STUDIES ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SOYBEAN [*Glycine max* (L.) Merrill] GENOTYPES TO INDUCED WATER STRESS

By

CH. RAMESH

B.Sc. (Ag.)

THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
RAJENDRANAGAR, HYDERABAD-500 030 (A.P.)

October, 2006

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SOYBEAN [*Glycine max* (L.) Merrill] GENOTYPES TO INDUCED WATER STRESS"** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the **Acharya N.G. Ranga Agricultural University, Hyderabad**, is a record of the bonafide research work carried out by **Mr. CH. RAMESH** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigation have been duly acknowledged by the author of the thesis.

(Dr. V. PADMA)
Chairman of the Advisory Committee

Thesis approved by the Student's Advisory Committee

Chairman **(Dr. V. PADMA)** _____
Associate Professor
Department of Plant Physiology
College of Agriculture, ANGRAU
Rajendranagar, Hyderabad-500 030

Member **(Dr. T.Y. MADHULETY)** _____
Professor
Department of Plant Physiology
College of Agriculture, ANGRAU
Rajendranagar, Hyderabad-500 030

Member **(Dr. K. MANORAMA)** _____
Associate Professor
Department of Agricultural Biotechnology
College of Agriculture, ANGRAU
Rajendranagar, Hyderabad-500 030

CERTIFICATE

Mr. CH. RAMESH has satisfactorily prosecuted the course of research and that the thesis entitled **"STUDIES ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SOYBEAN [*Glycine max* (L.) Merrill] GENOTYPES TO INDUCED WATER STRESS"** submitted is the result of original research work done and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date :
Place : Hyderabad

(Dr. V. PADMA)
Chairman of the Advisory Committee

DECLARATION

I, **CH. RAMESH** hereby declare that the thesis entitled **"STUDIES ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SOYBEAN [*Glycine max* (L.) Merrill] GENOTYPES TO INDUCED WATER STRESS"** submitted to the **ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY** for the degree of **MASTER OF SCIENCE IN AGRICULTURE** is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier in any manner.

Date :
Place : Hyderabad **(CH. RAMESH)**

Author	:	**CH. RAMESH**
Title of the thesis	:	**STUDIES ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SOYBEAN [*Glycine max* (L.) Merrill] GENOTYPES TO INDUCED WATER STRESS**
Degree	:	**MASTER OF SCIENCE IN AGRICULTURE**
Faculty	:	**AGRICULTURE**
Discipline	:	**PLANT PHYSIOLOGY**
Major Advisor	:	**Dr. V. PADMA**
University	:	**ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY**
Year of submission	:	**2006**

ABSTRACT

Field experiments with soybean varieties were conducted during post-rainy seasons of 2005-2006 at College Farm, College of Agriculture, Rajendranagar, Hyderabad to evaluate the physiological and biochemical responses of soybean genotypes to induced water stress. The experiment was laid out in a split-plot design replicated thrice with three main treatments *viz*., control or No water stress, water stress at flowering phase and water stress at pod filling phase, with five soybean genotypes *viz*., MAUS-47, JS-335, JS-93-05, PK-1029 and NRC-37 as sub-plot treatments.

The results obtained in the present investigation revealed that plant height, number of branches, number of leaves, dry weights of leaves, stem, pods and total dry weight, seed yield, pod per plant, seeds per pod and 100 seed weights were decreased with the water stress in soybean genotypes. The decrease was more when

stress was imposed at flowering phase than that at pod filling phase. Among soybean genotypes, JS-93-05 was found relatively more tolerant to water stress, while MAUS-47 was relatively susceptible.

Among all soybean genotypes tested, maximum drought index and drought tolerance efficiency were recorded in JS-93-05, while the lesser values were recorded in MAUS-47.

Biochemical studies revealed that catalase, SAMS, GST, glycine betaine and proline content increases, while decrease in Ascorbic acid content and expression of isomorphs viz., SOD, POX and trehalase observed in water stress conditions in soybean genotypes.

Based on physiological and biochemical studies it could be concluded that water stress at flowering and pod filling phases decreases the seed yield 22.76 % and 17.74 % respectively. Among the five cultivars tested Cv. JS-93-05 performed better in all main treatments *viz.*, no water stress, water stress at flowering and water stress at pod filling phase..

