minimum tuber forming root protein content in S-1464. During protein profiling (leaf, tuber and tuber forming root) in the case of tuber forming root and tuber, clear bands were observed in control condition indicating high protein content in the control condition. For leaf protein no bands were obtained in three conditions due to presence of high mucilage in leaves. Physiological parameters like Relative water content (RWC), chlorophyll and carotenoid content was maximum in stress free condition and gradual reductions were observed in treatment conditions.

Under WDS condition APX activity has increased in Pale Green and the variety Sree Arun had the minimum enzyme activity. WDS condition is showed more APX enzyme activity compare to HTS and control condition. Under WDS Sree Arun showed maximum catalase activity in HTS whereas Pale Green had highest SOD activity in WDS condition. SOD activity was high in all the varieties under HTS condition. GR activity has increased in purple and Sree Kanaka in HTS condition.

Enzymatic antioxidants (GR, SOD, CAT, APX and AR) showed maximum activity in treatment condition (WDS and high temperature stress) compared to stress free condition. The non-enzymatic antioxidants (ascorbic acid and glutathione) in five varieties/ genotypes increased when WDS conditions. Present study revealed that the glutathione content in of sweet potato leaves was negligible or could not be determined under both stress and control conditions in all the varieties.

Under HTS condition SOD activity increased in Sree Arun and S-1466 alone whereas all the five varieties had increased SOD activity under WDS. The variety Kanjangad had maximum APX activity in HTS whereas S-1464 had higher APX activity in WDS. From the present study, it is inferred that SOD activity can be used as a biochemical marker for developing HTS tolerant varieties as it had consistent changes under both WDS and HTS. APX activity can be utilised as marker for identifying WDS tolerant varieties. Detailed molecular biochemical and physiological information will be necessary using different varieties for getting appropriate activities as the marker to screen abiotic stress tolerant varieties.

REFERENCE

7. REFERENCE

Adhanom, N., Tsedeke, A. and Emana, G. 1985. A Review of Crop Protection Research in Ethiopia: Proceedings of the first Ethiopian Crop Protection Symposium, 4-7 February, Institute of Agricultural Research, Addis Ababa, Ethiopia.

Agami, A. R. 2013. Salicylic acid mitigates the adverse effect of water stress on lettuce (*Lactuca sativa* L.) *J. Applied Sci. Res.* 9(11): 5701-5711.

Alscher, R. G. and Hess, J. L. 1993.Antioxidants in higher plants. Boca Raton: CRC press. pp. 77-104.

Apel, K. and Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev. Plant Biol.* 55: 373–399.

Arriaga, C., Boyer, J. S. and Hageman, R. H. 1972. Nitrate reductase activity and polyribosome content of corn at low leaf water potential. Plant Physiol. 49: 49-57.

Arrigoni, O., Zacheo, G., Arrigoni-Liso, R., Bleve-Zacheo, T. and Lamberti, F. 1979. Relationship between ascorbic acid and resistance in tomato plants to Meloidogyne incognitu. Phytopathology 69: 579-581.

Arrigoni, O., Dipierro, S. and Borraccino, G. 1981.Ascorbate free radical reductase, a key enzyme of the ascorbic acid system.*FEBS Lett.* 125: 242-244.

Asada, K. 2000. The water-water cycle as alternative photon and electron sinks. *Phil Trans R Soc Lond B Biol Sci.* 355: 1419–1431.

Asada, K. 1994. Production and action of activated oxygen species in photosynthetic tissues. In: Foyer, C. H. and Mullineux, P. M. (eds), *Causes of photooxidative stress and amelioration of defence systems in plants*. Boca Raton, CRC Press. pp. 77-104.

Asada, K. and Takahashi, M. 1987. Production and scavenging of active oxygen in photosynthesis. In photo inhibition (Topics in Photosynthesis) Kyle, D. J. (eds), *Elsevier* – 2, 9: 227 – 287.

Babu, C. R., Zhang, J., Blum, A., Hod, T. H. D., Wue, R. and Nguyen, H. T. 2004. HNA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) *via* cell membrane protection. *Plant Sci.* 166: 855-862.

Bai, J., Gong, C. M., Chen, K., Kang, H. M. and Wang, G. 2009.Examination of antioxidative system's responses in the different phases of drought stress and during recovery in desert plant *Reaumuria soongorica* (Pall.) Maxim. *J Plant Biol.* 52:417–425.

Bartoli, C. G., Simontacchi, M., Tambussi, E. A., Beltrana, J., Montaldi, E. R. and Puntarulo, S. 1999. Drought and watering dependent oxidative stress. Effect of antioxidant content in *Triticum aestivum* L. leaves. *J. Exp. Bot.* 50:375-383.

Bartosz, G. 1997. Oxidative stress in plants.*Acta Physiol. plant.* 19: 47-64.

Bates, L. S., Waldeen, R. P. and Teare, I. D. 1973.*Plant Soil.* 39:199-205.

Beauchamp, C. and Fridovich. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal.Biochem.44: 276.

Bielski, B. H. J. 1982. Chemistry of ascorbic acid radicals.In Seib, P. A. ans Yolbert. B. M. (eds.), Ascorbic Acid: Chemistry, Metabolism and Uses. American Chemical Society, Washington, DC, pp. 81-100.

Boo, Y. C. and Jung, J. 1999. Water deficient induced oxidative stress and antioxidative defenses in rice plants. *J. Pl. Physiol.* 155:255-261.

Booker, F. L. and Fiscus, L. E. 2005.*J. Exp. Bot.*, 56(418): 2139-2151.

Bosch, M. and Alegre, L. 2002. Interplay between ascorbic acid and lipophilic antioxidants defenses in chloroplasts of water-stressed Arabidopsis plants. *FEBS Lett.* 31: 145-148.

Bourke, R. M. 1985. Sweet potato (*Ipomoea batatas*) production and research in Papua New Guinea, Papua New Guinea *J. Agric.*, For., *Fish.* Vol. 33: 89-108.

Bowler, C., Van Camp, W., Van Montagu, M. and Inze, D. 1994.Superoxide dismutases in plants.*Critical rev in plant sci.* 13: 199-218.

Bowler, C. 1991. Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *EMBO J.* 10: 1723 – 1732.

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

Brandt, C., Balko, B. and Eichler-Lobermann, L. 2011. Interactive effects of soil water content and phytin supply on phosphorus nutrition of different crops species. *Agric. For. Res.* 4(61):317-326.

Bray, E. A., Bailey-Serres, J. and Weretilnyk, E. 2000. Responses to abiotic stresses.In W Gruissem, B. Buchannan, R. J. (eds.), Biochemistry and Molecular Biology of plants. American Society of Plant Physiologists, Rockville, MD, pp. 1158-1249.

Buchanan,B., Kutchan, T. M. and Lewis, N. G. 2000.Natural Products (Secondary metabolites). Biochemistry and Molecular Biology of Plants (eds), Buchanan, B., Gruissem, W. and Jones, R. American Society of Plant Physiologists, Rockville, Maryland. pp. 1250-1318.