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

ACKNOWLEDGEMENTS

*I bow with greatfulness before **LORD ANJANEYA** and **LORD SRIKRISHNA** for having bestowed upon me their grace and blessings, giving me stamina, patience and strength to bring out this humble piece of work into light.*

*With immense pleasure and deep respect, I express my profound sense of gratitude, indebtedness and heartful thanks to my revered teacher, Major Advisor and Chairman of advisory committee, **Dr. V. PADMA**, Associate Professor, Department of Plant Physiology, College of Agriculture, Rajendranagar, Hyderabad for her valuable suggestions, keen interest, invaluable guidance, constant encouragement, wise direction, wholehearted cooperation and immense support throughout the study period and in the successful completion of present study.*

*I extend my deep sense of reverence and gratitude to **Dr. T.Y. MADHULETY**, Professor, Department of Plant Physiology and member of my Advisory Committee for his constant encouragement, incisive criticism and constructive suggestions during the course of investigation and in shaping the thesis.*

*I humbly place on my record and gratitude to **Dr. K. MANORAMA**, Associate Professor, Department of Agricultural Biotechnology and Member of my Advisory Committee for her valuable guidance and whole-hearted help which have greatly facilitated the production of this thesis.*

*I humbly express my profound gratitude to **Dr. B. GOPAL SINGH**, Professor and University Head, Department of Plant Physiology, College of Agriculture, Rajendranagar, Hyderabad for providing the required facilities for my research work and for his marked advice, affectionate encouragement and cooperation during the course of this study.*

*With respectful regards and immense pleasure. I express my deep sense of gratitude to **Dr. P.V. Rao, Dr. A. Sivasankar** and **Dr. Suryaprakash Rao** for their constant encouragement and cooperation during the course of my study.*

*I wish to express my sincere thanks to **DR. Ramasubba Reddy,** Farm Superintend, **Mr. Katti**, Agronomist and Sub-Assistants for their help and cooperation during the period of my field research.*

*From innermost of my heart I express my deep sense of gratitude and love to my grand parents, **Sri Rajaram, Smt. Kashmma,** my parents, **Sri***

***Venkataswamy, Smt. Vijayalaxmi**, ever loving sister **Vanitha** and other family members for their constant encouragement and help in the pursuit of my post-graduate studies*

*Above all, this acknowledgement would be out of tune if I fail to mention my beloved **Sri B. Venkateshwarlu**, **Sri R. Murali**, **Mr. V. Rajaram, Mr. A. Jayaprakash** and **Dr. Varalaxmi** who are an everlasting source of inspiration to achieve higher in each one of my endeavours. The words fail me in expressing my feeling to my beloved, who stood by my side in sorrows and joys alike and there is no match to the affection and encouragement given constantly.*

*I am also thankful to my best friends, **Chilumula.Ramakrishna, Praveen Yadav** and **T.V.R.R. Prashanth**, my colleagues **Bhaskar, Bharghavi, Raju, Ramakrisna Prasad,** my seniors, **Umamaheshwar, Kiran, Madhukar Rao, Mohandhas, Murali, Hassan , Ravicharan,** my junior **Anupam** and all 2004-06 batch P.G. Students who helped me in numerous ways during my research work.*

*I would like to thank the computer centre of **ANGRAU** for the help in statistical analysis of data.*

The financial support rendered by the Acharya N.G. Ranga Agricultural University is greatefully acknowledged.

I wish to extend my thanks to one and all who have contributed even in a small way in completing of my research work.

*I am thankful to **Sasi Graphics**, Bhavani Colony, Rajendranagar, Hyderabad and **Mr. Mohith** for their neat and timely computer typesetting of this thesis.*

Date :
Hyderabad ***(Ch. RAMESH)***

ABBREVIATIONS

%	:	Per cent
°C	:	Degree centigrade
AOSS	:	Active oxygen-scavenging system
APS	:	Ammonium persulphate
cm	:	Centimeter
cv.	:	cultivar
DAS	:	Days after sowing
g	:	gram
GST	:	Glutathione-S-transferase
hrs	:	hours
kg/ha	:	Kilogram per hectare
LAI	:	Leaf Area Index
mg	:	Milligram
mM	:	milli molar
mm	:	millimeter
MSF	:	Moisture stress during flowering phase
MSP	:	Moisture stressduring pod filling phase
nm	:	nano metre
PAGE	:	Polyacrylamide Gel Electrophoresis
POX	:	Peroxidase
Rm	:	Relative migration
ROS	:	Reactive oxygen species
rpm	:	Revolutions per minute
SAMS	:	S-adenaxyl L-methionine synthetase
SAT	:	Semi-arid tropics
SOD	:	Super oxide dismutase
TEMED	:	(N, N, N, N-tetramethylene diamine)
TTC	:	Tri Phenyl tetrazolium chloride
UV	:	Ultra violet
µl	:	micro litre

CHAPTER I

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) has been cultivated and consumed as a food for centuries in the far east though the superiority of soy protein was scientifically established only in the more recent times. It is mostly grown in USA, China, Japan and West European countries but recently introduced in India. India has imported edible oils to the extent of 2.7 million tones during 2004-05, which had an impact on the present foreign exchange situation of the country.