Buchanan-Wollaston, V. 1997.The molecular biology of leaf senescence.*J of Exp.* Bot. 48: 181-199.

Cakmak, I., Strbac, D. and Marschner, H. 1993. Activities of hydrogen peroxidase-scavenging enzymes in germinating wheat seeds.*J. Exp. Bot.* 44: 127-132.

Chen, W. D. and Xu, J. S. 1982.Breeding the new sweet potato variety Tainang 18.*Taiwan Agr.*Biomonth. 18: 48-54.

Chen, L. M. J. C. 1988. IAA and IAA oxidase levels in developing sweet potato (*Ipomoeabatatas* (L.) Lam.) root systems. *Diss.Abstr.Int. B. Sci. Eng.* 49: 5968.

Chukwu, G. O. 1995. Crop irrigation water needs to sweet potato. *Afr. J. Root Tuber Crops*. 1: 35-38.

Chowdhury, S. R. 1996. Effect of different irrigation treatments on water requirements in sweet potato.*J. Root Crops* 22: 50-53.

Chowdhury, S. R. and Naskar, S. 1993. Screening of drought tolerance traits in sweet potato: role of relative water content.*Orissa J. Hort.* 21: 1-4.

Chowdhury, S. R. and Ravi, V. 1987.Physiology of tuberization in sweet potato with reference to moisture stress and seasonal influence.*Annu. Rep.* CTCRI, Trivandrum, India, pp. 73-75.

Chowdhury, S. R. and Ravi, V. 1988.Physiology of tuberization in sweet potato with reference to moisture stress and seasonal influence.*Annu. Rep.* CTCRI, Trivandrum, India, pp. 89-90.

CIP. 1998. International Potato Center (CIP) Annual Report, Lima, Peru, pp. 68.

Collins, A. 2001.Carotenoids and genomic stability.*Mutat Res.* 475:1–28.

Collins, W. W. 1993. Root vegetables: New uses for old crops In Janick, J. and Simon, J.E. (eds), New Crops, Wiley, New York, pp. 535-537.

Contour-Ansel, D., Torres-Franklin, M. L., Cruz, H., de Carvalho, M., D'arcy-Lameta, A. and Zuily-Fodil, Y. 2006. Glutathione reductase in leaves of cowpea: cloning of two cDNAs, expression and enzymatic activity under progressive drought stress, desiccation and abscisic acid treatment. *Ann Bot*. 98:1279–1287.

Corpas, F. J., Palma, J. M., Sandalio, L. M., Lopez- Huertas, E., Romero-Puertas, M. C., Barroso, J. B. and Del Rio, L. A. 1999. Purification of catalase from pea leaf peroxisomes: identification of five different isoforms. *Free Radic Res.* 31:235–241.

D' Arcy-Lameta, A., Ferrari-IIiou, R., Contour-Ansel, D., Pham-THI, A. T. and Zuily-Fodil, Y. 2006.Isolation and characterization of four ascorbate peroxidase cDNAs responsive to water deficit in cowpea leaves.*Ann Bot.* 97: 133-140.

Dat, J., Vandenbeele, S. E., Vranova, E. M., Van Montagu, M., inze, D. and Van Breusegem, F. 2000. Dual action of the active oxygen species during plant stress responses. *Cell.and mol. life sci.* 57: 779-795.

Dalton, D. A., Baird, L. M., Langeberg, L., Taugher, C. Y., Anyan. W. R., Vance, C. P. and Sarath, G. 1993. Subcellular localization of oxygen defence enzymes in soybean (*Glycine max* [L.] *Merr.*) Root nodules. *Plant physiol.* 102: 481-489.

Del Rio, L., Pastori, G. M., Palma, J. M., Sandalio, L. M., Sevilla, F., Corpas, F.J., Jiminez, A., Lopez-Huartas, E. and Hernandez, J. A.1998.The activated oxygen role of peroxisomes in senescence.*Plant Physiol.* 116: 1195-1200.

Delgado, E., Mitchell, R. A. C., Parry, M. A. J., Driscoll, S. P., Mitchell, V. J. and Lawlor, D. W. 1994. Interacting effects of CO_2 concentration, temperature and nitrogen supply on the photosynthesis and composition of winter wheat leaves. *Pl., cell environ.* 17(11):1205-1213.

Droillard, M. J. and Paulin, A. 1990. Isozymes of superoxide dismutase in mitochondria and peroxisomes isolated from petals of carnation (*Dianthus caryophyllus*) during senescence. *Plant physiol.* 94: 1187-1192.

Elstner,E. F. 1982. Oxygen activation and oxygen toxicity.*Annu Rev of Plant Physiol.* 33: 131-154.

Elstner, E. F. 1991. Mechanisms of oxygen activation in different compartments of plant cells.In *Active Oxygen/ OxidativeStress in Plant Metabolism*, Pelland,E. J.and Steffen,K. L. (eds), Rockville, M. D. American society of plant physiologists. pp. 13-25.

Eskling, M., Arvidsson, P. O. and Akerlund, H. E. 1997. The Xanthophyll cycle, its regulation and components, *Physiologia Pl.* 100: 806-816.

Fan, F. H., Ding, L., Du, X. C. and Wu, X. 2014.Effect of short-term water deficit stress on antioxidative systems in cucumber seedling roots.*Bot. Stud.*55: 46. Available: http://www.as-botanicalstudies.com-/content/55/1/46.

FAO: 2005. Production Year Book for 2004, Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy Shiotani, I. T., Huaman, Z. Z., Sakamoto, S. and Miyazald, T. 1991. The role of wild *Ipomoea batatas* germplasm in sweet potato breeding In: Proceedings of the 9th Synposium of the International Society for Tropical Root Crops (ISTRC), 20-26. October. 1991. Accra, Ghana.

Fathima, K., Khan, M. H. and Panda, S. K. 2002.Active oxygen metabolism as influence by NaCl in Palmarosa.*J. Pl. Bio.* 29:1177-1180.

Feder, M. E. 2006. Integrative biology of stress: molecular actors, The Fravi ecological theater, and the evolutionary play. International symposium on Environmental Factors, Cellular Stress and Evolution, Varanasi, India, October 13-15, 2006, P. 21.

Ford, C. W. and Wilson, J. R. 1981. Changes in levels of solutes during osmotic adjustment to water stress in leaves of four tropical pasture species. *Aust. J. Plant Physiol.* 8: 77-91.

Foster, J. G. and Edward, G. E. 1980. Localization of superoxide dismutase in leaves from C3 and C4 plants.*Plant Cell Physilo.* 21: 895-906.

Foyer, C. H. and Halliwell, B. 1977.Purification and properties of dehydroascorbate reductase from spinach leaves.Phytochemistry. 16: 1347-1350.

Foyer, C. H. and B. Halliwell. 1976. The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Plant 133:21–25.

Foyer, C. H., Alscher, R. G. and Hess, J. L. 1994." Ascorbic acid". In antioxidants in higher plants. CRC Press, Boca Raton, FI. pp. 31-58.