In India, soybean is grown in an area of 7.46 million hectares with an annual production of 7.51 million tonnes. Madhya Pradesh is the leading state in area and production of soybean followed by Maharashtra, Rajasthan, Karnataka and Andhra Pradesh. In Andhra Pradesh, it is grown in 0.76 lakh hectares with an average productivity of 15.6 q/ha (Ministry of Agriculture, Govt. of India, 2005).

Soybean is called wonder bean as it is triple beneficiary crop with proteins (40-45%), oils (20-25%) and vitamins like A, B, C and D. It also enriches the soil by fixing atmospheric nitrogen (65-100 kg/ha). It is used as a pulse, oil seed, vegetarian meat and milk. Soy protein is needed to prevent protein malnutrition of India's underfed population.

In the recent past, several industries are coming up around Hyderabad for processing soybean. Though the crop is mostly grown in Madhya Pradesh and Maharastra, its introduction to Andhra Pradesh is of recent one and it is being recommended as an alternate crop to cotton in coastal areas of Andhra Pradesh.

Since soybean is a short day plant, it comes up well in post rainy season under irrigation. However, reproductive phase is highly sensitive to water stress in soybean (Doss *et al.*, 1974 and Eck *et al.*, 1987). Soybean crop if taken up during *rabi* in rice fallows may have to face drought or water stress during reproductive phase. So, most sensitive stage for water stress if identified can be taken up under assured or limited irrigation facilities. Also, the varieties which are tolerant to water stress during reproductive phase if identified can be recommended as an alternative to pulse crops in rice fallows in *rabi* season.

In this context various physiological and biochemical parameters responsible for drought tolerance are to be studied and the information will be useful to screen the varieties for drought tolerance.

Keeping these facts in view, an experiment was conducted with the following objectives.

1. Estimation of various physiological parameters under induced water Stress conditions.

2. Bio-chemical studies (study the expression of isozymes of superoxide dismutage, Ascorabate, Peroxidase, Catalase, Trehalase etc. and assess the accumulation of Glutathione-S-Transferase, proline, Glycine betaine and S-Adenosyl-L-Methionine synthetase).

3. To characterize susceptibility and tolerance of genotypes.

CHAPTER II

REVIEW OF LITERATURE

The major factor limiting the crop yield in semi arid tropics (SAT) is the amount of moisture available to the crop during the growing season. Lack of moisture hamper physiological processes related to yield and therefore reduce yield.

The survival and production of the plants under stress is conditioned by many complex mechanisms (Boyer and Phearson, 1975; Begg and Tumer, 1976). Owing to the genetic complexity of stress response, various screening strategies rely mainly upon the biochemical pathways or endpoints of signalling pathways (Cushman and Bohnert, 2000).

2.1 EFFECT OF WATER STRESS ON MORPHO-PHYSIOLOGICAL CHARACTERS OF PLANT

Patterson and his associates (1977) reported that drought stress significantly reduced soybean plant height. The response of plant height to the water gradient was linearly increasing on an average 8.5 and 6.8 cm per 10 cm of water applied in 1983 and 1984, respectively (Specht *et al*., 1986). Eck and his associates (1987) reported that early season stress reduced plant height. Drought at flowering greatly reduced plant height, while at pod enlargement

caused some decrease and at seed enlargement has no effect on plant height (Korte *et al*., 1983).

Momen and his associates (1979) reported that during drought stress the branch primordia are aborted thus reducing the branch number.

Silvius and his associates (1977) found that in soybean reduced leaf turgidity led to reduced leaf expansion rates and leaf area. Leaf water potentials within the developing cells are an important determinant of leaf enlargement rate and leaf area. At its full development, rapid soybean leaf enlargement occurs when leaf turgour is high (Heatherly *et al*., 1977). Moderate day time water deficit may not limit leaf growth (Wenkert and his associates,1978) in soybean. Boyer (1970) observed differing sensitivity levels to drought stress for the major metabolic and growth process in soybean leaves with cell growth being the most sensitive process. Similar observations were made by Levitt (1972). Because increase in leaf area includes both cell division and cell enlargement, one of the consequences of water deficits is a reduction of leaf area (Begg and Turner, 1976). Ramseur *et al*. (1985), Patterson *et al*. (1979) and Scott and Batchelor (1979) observed that soybean leaf area was reduced during drought stress periods. Leaf senescence was very pronounced under severe moisture stress conditions (Muchow *et al*., 1986).