Gargi, G. 2014. Physiological, phytochemical and molecular studies on abiotic stress mediated antioxidant accumulation in *Amaranthus spinosus* Linn. M.Sc. (Ag) Thesis, Kerala A gricultural University. P. 67

Ghezzi, P., Bonetto, V.and Fratelli, M. 2005. Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation.Antioxidants and Redox Signaling 7: 964–972.

Gogorcena, Y., Ormaetxe, H. I., Escureds, P. R. and Becana, M. 1995. Antioxidant defenses against activated oxygen in pea nodules subjected to water stress. *Pl. Physiol.* 108:753-759.

Gong, M., Chen, S. N., Song, Y. Q., Li, Z. G. 1997. Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedling. Australian J. Plant Physiol. 24: 371-379.

Groden, D. and Beck, E. 1979.H_2O_2 destruction by ascorbate dependent systems from chloroplasts. Biochimica et biophysica Acta 546: 426-435.

Gulnur, Arabaci. 2011. Partial Purification and Some Properties of Catalase from Dill (*Anethum graveolens* L.)*J. of Biology and Life Sci.* 2:11-15.

Gupta, S. C., Sharma, A., Mishra, R. and Chowdhuri, D. K. 2010. Heat Shock proteins in toxicology: how close and how far. *Life Sci.* 86: 377-384.

Habtu, A. 1995. Proceeding of the 25th anniversary of Nazareth agricultural research center: 25 years of experience in lowland crops research, 20-23 September 1995. *Nazareth Agric Res cent.*p.36.

Hahn, S. K. 1977. Sweet potato, Ecophysiology of Tropical Crops, Academic Press, *Inc.* New York, pp. 237-248.

Hahn, S. K. and Hozyo, Y. 1984. Sweet potato. In: Goldsworthy, P. R. and Fisher, N. M. (eds.), The physiological and tropical field crops. Wiley, New York. P. 551-562.

Halliwell, B. 2006. Oxidative stress and neurodegeneration: where are we now? *J Neurochem* 97:1634–1658.

Hammett, H. L., Constantin, R. J. and Hernandez, T. P. 1982.The effect of phosphorous and soil moisture levels on yield and processing quality of 'Centennial' sweet potatoes.*J. Am. Soc. Hort. Sci.* 107: 119-122.

Hanson, A. D. and Hitz, W. D. 1982. Metabolic response of mesophytes to plant water deficits. *Annu. Rev. Plant Physiol.* 33: 163-203.

Hernandez, T. P. and Barry, J. R. 1966.The effect of different soil moisture levels and rates of nitrogen on production and quality of sweet potatoes.*Proc. Int. Hort. Congr.* 17: 327-335.

Hertwig, B., Streb, P. and Feierabend, J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant physiol.* 100: 1547-1553.

Hernández, J. A., Ferrer, M. A., Jiménez, A., Barceló, A. R. and Sevilla, F. 2001.Antioxidant systems and $O^{2\cdot-}/H_2O_2$ production in the apoplast of pea leaves.Its relation with salt-induced necrotic lesions in minor veins.*Plant Physiol.* 127:817–831.

Horemans, N., Foyer, C. H., Potters, G. and Asard, H. 2000.Ascorbate function and associated transport systems in plants. *PlantPhysiol Biochem* 38:531−540.

Hossain, M. A., Nakano, Y. and Asada, K. 1984.Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide.*Plant Cell Physiol* 25: 385-395.

Hossain, M. A. and Asada, K. 1985. Monodehydroascorbate reductase from cucumber is a flavin adenine dinucleotide enzyme. *J. Biol. Chem.* 260:12920-12926.

Hsien-Jung,Chen., Mufidah, Afiyanti., Guan- Jhong, Huang., Shyh-Shyun, Huang.and Yaw-Huei, Lin. 2011. Characterization of a leaftype catalase in sweet potato (*Ipomeabatatas* Lam. (L.))*Bot Stud.*52: 417-426.

Huaman, Z. 2002. Systematic botany and morphology of sweet potato plant, Sweet potato germplasm management (*Ipomoea batatas*) CIP Training manual section.

Huett, D. O. 1976. Evalution of yield, Variability and quality of sweet potato cultivars in sub-tropical Australia. *Expt. Agr.* 12: 9-16.

Huett, D. O. and O'Neill, G. H. 1976.Growth and development of short and long season sweet potatoes in sub-tropical Australia.*Expt. Agr.* 12: 385-394.

Huffaker, R. C., Radin, T., Kleinkopf, G. E. and Cox, E. L. 1970.Effect of mild water stress on enzymes of nitrate assimilation and of the carboxylation phase of photosynthesis in barley.*Crop Sci.* 10: 471-474.

Indira, P. and Kabeerathumma, S. 1988. Physiological response of sweet potato under water stress: 1. Effect of water stress during different phases of tuberisation. *J. Root Crops* 14: 37-40.

Indira, P. and Kabeerathumma, S. 1990. Physiometabolic changes in sweet potato grown under different levels of soil moisture. *J. Root Crops* 16: 28-32.

Jackson, C., Cench, J., Moore, A. L., Halliwel, B., Foyer, C. H. and Hall, D. O. 1978.Subcellular localization and identification of superoxide dismutase in the leaves of higher plants.*Eur J of Biochem.* 91: 339-344.

James, A. W. 1994. Sweet potato research at IITA: 1971-1987 In: International Potato Center (CIP), Sweet potato situation and priority research in West and Central Africa, Lima, Peru, pp. 47 - 58.

Jansen, M. A. K., Gaba, V. and Greenberg, B. M. 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends in *Plant Sci.* 3: 131 - 135.

Jimenez,A., Hernandez, J., Pastori, G., del Rio, L. and Sevilla, F. 1998. Role of the ascorbate±glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves. *Plant Physiol.* 118: 1327 - 1335.

John, C. F., Morris, K., Jordan, B. R., Thomas, B. and A-H-Mackerness S. 2001. Ultraviolet-B exposure leads to upregulation of senescenceassociated genes in Arabidopsis thaliana. *J of Exp Bot.* 52: 1367 - 1373.

Karuppanapandian, T., Sinha, P. B., Kamarul Haniya, A. and Manoharan, K. 2006a. Differential antioxidative responses of ascorbate-glutathione cycle enzymes and metabolites to chromium stress in green gram (*Vigna radiata* L. Wilczek) leaves. *J Plant Biol.* 49: 440 - 447.

Karuppanapandian, T., Sinha, P. B, Kamarul Haniya, A., Premkumar, G. and Manoharan, K. 2006b. Aluminium-induced changes in antioxidative enzyme activities, hydrogen peroxide content and cell wall peroxidase activity in green gram (*Vigna radiata* L. cv. Wilczek) roots. *J Plant Biol.* 33: 241 - 246.

Karuppanapandian, T., Sinha, P. B., Premkumar, G. and Manoharan, K. 2006c. Chromium toxicity: Correlated with increased in degradation of photosynthetic pigments and total soluble protein and increased peroxidase activity in green gram (*Vigna radiata* L.) seedlings. *J Swamy Bot-Cl.* 23: 117 - 122.

Karuppanapandian, T. and Manoharan, K. 2008.Mechanism, control and regulation of leaf senescence in plants.*J Plant Biol* 35: 141 - 155.

Karuppanapandian, T., Sinha, P. B., Kamarul Haniya, A. and Manoharan, K. 2009. Chromium-induced accumulation of peroxide content, stimulation of antioxidative enzymes and lipid peroxidation in green gram (*Vigna radiata* L. cv. Wilczek) leaves. *Afr J Biotechnol* 8:475–479.

Karuppanapandian, T., Wang, H. W., Prabakaran, N., Jeyalakshmi, K., Kwon, M., Manoharan, K. and Kim, W. 2011.2,4-dichlorophenoxyacetic acid-induced leaf senescence in mung bean (*Vigna radiata* L. Wilczek) and senescence inhibition by co-treatment with silver nanoparticles.*Plant Physiol Biochem* 49: 168 - 177.

Kawakami, S., Matsumoto, Y., Matsunaga, A., Mayama, S. and Mizuno, M. 2002.Molecular cloning of ascorbate peroxidase in potato tubers and its response during storage of low temperature.*Plant Sci.* 163: 829-836.

Katiyar – Agarwal, S., Agarwal, M. and Grover, A. 2003. Heat – tolerant basmati rice engineered by over – expression of hsp 101. *Plant Mol. Biol.* 51: 677 - 686.

Kaur, C. and Kapoor, H. C. 2001.Antioxidants in fruits and vegetables – the millennium's health.*Int. J. Food Sci. Technol.* 36: 703 – 725.

Kay, D. E. 1987. Root Crops, Crop and Product Digest no. 2 (2nd edition) revised by Gooding, EGB, Tropical Development and Research Institute, *Lond.*pp. 380.

Keller, T. 1998. A plant homolog of the neutrophil NADPH oxidase gp91 phox subunit gene encodes a plasma membrane protein with Ca_2C binding motifs. *Plant cell.* 10: 255 – 266.

Kimball, B. A. and Mitchell, S. T. 1981.Effects of CO_2 enrichment, ventilation, and nutrient concentration on the flavor and vitamin C content of tomato fruit.*HortScience,* 16: 665-666.

King, G. A. 1985. The effect of time of planting on yield of six varieties of sweet potato in the southern coastal lowlands of Papua New Guinea.*Trop. Agr.* 62: 225-228.

Kosakivska, D., Klymchuk, V., Negretzkyl, D. and Blumal, A. U. 2008. Stress proteins and ultrastructural characteristics of leaf cells of plants with different types of ecological strategies, Gen. Appl. *Plant Physiol.* 35: 405-408.

Kuroda, H., Sagisaka, S., Asada, M. and Chiba, K. 1991. Peroxidase-scavenging enzymes during cold acclimation of apple callus in culture. *Plant Cell Physiol.* 32: 635-641.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage. T4. *Nat.* 227: 680-685.

Lambers, S.K., Chaplin, F. and Pons, T. L. 1998.*Plant Physiol. Ecol.*, Springer – Verlag, New York, P. 540.

Lara-Ortiz, T. 2003. Reactive oxygen species generated by microbial NADPH oxidase NoxA regulated sexual development in *Aspergillus nidulans. Mol. Microbio.* 50: 1241 – 1255.

Lee, J. H. and Schoff, F. 1996. An Hsp70 antisense gene affects the expression of HSP70/HSC70, the regulation of HSF and the acquisition of thermotolerance in transgenic *Arabidopsis thaliana. Mol. Gen. Genet.* 252: 11-19.

Lindquist, S. and Crig, E. A., 1988.The heat shock proteins.*Annu. Rev. Genet.*22: 631-677

Logan, B. A., Grace, S. C., Adams, W. W. and Demming, A. 1998. Seasonal differences in Xanthophyll cycle characteristics and antioxidants in Mahonia ripen growing in different light environments. *Oecolgia*, 116:1191-1201.

Luck, H. 1974.Methods in *Enzymatic Analysis*, (2nd Edn.) Bregmeryer Academic Press, New York, P. 805.

Luwe, M. F. W., Takahama, U. and Heber, U. 1993. Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinuciu oleracea* L.) leaves. *Plant physiol.* 101: 969-976.

Madamanchi, N., Alscher, R., Hatzion, K. and Cramer, C. 1994. Acquired resistance to herbicides in pea cultivars by exposure to sulfur dioxide.Pest.Biochem. Physiol. 48: 31-40.

Malan, C., Greglng, M. and Gressel, J. 1990. Correlation between Cu/Zn superoxide dismutase and glutathione reductase and environmental and xenobiotic stress tolerance in Maize inbreeds. *Pl. Sci.*, 69:157-166.

May, M. J. and Leaver, C. J. 1993.Oxidative stimulation of glutathione synthesis in Arabidopsis thaliana suspension cultures.*Plant Physiol.* 103: 621-627.

Mehlhorn, H., Seufert, G., Schmidt, A. and Kunert, K. J. 1986.Effect of SO_2 and O_3 on production of antioxidants in conifers.*Plant Physiol.* 82: 336-338.

Metwally, A., Safronova, V. I., Belimov, A. A. and Dietz, K. J. 2005. Genotypic variation of the response to cadmium toxicity in *Pisum sativum L. J Exp Bot.* 56:167–178.

Michael, P. I. and Krishnaswamy, M. 2011. The effect of zinc stress combined with high irradiance stress on membrane damage and antioxidative response in bean seedlings. *Environ.Exp. Bot.*, 74:171-177.

Mitra, R. and Bhatai, C. R. 2008. Bioenergetic cost of heat tolerance in wheat crop. *Curr. Sci.* 94: 1049-1053.

Mittler, R., Vanderauwera, S., Gollery, M. and Van Breusegem, F. 2004.Reactive oxygen gene network of plants.*Trends Plant Sci.* 9: 490-498.

Moron, M. S., Dipierre, J. W. and Mannervik, B. 1979.Levels of glutathione reductase and glutathione-S-transferase activities in rat lungs and liver.*Biochem.Biophy.Acta.* 582: 67 - 82.

Moran, J. F., Becna, M., Iturbe, O. J., Frechilla, S., Klucas, R. V. and Apariciao-Tejo, P. 1994. Drought induced oxidative stress in pea plant. *Planta*, 194:346-352.

Morel, J. and Dangl, J. 1997.The hypersensitive response and the induction of cell death in plants. Cell Death and Differentiation 4: 671 - 683.

Morimoto, R. I. 1993. Cells in stress: The transcriptional activation of heat shock genes. Sci. 259: 1409-1410.

Mortensen, A., Skibsted, L. H. and Truscott, T. G. 2001.The interaction of dietary carotenoids with radical species.*Arch Biochem Biophys* 385:13–19.

Mullineaux, P. M. and Rausch, T. 2005. Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression.*Photosynth Res.* 86: 459–474.

Murgia, I., Tarantino, D., Vannini, C., Bracale, M., Carravieri, S. and Soave, C. 2004.*Arabidopsis thaliana* plants overexpressing thylakoidal ascorbate peroxidase show increased resistance to paraquat-induced photo-oxidative stress and to nitric oxide-induced cell death, *Pl. J.*, 38:940-953.