The maximum LAI in well watered treatment averaged 5.0 when compared with 3.8 and 1.7 in the deficit and dry land treatments respectively (Cox and Jolliff, 1986). Soybean recorded a peak LAI of 5 by day 62 and then

declined in all treatments but most rapidly in the rain fed plots. The loss of leaf in irrigated treatments occurred slowly by leaf abscission but in no irrigation treatments the leaf lamina dried and shriveled but did not immediately fall off the plant, possibly because desiccation and heating prevented the formation of abscission layer (Constable and Heason, 1978). Both Leaf number and LAI were affected by soil water deficits in soybean crop (Sivakumar and Shah, 1978; Neyshaboun and Hatfield, 1986). Cox and Jolliff (1987) reported that unfavorable plant water status in dry land soybean was accompanied by marked reduction (67%) in its LAI.

2.2 Dry matter production

The water deficit treatments produced significantly less dry matter when compared with the well-irrigated treatments (Cox and Jolliff, 1986). Fellows and his associates (1987) reported that during the stress period the rate of accumulation of dry matter in leaves and roots declined to nearly zero. By the time of maximum stress, the percentage of the total dry matter present in leaves had declined while it increased in stems. Constable and Heason (1978) found that rain fed soybeans recorded less dry weights of plant parts than irrigated soybean. Significantly decrease in dry weights of leaves and stems and increase in root weight was reported due to moisture stress (Finn and Brun, 1980). Kirda and his associates (1989) observed that the regularly watered soybean plant gave the highest above ground dry matter when compared to the non-irrigated once. Silvius *et al*. (1977) showed that the dry weights of controls were 20 – 40 per cent more than that of water stressed plants. At – 4 bars water potential the

partitioning of current photosynthates is more towards root and nodules than leaves. Under severe stress condition remobilization of stored carbohydrates coupled with partitioning of current photosynthates probably accounted for the changes in the partitioning of C^{14} favoring the below ground parts (Finn and Brun, 1980).

2.3 Yield

Specht *et al.* (1986) showed that there is a substantial response of seed yield to the irrigation gradient. Mean yield of irrigated treatments were significantly above the non-irrigated treatments (Prasad, 1988; Caviness and Thomson, 1980). Huck and His associates (1983) demonstrated that differences in final seed yield depended markedly on developmental stage at which water stress was imposed. A single irrigation at flowering had essentially no effect on seed yield, where as, a single irrigation at pod elongation or at seed enlargement significantly enhanced seed yield relative to non- irrigated check (Korte *et al.*, 1983a). Seed yield was severely affected by moisture stress, especially at pre flowering and full bloom stages (Rodriguer and Shbles, 1985). Rincosky and Deaton (1979) reported that the 34 days of drought period during the flowering and pod filling stages decreased the soybean yields. Water stress imposed during the reproductive stage was most detrimental to the yield (Ashley and Ethrudge, 1978). Korte *et al.* (1983a) worked with eight cultivars of soybean crop and concluded that irrigation during pod elongation was the most beneficial to increase seed yield. Soybean yields are often reduced because of periods of moisture stress which occur during critical seed filling periods (Albrecht *et al.*,

1984; Boyer *et al*., 1980). Studies of Doss *et al*. (1974) have revealed that yield from adequately watered soybean were up to 28 to 52 per cent greater than those under drought stress occurred during the flowering and pod filling periods. Stress during seed filling reduced the yields by 21 per cent and 25 per cent in 1982 and 1983, respectively (Eck *et al*., 1987). Seed yield in water deficit and dry land treatments recorded 20 per cent and 87 per cent yield reduction compare to from the well irrigated treatment (Cox and Jolliff, 1986).

2.4 Yield components

Brown and his associates (1985) observed that moisture stress treatments did not significantly affect flower and pod shedding. Irrigation significantly increased soybean grain yield and water use efficiency (Deniell and Scottt, 1991).