Murthy, S. S. and Zilinskas, B. A. 1994. GenBank accession number U06461.

Nagesh Babu, R. and Devaraj, V. R. 2008. High temperature and salt stress response in French bean (*Phaseolus vulgaris*). *Aust J Crop Sci.* 2:40–48.

Nair, G. M., Nair, V. M. and Sreedharan, C. 1996. Response of sweet potato to phasic stress irrigation in summer rice fallows. *J. Root Crops* 22: 45-49.

Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts.*Plant and cell physiol.* 22: 864-880.

Nakano, Y. and Asada, K. 1987. Purification of ascorbate peroxidase in spinach chloroplasts: its inactivation in ascorbate – depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiol.* 28: 131 - 140.

Nakatani, M. 1989. Recent studies on dry matter production physiology. *Rep. Workshop* Sweet *potatoImprovement in Asia, ICAR, India*, pp. 147-159.

Naskar, S. K. and Chowdhury, S. R. 1995.Genotypic variation in relative water content and nitrate reductase activity in sweet potato under field drought condition. *J. Root Crops* 21: 46- 49.

Nautiyal, S., Pal, M. and Nagi, S. 1993. Effect of water stress and antitranspiranance on the chlorophyll contents of the leaves of Grevellia robusta (A. Cunn.).*Ann. For.* 1:85-89.

Negeve, J. M., Hahn, S. K. and Bouwkamp, J. C. 1992. Effects of altitude and environments on sweet potato yield in Cameroon. *Trop. Agric. Trinidad* 69: 43-48.

Niu, Y., Wang, Y. P., Li, P., Zhang, F., Liu, H. and Zheng, G. C. 2013. Drought stress induces oxidative stress and the defence system in ascorbate – deficient *vct l* mutants of *Arabidopsis thaliana. Acta. Physiol. Pl.* 35:1189-1200.

Noctor, G. and Foyer, C. H. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Physiol Plant Mol Biol* 49:249–279.

Noctor, G., Gomez, L., Vanacker, H. and Foyer, C. H. 2002.Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling.*J of Exp Bot.* 53: 1283–1304.

Noreen, S., Athar, R. U. B. and Ashraf, M. 2013. Interactive effects of watering regimes and exogenously applied osmoprotectants on earliness indices and leaf area index in cotton (Gossypium hirsutum L.) *crop. Pak. J. Bot.*, 45(6): 1873-1881.

NRCRI: 1983. Annual Report, National Root Crop Research Institute (NRCRI), Umudike, Nigeria, vol. 1, pp. 2-3.

Oliva, M., Theiler, G., Zamocky, M., Koua, D., Margis-Pinheiro, M., Passardi, F. and Dunand, C. 2009. Peroxibase: A powerful tool To collect and analyse peroxidase sequences from Viridiplantae. *J. Exp. Bot.* 60: 453-459.

Onwueme, I. C. and Sinha, T. D. 1991. Field Crop Production in Tropical Africa: Principles and Practice, Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, *Neth.* pp.480.

Onyekwere, P. S. N. and Nwinyi, S. C. O. 1989.Water requirements of sweet potato.*In Annu. Rept. Nat. Root Crops Res. Inst.* Umudike, Nigeria. P. 48-51.

Park, S. Y, Ryu, S. H., Jang, I. C., Kwon, S. Y., Kim, J. G., Kwak, S. S. 2004. Molecular cloning of a cytosolic ascorbate peroxidase cDNA from cell cultures of sweet potato and its expression in response to stress. *Mol. Genet Genomics*. 271: 339-346.

Parsell, P. A. and Lindquist, S. 1993. The function of heat shock proteins in stress tolerance degradation and reactivation of damaged proteins.*Annu. Rev. Genet*. 27: 437-496.

Parthasarathi, V.2004.Effect of abiotic stress on the physiology and andrographolide content in *Andrographis paniculata Nees*. M.Sc. (Ag.) Thesis, Kerala agricultural university.P.34.

Passardi, F., Bakalovic, N., Teixeira, F. K., Margis-pinheiro, M., Penel, C. and Dunand, C. 2007.Prokariyotic origins of the non animal peroxidase superfamily and originelle-mediated transmission to eukaryotes. *Genome.*89: 567-579.

Pell, E. J., Schlagnhaufer, C. D. and Arteca, R. N. 1997. Ozone-induced oxidative stress: mechanisms of action and reaction. *Physiologia Plant*. 100: 264 - 273.

Pietrini, F., Iannelli, M. A., Pasqualini, S. and Massacci, A. 2003.Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin.Ex Steudel.*Plant Physiol*. 133:829–837.

Prasad, T. K., Anderson, M. D., Martin, B. A. and Stewart, C. R. 1994. Evidence for chilling induced oxidative stress in maize seedling and a regulatory role for hydrogen peroxide. *Pl. Cell*. 6:65-74.

Pratima, K. 1999. Chlorophyll as an indicator of light Intensity in andrographis paniculata.*Indian J. Pl. Physiol*. 4:15-19.

Prochazkova, D., Sairam, R. K., Srivastava, G. C. and Singh, D. V. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Sci.* 161: 765 - 771.

Rahimi, A., Hossenini, M. S., Pooryoosef, M. and Fateh, I. 2010.Variation of leaf water potential, relative water potential, relative water content and SPAD under gradual drought stress and stress recovery in two medicinal species of *plantago ovato and P. psyllium.Pl. Ecophysiol.*2: 53-60.

Rajasekar, R., Cox, S. and Satyanarayana, N. 1991. Evalution of certain morphological factors in tea (Camellia sinensis) cultivars under stress.*J. plant Crops*. 18:83-89.

Ramanujam, T. 1990. Effect of moisture stress on photosynthesis and productivity of cassava photosynthetica 24: 217-224.

Ravi, V. and Indira, P. 1995. Investigation on the physiological factors limiting yield potential in sweet potato under drought stress.*Annu. Rep.* CTCRI, Trivandrum, India, pp. 37-38.

Ravi, V. and Indira, P. 1996. Investigation on the physiological factors limiting yield potential in sweet potato under drought stress.*Annu. Rep.* CTCRI, Trivandrum, India, pp. 23-24.

Ravi, V. and Indira, P. 1997. Investigation on the physiological factors limiting yield potential in sweet potato under drought stress.*Annu. Rep.* CTCRI, Trivandrum, India, pp. 29.

Ravi, V,. and Indira, P. 1999. Crop physiology of sweet potato.*Hort. Rev.* 23: 227-338.

Ravi, V. and George, J. 2003.Studies on drought management in cassava. In: Annual Report, Central Tuber Crops Research Institute, Trivandrum. pp. 41-42.

Reddy, C. D. and Venkaiah, B. 1982.Studies on isoenzymes of superoxide dismutase from mung bean (*Vigna radiata*) seedlings.*J of plant physiol.* 116: 279-284.

Sano, S. and Asada, K. 1994.cDNA cloning od monodehydroascorbate radical reductase from cucumber: a high degree of homology in terms of amino acid sequence between this enzyme and bacterial flavor enzymes. *Plant Cell Physiol.* 35: 425-437.