Rodrigquer and Shibbles (1985) reported that the differential response in seed yield is best explained by changes in pods per node than any other yield component. Podding is the yield component most responsive to moisture stress (Vidal *et al*., 1981a, b) and continuous stress reduced pod number and number of seeds per pod (Khodhambashi *et al*., 1988; Brown *et al*., 1985). Feher and Cavinese (1980) found that the reduction in the number of pods per plant that occurred when stress coincided with flowering was compensated by increase in the number of seeds per pod and mean seed weight. Both pod abortion and subsequent ovule abortion within the developing pods are characteristic

response of soybean to moisture stress during reproductive ontogeny (Shaw and Loung, 1976).

Cox and Jolliff (1986) reported that seed weight was 20 per cent lower in stressed treatments but seeds per pod were not affected by water stress. They also stated that pod number was the yield component most sensitive to soil water deficits. The deficit and dry land treatments had 20 per cent and 77 per cent fewer pods respectively than well irrigated treatment.

Eck and his associates (1987) observed that early season stress reduced seed number, but the plants compensated yield by increasing seed weight. He also stated that stress during seed filling reduced the seed weight by 13.7 and seed number by 18 per cent. Drought stress that occurred during the seed formation reduced yield due to decrease in seed number (Smiciklas *et al*., 1989).

BIOCHEMICAL STUDIES

2.5 Trehalase in imparting stress tolerance

Trehalose, a non-reducing disaccharide ubiquitous among microorganisms (Crowe *et al.*, 1984; 1998) is also found in some invertebrates and vascular plants (Elbein, 1974). It serves as an osmoprotectant and effectively stabilizes dehydrated enzymes and lipid membranes in some plants (Datta, 2002) in water stress conditions. Trehalase, the hydrolyzing enzyme of trehalose is involved in alteration of carbohydrate allocations.

Trehalase (α-D-glucopyranosyl-1 (1, 1) - α - D – glucopyranoide) (E.C 3.2.1.28) plays a role in stress protection, particularly with regard to dessication (Crowe *et al.*,1998; Wiemken, 1990; Robero *et al*., 1997). Trehalose has so far, not been conclusively identified as an endogenous compound in vascular plants, except for the two well documented cases of resurrection plants *Selaginella lepidophylla and Myrothamnus flabellifolia* (Muller *et al*., 1995). However, the presence of functional genes encoding the enzymes of trehalase synthesis indicates that higher plants potentially have the ability to synthesize trehalose (Blazuez *et al*., 1998; Goddijn and Van Dunk, 1999; Muller *et al*., 1999).

Although exogenously supplied trehalose has been found to be toxic for plant tissues (Veluthambi *et al*., 1981), plants were evaluated for their suitability as organisms for accumulation of trehalose, both to exploit plants as production systems (Goddijn and Pen, 1995) and to evaluate the potentiality of trehalose as an osmoprotectant (Holmstrom *et al*., 1987). However, attempts made to exploit trehalose as stress protectant in plants, by over expressing bacterial or yeast trehalose synthesis genes has led to plants with severe growth defect such as aberrant root growth and dwarfism (Goddijn *et al*., 1997; Romero *et al*., 1997).

Trehalase, the enzyme that hydrolyzes trehalose is ubiquitous in higher plants (Muller *et al*., 2001) and is present in all organs of higher plants with the highest activities in reproductive parts (Muller *et al*., 1995; 1999). In *Arabidopsis*, a putative trehalose isolog has been identified. This gene, T 19 F

6.15 is closely homologous to various trehalases, including Trehalase GMTRE 1 from soybean (Aeschbacher *et al.*, 1999). Thus both trehalose and trehalase might be involved in carbohydrate allocations in plants (Muller *et al.*, 2001).

2.6 Active oxygen-scavenging system (AOSS) in imparting stress tolerance

Plants have evolved various enzymatic and non-enzymatic protective mechanisms in order to scavenge active oxygen species. Among the enzymatic mechanisms an active oxygen-scavenging systems AOSS *viz.*, Superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) (Kumar and Knowles, 1993; Scandalios, 1993) protects the cell integrity by mopping up the excessive free radicals and thereby lowering active oxygen/hydroxyl radical formation.

Active oxygen scavenging systems (AOSS), viz., superoxide dismutase (SOD) peroxidase (POX) and catalase (CAT) are involved in secondary protection of cell by quenching the reactive oxygen species (ROS) that are triggered during oxidative stress (Kumar and Knowles, 1993; Scandalios, 1993).