Sano, S., Ueda, M., Kitajima, S., Takeda, T., Shigeoka, S., Kurano, N., Miyachi, S., Miyaki, C. and Yokota, A. 2001.Characterization of ascorbate peroxidase from unicellular red alga Galdieria partita.*Plant Cell Physiol.* 42: 433-440.

Sadasivam, S. and Manickam,A. 1992. In: Biochemical Methods for Agricultural Sciences, *Wiley Eastern Ltd.*, New Delhi, pp. 184-185.

Sadasivam, S. and Manickam, A. 2008.*Biochemical methods*, (second edition). New Age Int Publishers, New Delhi,pp. 256.

Sairam, R. K. and Srivastava, G. C. 2002.Changes in antioxidant activity in sub-cellular fraction of tolerant and susceptible wheat genotypes in response to long term salt stress.*Plant Sci* 162:897–904.

Sairam, R. K.,Deshmukh, P. S. and Saxena, D. C. 1998. Role of antioxidant system in wheat genotypes tolerance to water stress. *Biologia Pl.*, 41:387-394.

Sandalio, L. M. and Del Rio, L. A. 1987. Localization of superoxide dismutase in glyoxysome from Citrullus vulgaris: functional implications in cellular metabolism. *J of plant physiol.* 127: 395-405.

Sarowar, S., Kim, E. U., Kim, J. Y., Ok, S. H., Kim, D. K., Hwang, K. B. and Shin, S. J. 2005. Overexpression of a pepper ascorbate peroxidase-like 1 gene in tobacco plants enhances tolerance to oxidative stress and pathogens. *Pl. Sci.*, 169:55-63.

Saruyama, H., Onodera, H. and Uemura, M. 2005. Transgenic rice plants expressing wheat catalase show improved tolerance for chilling-induced damage in membranes. Session 3: Opportunities and challenges of transgenic rice. *Proc. World Rice Res. Conf.*, pp. 1108-1109.

Scandalios,J. G. 1993.Oxygen stress and superoxide dismutases.*Plant Physiol.* 101: 7-12.

Scandalios, J. G. 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz. J. Med. Biol. Res. 38: 995-1014.

Schoner, S. and Krause, G. H. 1990. Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. *Planta.* 180: 383-389.

Shamsuddin, S.A.K. and Paul,N.K. 1988.Environmental effects on growth and dry matter production of sweet potato.Bangladesh *Hortic.* 16(2): 17-24.

Shigeoka, S., Nakano, Y. and Kitaoka, S. 1980b. Purification and some properties of L-ascorbic acid – specific peroxidase in euglena gracilis Z.Archives of biochemistry and biophysics 201: 121-127.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y. and Yoshimura, K. 2002.Regulation and function of ascorbate peroxidase isoenzymes.*J. Exp. Bot.* 53: 1305-1319.

Slabbert, M. M. and Kruger, G. H. J. 2014. Antioxidant enzyme activity, proline accumulation, leaf area and cell membrane stability in water stressed Amaranths leaves. *S. Afr. J.Bot.*, 95:123-128.

Smirnoff, N. and Colombe, S. V. 1988. Drought influences the activity of enzymes of the chloroplast hydrogen peroxide scavenging system. *J. Exp. Bot.* 39: 1097-1108.

Smirnoff, N. 2000. Ascorbic acid: metabolism and functions of a multi-facetted molecule. *Curr Opin Plant Biol* 3:229–235.

Smitha, K. R. and Sudha, G. 2011.Antioxidant activity of Spergula arvensis, *Amaranthus spinosus* and *spinacia oleracea.Int. J. Univ. Pharm. Lif.* Sci., 1(3). Available: http://:www.ijupls.com.

Song, X. S., Hu, W. H., Mao, W. H., Ogweno, J. O., Zhous, Y. H. and Yu, J. Q. 2005. Response of ascorbate peroxidase isoenzymes and ascorbate regeneration system to abiotic stresses in *Cucumis sativum* L. *Plant Physiol. Biochem.* 43: 1082-1088.

SPC: 1986. South Pacific Foods and Community Health Services, Leaflet no 13, Sweet potato, South Pacific Commission (SPC), Stredder Print Ltd, Auckland, New Zealand.

Spence, J. A. and Humphries, E. C. 1972. Effect of moisture supply root temperature and growth regulators on photosynthesis of isolated rooted leaves of sweet potato (*Ipomoeabatatas*). *Annu. Bot.* 36: 115-121.

Sun, P., Mantra, N., Moller, M., Shen, J., Shen, Z., Jiang, B., Chen, C., Miao, Q. and Lu, F. 2012. Influence of light and salt on the growth of alien invasive tropical weed Ageratum conyzoides. *Aust. J. crop Sci.*, 6(4):739-748.

Takeda, T., Yoshimura, K., Ishikawa, T. and Shigeoka, S. 1998.Purification and characterization of ascorbate peroxidase in *Chlorella vulgaris.Biochem.* 80: 295-301.

Takeda, T., Yoshimura, K., Yoshii, M., Kanahoshi, H., Miyasaka, H. and Shigeoka, S. 2000. Molecular characterization and physiological role of ascorbate peroxidase from halotolerant Chlamydomonas sp. W80 strain. *Arch. Biochem. Biophys.* 376: 82-90.

Tanaka, K., Suda, Y., Kondo, N. and Sugahara, K. 1985. O_2 tolerance and the ascorbate dependent H_2O_2, decomposting system in chloroplasts.*Plant Cell Physiol.* 26: 1425-1431.

Teixeira, F. K., Menezes-Benavente, L., Margis, R. and Margis-Pinheiro, M. 2004. Analysis of the molecular analysis history of the ascorbate peroxidase gene family: inferences from the rice genome. *J. Mol. Evol.* 59: 761-770.

Teixeira, F. K., Menezes-Benavente, L., Galvao, V. C., Margis, R. and Margis-Pinheiro, M. 2006. Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. *Planta.* 224: 300-314.

Terry, E. R. 1987. Tropical root crops: Root crops and the African food crisis, Proceedings of the 3rd Triennial Symposium of the International Society for Tropical Root Crops (ISTRC)- African Branch, Owerri, Nigeria, August 1986, 197 pp. 17-23.

Thompson, J. E., Froese, C. D., Madey, E., Smith, M. D. and Hong, Y. 1998. Lipid metabolism during plant senescence. Progress in Lipid Research 37: 119-141.

Thompson, J. E., Legge, R. L. and Barber, R. F.1987.The role clf free radicals in senescence and wounding. New PhytollO_5 317-344.

Torres, M. A. 1998. Six Arabidopsis thaliana homologues of the human respiratory burst oxidase (gp91 phox). *Plant J.*, 14: 365 – 370.

Trindade, H., Karmali,A. and Pais,M.S. 1998. One step purification and properties of catalase from *Zantedeschia aethiopica*. *Bio chimie*; 40:17-59.

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

Tsubo, M., Mukhala,E. Ogindo,H. O. and Walker,S. 2003. Productivity of maize-bean intercropping in a semi-arid region of South Africa. Water SA, 29(4): 381-388.