Since, the osmolytes or putative cyto salts are synthesized by plants denovo by expressing relevant genes under threshold signaling of stress, the isozymes of these key enzymes (Trehalase, (SOD), (POX)) stand as the indicators for the degree of stress tolerance in such plants. Since each isozyme represent a gene or a group of genes, appearance of such isomorphs of key enzymes indicate the expression of genes for stress tolerance.

Superoxide dismutase (SODs : EC 1.15.1.1) are enzymes strongly implicated in the response to oxidative stress. SODs catalyzes the disproportion of superoxide radical anions to hydrogen peroxide and molecular oxygen (Bowler *et al*., 1992). Different isoforms of SOD exist in the cytosol, choroplasts and mitochondria of plant cells.

Potato plants over expressing tomato chloroplast Cu/Zn SOD and tobacco plants over expressing pea chlorplastic Cu/Zn SOD or Mn SOD showed improved tolerance to oxidative stress (Perl *et al.*, 1993; Schake *et al*., 1995; Sen Gupta *et al.*, 1993). Mc Kersie *et al*. (1996) reported that transgenic alfalfa expressing Mn SOD suffered reduced injury from water deficit stress.

Fambrini *et al*. (1999) characterized the electrophoretic variants for Cu/Zn SOD in sunflower in response to oxidative stress, SODs and abscissic acid (ABA) are in general implicated in response to environmental stresses. The susceptibility of W-1, a mutant with ABA deficiency and variant isoforms of chloroplastic Cu/Zn SOD, to oxidative stress was evaluated. The results indicated that W-1 leaves were less damaged by paraquat (methyl violagen) and H_2O_2 treatments compared to control. However, the spectrophotometric assays did not provide evidence for a higher level of SOD activity in W-1 extracts conforming that ABA concentration does not modify SOD activity. The better response of W-1 leaves to methyl violagen treatment could not be attributed to a higher efficiency of the chloroplastic isoform variant to SOD.

Peroxidase activity was found to increase with an increase in the concentration of salinity and waterstress (Strogonov, 1964; Rakov *et al*., 1969; Maliwal and Paliwal, 1972). Sheoran and Garg (1979) studied the quantitative and qualitative changes in peroxidase during germination of moong bean under salt stress.

Venkateshwar Rao (1992) reported a variation of electrophoretic profiles of peroxidase isozymes which varied significantly by the genotype and stress treatments imposed in the rice callus. More number of low mobility bands were observed in control callus of all the cultivars. However, in platelets derived from salt and drought tolerant CV of rice, pokkali, more number of high mobility bands were noticed with 1 per cent NaCl stress.

2.7 Glutathione-S-transferase (GST)

Glutathione-s-transferase (EC 2.5.1.18) have been involved in many biotic and abiotic interaction of plants with their environment, but very little information is available on their regulation and possible role in drought tolerance. The GST gene of arabidopsis thaliana encodes as assessed by phylogenetic analysis, the homologue of an extremely conserved sub-group of Tau GSTs. GST transcripts accumulate in response to a wide range of stimuli, such as xenobiotics, high doses of hormones, pathogens and ozone (Marrs, 1996). This apparently unspecific response certainly betrays differential regulation of GST genes (Takahashi and Nagata, 1992, Mc Gonigle *et al*., 2000), but also a partial knowledge of physiological reactions to experimental conditions. A common

element to some of these conditions is the build-up of reaction oxygen species (ROS), which are potent induces of GST accumulation in animals and plants. This suggests that some plant GSTs play a direct role in oxidative stress tolerance, a view supported by the demonstration of a dual GST and peroxidase activity for some enzymes (Cummins *et al*, 1999; Roxas *et al*., 2000).

2.8 Proline

Free proline accumulates in the attached and detached leaves of almost all the crops subjected to moisture stress (Waldren *et al*., 1974). Accumulation of proline in water deficit plants was considered to be an important character responsible for drought tolerance. Proline may act as a source of respiratory energy in the recovery of plants. In severely stressed plants, proline is typically the major free aminoacid, which accounts upto 30 per cent of the total soluble nitrogen in the leaves. The precursors of proline are glutamic acid, arginine, ornithine and n-acetyl glutamic acid. The conversion of these precursors to proline was known to be enhanced under moisture stress (Steward and Boggess, 1977). The possible adaptive significance of proline accumulation under moisture stress is based on the hypothesis that it acts as an osmaticum *i.e.*, for storage of Nitrogen in a nontoxic form and / or for storage of energy during moisture stress and subsequent use upon relief from stress (Mukherjee, 1974).