Tsugane, K., Kobayashi, K., Niwa, Y., Obha, Y., Wada, K. and Kobayashi, H. 1999. A recessive Arabidopsis mutant that grows enhanced active oxygen detoxification. *Pl. Cell.*, 11:1195-1206.

Umebese, C. E., Olatimilehin, T. O. and Ogunsusi, T. A. 2009.Salicyclic Acid Protect Nitrate Reductase Activity, Growth and Proline in Amaranth and Tomato plants during Water Deficit.*Am. J. Agric. Biol. Sci.*, 4 (3):224-229.

Van Camp, W., Willekens, H., Bowler, C., Van Montagu, M., Inze, D., Langebartels, C. and Sandermann, H. 1994. Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. *Biotechnol* 12: 165-168.

Vierling, E. 1991.The role of heat shock proteins in plants.Annu. Rev. Plant Phys. 42: 579-620.

Wahid, A., 2007. Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane sprouts.*J. Plant Res.* 120: 219-228.

Wahid, A., Gelani, S., Ashraf, M. and Foolad, M. R. 2007. Heat tolerance in plants: an overview. Environ. Exp.Bot.61: 199-223.

Walter, S. H. 1995. Physiological Plant Ecology, Springer Verlag. New York, P. 508.

Wang, W., Vinocur, B. and Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218: 1-14.

Wang, W., Vinocur, B., Shoseyow, O. and Altman, A. 2004.Role of plant heat shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci.* 9: 244-252.

Wang, J. P., Li, Y. L. and Zhang, J. G. 2008.Effect of high-temperature and excessive-light stress on APX activity in apple peel. Acta Agri Boreali-Sin 23:144–147.

Wilkinson, S. R., Obado, S. O., Mauricio, I. L. and Kelly, J. M. 2002.*Trypanosoma cruzi* expresses a plant-like ascorbate dependent hemoperoxidase localized to the endoplasmic recticulum. *Proc Natl Acad Sci.* 99: 13453-13458.

Wingsle, G. and Karpinski, S. 1996. Differential redox regulation by glutathione of glutathione reductase and CuZn-superoxide dismutase gene expression in Pinus sylvestris L. needles.*Planta* 198: 151–157.

Xiang, C., Werner, B. L., Christensen, E. M. and Oliver, D. J. 2001. The biological function of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. *Plant Physiol* 126:564–574.

Xunzhong, Z. and Schmidt, R. E. 2000. Hormone – containing products impact on antioxidant status of tall fescue and creeping bent grass subjected to drought. *Crop Sci.*, 40: 1344-1349.

Yamaguchi, J. and Nishimura, M. 1984.Purification of glyxoxysomal catalase and immunochemical comparison of glyoxysomal and leaf peroxisomal catalase in germinating pumpkin cotyledons.*Plant Physiol*; 74:261-267.

Yamazaki, I. and Piette, L. H. 1961.Mechanism of free radical formation and disappearance during the ascorbic acid oxidase and peroxidase reactions. *Biochem.Biophys.Acta.* 50: 62-69.

Yang, R. Y. and Keding, G. B. 2009. Nutritional contributions of important African indigenous vegetables In: Shackleton, C. M., Pasquini, M. W. and Drescher, A.W. (eds.), African Indigenous Vegetables in Urban Agriculture, Earthscan, UK, pp. 105-143.

Yang, Y., Han, C., Liu, Q., Lin, B. and Wang, J. 2008.Effect of drought and low light on growth and enzymatic antioxidant system of *Picea asperata* seedling.*Acta. Physiol. Pl.*, 30:433-440.

Yong, T., Zongsuo, L., Hongbo, S. and Feng, D. 2006. Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of *Radix astragalia* at seeding stage.*Colloids and surfaces B:Biointerfaces*, 49:59-64.

Yoruk ,I.H.,Demir,H., Ekici, K. and Savran , A. 2005.Purification and Properties of Catalase from Van Apple (*GoldenDelicious*).*Pakist J of Nutr*.4: 8-10.

Zhang, J. and Kirkham, M. B. 1996.Antioxidant responses to drought in sunflower and sorghum seedlings.*New Phytology*., 132:361-373.

Zhang, H., Wang. J., Nickel, U., Allen, R. D. Goodman, H. M. 1997. Cloning and expression of an Arabidopsis gene encoding a putative peroxisomal ascorbate peroxidase.*Plant Mol Biol.* 34: 967-971.

Zhu, J. K. 2001. Plant salt tolerance. *Trends Plant Sci.* 6: 66-71.

APPENDIX

APPENDIX - I

Reagent for total protein extraction and quantification

a. Bradford's reagent

Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol 100 ml 85% (w/v) phosphoric acid Whatman filter paper

b. Phosphate buffer

PH - 7.0

Volume of 1M K_2HPO_4- 61.5 ml

Volume of 1M KH_2PO_4 – 38.5 ml

Make up to 100 ml

Appendix-II

Reagent for protein profiling using SDS PAGE

a. Resolving gel (12%)

Distilled water - 6.7 ml

1.5M Tris-HCL - 5.0ml (PH 8.8)

10% SDS - 0.2ml

Acrylamide Stock - 8.0 ml

Freshly prepared ammonium persulphate - 0.1 ml

TEMED - 0.01ml

b. Stacking Gel (4%)

Distilled water - 6.1ml

1.5M Tris-HCL 2.5 ml (PH 6.8)

10% SDS - 0.1 ml

Acrylamide Stock - 1.3 ml

Freshly prepared ammonium persulphate - 0.05 ml

TEMED - 0.01ml

c. Acrylamide stock solution (30%)

Acrylamide - 30g

Bisacrylamide - 0.8g

100 ml of distilled water

d. Protein Extraction buffer

Tris - 50mM

Sodium chloride - 50mM

Ethylenediaminetetraacetiacid - 2mm

β -mercaptoethanol - 5mM

PhenylmethylsulphonylIfluoride - 1mM

Polyvinylpyrrolidone - 0.5% (pH 8.0)

e. SDS solution (l0%)

SDS - l0 g

100 ml of distilled water

f. Electrophoresis buffer (pH.8.3)

Tris (0.025M) - 3.02 g

Glycine (97M) - 14.428g

SDS (0.1%) - 1.0 g

Distilled water - 1.0 litre

g. Coomassie blue staining solution

CBB R-250 - 0.25 g

Methanol: water (1: l) - 90 ml

Glacial acetic acid - 10 ml

h. Destaining solution

Methanol: water (1:1) - 90 ml

Glacial acetic acid - 10 ml

Appendix – III

Reagent for estimation of Proline

a. Aqueous sulphosalicylic acid (3%)

Sulphosalicylic acid - 3 g

Distilled water - 100 ml

b. Acid Ninhydrin

Ninhydrin - 1.25 g dissolved in a warm mixture of 30 ml of glacial acetic acid. Phosphoric acid - 20 ml (6 M) Stored 24 hours at 4°C

Appendix – IV

Reagent for glutathione reductase assay

a. Glutathione Reductase Extraction buffer

Phosphate buffer - 50 mM (pH7.0)