Free proline accumulates in response to moisture stress conditions (Singh *et al*, 1973). Palfi and his associates (1978) reported that higher plants can be classified as “Proline-accumulating type” or “non-Proline accumulating

type" according to whether they could accumulate proline to a concentration greater than 1 per cent of dry weight under severe stress conditions.

Total proline increased under severe water stress (- 2.6 M Pa) attaining a level of 150 per cent that of control plants (Fukutoku and Yamada, 1981).

2.9 Role of glycine betaine in imparting stress tolerance

Stress tolerance comprises of a series of both enzymatic and non enzymatic mechanisms. Glycine betaine is a major cytoplasmic osmaticum accumulated under water or salt stress in higher plants, bacteria, algae and marine animals (Rhodes and Hanson, 1993). The activity of the terminal enzyme of Glycine betaine biosynthetic pathway, betaine aldhyde dehydrogenase (BADH) was induced by three fold in spinach chloroplasts (dark and light) when exposed to 300 mM NaCl salt stress conditions (Weigel *et al*., 1986), while in pea that lacks betaine, BADH activity was not seen.

Glycine betaine (N, N, N-trimethyl glycine) is a quarternary ammonium compound naturally occurs in a wide variety of plants, animals and micro-organisms. Glycine betaine is known to preserve thylakoid and plasma membrane integrity after exposure to saline and drought conditions (Rhodes and Hanson, 1993).

Glycine betaine accumulates naturally in response to salinisation or to water deficit in members of chenopodeaceae and poaceae like sugar beet,

spinach, barley and maize (Hanson *et al*., 1985) and wheat (Grattan and Grieve, 1985). Some prokaryotes also have the capacity to synthesize Glycine betaine(Imhoff and Valera, 1984 and Mohammad, 1983). However, the ability to synthesize betaine occurs sporadically without a clear evolutionary pattern (Qiu *et al.*, 1998). The widespread but sporadic occurrence of Glycine betaine accumulation is strongly expressed by some living plants, while traces of Glycine betaine in diverse non accumulators like archetypal angiosperm is also seen (Rhodes and Hanson, 1993).In general, accumulation of Glycine betaine in salinity and drought conditions is significantly noted in plants.

However, *Brassica napus*, a crucifer and not an accumulater (in contrast to some chenopods) also expressed small amount of betaine (Qiu and Xiao, 1990).

Although these are very few examples of natural accumulators of Glycine betaine, exogenous supplementation of Glycine betaine has played a successful role in osmoprotection.

Harinasut *et al.* (1996) reported that an exogenous supplementation of 15 mM Glycine betaine for 4 days would help build tolerance in rice seedlings (28 days old) that were exposed to 150 mM NaCl for 6 days. An increase in seed yield following the application of 3 kg/ha Glycine betaine in soybean under water stress conditions was reported by Agboma *et al*. (1997).

2.10 S-Adenosyl-L-Methionine Synthatase

The enzyme S-adenosyl-L-methionine synthatase (EC2.5.1.6) is a ubiquitous enzyme that is well conserved from bacteria to few higher plants and mammals. The gene encoding SAM synthetase belong mostly to small multi gene families. SAM synthatase have been isolated from organisms including Escherichia coli, yeast, rat, human and in plants *viz*., Arabidopsis, actinidia, mustard, legumes, parsely. Carnation, tomato and rice (Arnon Mukhopadhyay *et al*., 2001).

2.11 DROUGHT INDICES

Sammons *et al*. (1987) grouped soybean cultivars into tolerant and susceptible varieties. Cultivars identified as tolerant to moisture stress were characterized by stable seed yield under the contrasting moisture stress treatments, but not necessarily stable vegetative growth. Cultivars termed susceptible to moisture stress exhibited decreased seed yield under sub optimal irrigation, but not necessarily decreased vegetative growth.

CHAPTER III

MATERIAL AND METHODS

The details of materials used and methods adopted during the course of the present investigation are summarized in this chapter under appropriate heads.

3.1 LOCATION

The present investigation entitled "Studies on physiological and Biochemical responses of soybean *(Glycine max (L.) merrill)* genotypes to induced water stress", was conducted at College Farm, College of Agriculture, Rajendranagar, Hyderabad (Latitude $17^{o}19`$N, Longitude $78^{o}$ 28`E and Altitude 543 m) during the post rainy season, 2005-06.

3.2 DETAILS OF EXPERIMENTAL SITE

The experiment was conducted on sandy clay loam. The field was uniform in level and fertility. Soil samples were drawn at random from 0-30 cm soil depth from the experimental field and composite samples were analyzed for their physico-chemical properties.