EDTA - 1mM

TritonX-100 - 0 05%

PVP-40 - 2%

Ascorbic acid - 1mM

b. Glutathione reductase assay mixture

Tris-buffer - 0.1M (pH7 8)

EDTA - 2mM

NADPH - 50mM

GSSG - 0.5mM

Enzyme extract - 20µ1

Appendix – V

Reagent for Superoxide Dismutase assay

a. SOD extraction buffer

Tris-HC1 - 20 mM (pH 7.5)

$MgCl_2$ - 5mM

NaC1 - l0mM

b. SOD Assay Mixture

Phosphate buffer - 50mM (pH **7.8)**

EDTA - 0.1mM

Methionine - 13mM

Nitroblue tetrazolium (NTB) - 75µM

Riboflavin - 2 µM

Supernatant - 100 µl

Appendix – VI

Reagent for Catalase assay

a. Catalase Extraction Mixture

Phosphate buffers - 50 mM (pH 7.0)

H_2O_2 – 3ml (2mM)

Appendix – VII

Reagent for ascorbate peroxidase assay

a. Ascorbate Peroxidase Extraction Buffer

Phosphate buffer - 1mM (pH 7.0)

Sorbitol - 70%

EDTA - 1mM

PMSF - 0.1%

b. Ascorbate peroxidase assay mixture

Ascorbate - 0.25 M

H_2O_2 - 1 mM

Phosphate buffer – 50 mM (pH 7.0)

Enzyme extract - 37.5 µl

Appendix VIII

Reagent for Ascorbate Free Radical Reductase assay

a. Ascorbate free radical reductase assay buffer

00.2 M potassium phosphate

PH 7.8, 1 mM EDTA

5 mM MgCl, 0.1% (w/v) BSA

10 mM 2-mercaptoethanol

0.005% (v/v) Triton X-100

Appendix IX

Preparing the field and planting

Make ridges at 60 cm apart having 25-30 cm height

Plant the cutting at 20 cm spacing on the ridges

Bury the vines horizontally with two to three nodes below the soil leaving the remaining portion above the soil leaving the remaining portion above the soil

Manuring and interculturing

Apply farm yard manure @ 5 tonnes/ ha before preparing the ridges apply fertilizers @ 50 kg N, 25 kg P2O5, 50 kg K2O/ha Apply urea (55 kg) or ammonium sulphate (125 kg), rock phosphate (125 kg) and muriate of potash (85 kg) per hectare at the time of planting. Top dress, a month after planting, with 55 kg urea or 125 kg ammonium sulphate along the side of the ridges

Earth up and weed along with top dressing

Crop protection

Sweet potato weevil (*cylas formicarius*)

Sweet potato weevil is the most important pest causing very severe damage to the crop. Adult weevil makes puncturing on vines and tubers. The gruds bore and feed by making tunnels. Even the slightly damaged tubers are unsuitable for consumption due to bitterness. Yield loss may go up to 100% in severe cases. On an average, 20-55% tuber loss occurs. The following integrated pest management will be effective for the control Dip the vine cutting in chlorpyriphos – 0.2% and monocrotophos – 0.05% solution for 10 minutes before planting.

Reridge the crop two months after planting

Harvesting

Remove the vines and dig out the tubers without injuring them.

ABSTRACT

OXIDATIVE STRESS AND PROTEIN PROFILING IN SWEET POTATO (*Ipomoeabatatas* (L.) Lam.) UNDER ABIOTIC STRESSES

AISWARYA N. S.

(2010-09-110)

Abstract of the thesis

Submitted in partial fulfilment of the

requirement for the degree of

MASTER OF SCIENCE (INTEGRATED) IN BIOTECHNOLOGY

Faculty of Agriculture

Kerala Agricultural University, Thrissur

B.S.c.-M.S.c. (Integrated) Biotechnology

Department of Plant Biotechnology

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2017

ABSTRACT

The present study an "Oxidative stress and protein profiling in sweet potato (*Ipomoeabatatas* (L.) Lam.) under abiotic stresses" was conducted during the period 2014-2015 in the Division of Crop Production, Central Tuber Crops Research Institute (CTCRI) Thiruvananthapuram. The objective of the study was to elicit information on the antioxidative enzyme activity, protein profiling, growth parameter and yield parameter of five varieties / genotypes of sweet potato under irrigated high temperature stress (HTS) (40°C during the day time) and water deficit stress (WDS) (2nd and 3rd month after planting) conditions and identify marker physiological parameter which can be used for identifying / developing sweet potato varieties for tolerance to abiotic stress conditions. The study indicated that the vein length of five different sweet potato varieties/ genotypes was not significantly affected by HTS and WDS. The leaf production of five sweet potato varieties was significantly affected by both HTS and WDS. The leaf retention of five varieties / genotype was differentially affected by both heat and water stress. There was negligible difference in yield parameters between stress and control condition. However, there was significant difference in yield observed for parameters among five varieties. The effect of abiotic stress on various physiological parameters like Relative water content (RWC), chlorophyll and carotenoid content significantly differed in control plant. Among the WDS and HTS treatments, the chlorophyll and carotenoid content decreased in all the five varieties. Chlorophyll and carotenoid content were significantly reduced in plants exposed to HTS.

RWC of leaves varied under both WDS and HTS conditions. A higher RWC was observed in plants exposed to HTS condition compared to plants was WDS condition. Total soluble protein content of leaf was found to be lower due to presence of high mucilage in leaves. Total soluble protein in tuber is increased in control and WDS conditions. Total soluble protein in tuber forming roots had increased under stress free condition. The effect of abiotic stress on biological parameter like proline status of plants under stress conditions followed an

increasing trend compared to stress free condition. The outcome of abiotic stress on various enzymatic antioxidant such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), ascorbate reductase, glutathione reductase (GR) was studied. Among the five different sweet potato varieties/ genotypes (Sree Arun, S-1464, S-1466, Sree Kanaka and Kanjangad) S-1466 and Sree Kanaka had maximum GR activity under HTS compared to WDS and control condition. Catalase activity of five varieties/ genotypes of sweet potato plant varied among different stress condition (WDS and HTS) compared to control. The GR activity was significantly reduced due to stress condition and SOD activity increased under stress conditions. The APX enzyme activity is more under WDS conditions compared to HTS and control conditions. Ascorbate reductase become there was no change in absorbance therefore the enzyme activity could not be measured. The AA content in sweet potato leaves increased under WDS condition compared to HTS and control. The glutathione content in sweet potato leaves was negligible or below detectable amount under both control and stress condition.

Under HTS condition SOD activity increased in Sree Arun and S-1466 alone whereas other varieties/genotypes had increased SOD activity under WDS. Kanjangad had maximum APX activity in HTS whereas S-1464 had higher APX activity in WDS. From the present study, it is inferred that SOD activity can be utilised as biochemical marker for developing HTS tolerant varieties as it showed consistent changes under both WDS and HTS. APX activity can be utilised as marker for identifying WDS tolerant varieties. Detailed molecular biochemical and physiological information will be necessary using different varieties for getting appropriate activities as the marker to screen abiotic stress tolerant varieties.