3.2.1 Mechanical analysis of Soil of experimental plots: (Bouyoucos hydrometer method, Piper, 1950)

	Percentage
Sand	64.82
Silt	26.23
Clay	9.95
Textural class	Sandy clay loam

3.2.2 Chemical analysis of Soil of experimental plots:

	Percentage	Method adopted
pH (1:2.5 soil : water)	7.2	Backmen pH meter (Jackson,1967)
Electrical conductivity (dS/m at 25^{o}C)	0.15	Solubrideg method (Jackson,1967)
Organic Carbon (%)	0.27	Walkey and Black (1934)
Available nitrogen (kg/ha)	141.00	Subbaiah and Asya (1956)
Available phosphorus (kg/ha)	14.10	Olsen *et al.* (1954)
Available potassium (kg/ha)	507.00	Flame photometer method (Jackson, 1967)

Chemical analysis of soil samples indicated that the soil is neutral in reaction, low in organic matter, available nitrogen and phosphorus and high in available potassium.

3.3 SEASONAL WEATHER

The meteorological data of post-rainy season, 2005-06 were presented in Table 1 and Fig 1.

3.3.1 Air temperature

The maximum monthly temperature recorded was 35.5 °C during standard week of 8 (February), 2006 and lowest minimum monthly temperature of 10.6 °C recorded during standard week of 4 (January), 2006. The average maximum and minimum temperature during crop period was 30.35 ^{o}C and 14.13 ^{o}C, respectively.

3.3.2 Rainfall

The total amount of rainfall received was 88.0 mm which was received on March, 2006.

3.3.3 Relative humidity

Relative humidity of air recorded at 0716 hrs was always higher than at 1416 hrs. The average humidity during the crop growth season at 0716 hrs was 84.16 per cent and at 1416 hrs was 31.27 per cent.

3.3.4 Sunshine hours

The maximum sunshine hours recorded was 10.3 hrs during standard week of 8 (February), 2006 and lowest minimum sunshine hours of 7.5 hrs recorded during standard week of 49 (December), 2005. The average sunshine hours during crop period was 8.61 hrs.

3.3.5 Wind speed

The maximum wind speed (kg/hr) recorded was 4.6 km/hr during standard week of 9th Mach, 2006 and lowest wind speed of 1.8 kg/hr recorded during standard week of 50 (December), 2005. The average wind speed during crop period was 2.62 km/hr.

3.3.6 Open pan evaporation

The maximum evaporation (mm) recorded was 5.7 mm during standard week of 9 (March), 2006 and lowest evaporation of 2.0 mm recorded during standard week of 51 (December), 2005. The average evaporation during crop period was 3.23 mm.

3.4 EXPERIMENTAL DETAILS

The experiment was laid out in a split plot design with three replications. The main plot treatments are (Three irrigation treatments):

M_1 : No moisture stress (or) control

M_2 : Moisture stress during flowering phase (MSF)

M_3 : Moisture stress during active pod filling phase (MSP)

The sub-plot treatments are (five cultivars)

C_1 : MAUS-47

C_2 : JS-335

C_3 : JS-93-05

C_4 : PK-1029

C_5 : NRC-37

The seeds were sown on flat bed of 4 x 5 m size plot with 30 cm x 10 cm spacing. The layout of the experiment was furnished in Fig 2.

3.5 PREPARATION OF LAND

The field was ploughed twice with a tractor and then harrowed to bring fine tilth. Dried weeds were removed, the land was leveled and the experiment was laid out.

3.6 APPLICATION OF FERTILIZERS

Nitrogen (N)	:	30 kg/ha as basal dose in the form of urea
Phosphorus (P_2O_5)	:	60 kg/ha applied as basal dose in the form of single super phosphate
Potash (K_2O)	:	30 kg/ha applied as basal dose in the form of muriate of potash.

3.7 SOWING

The seeds were treated with *Brodirhizobium japonicum* and sown in lines by dibbling 2 to 3 seeds per hill with a spacing of 30 cm between rows and 10 cm between plants within the row. A week after emergence the crop was thinned to a single plant per hill.

3.8 PLANT PROTECTION MEASURES

The crop was well protected from pests and diseases by timely spraying of monocrotophos (0.04%) and endosulfan (0.05%) against stem fly.

3.9 WEEDING AND INTERCULTURAL OPERATIONS

The first weeding was done on 15 days after sowing (DAS) with hand hoe and subsequent intercultural operations were done as and when necessary.

3.10 IRRIGATION

Immediately after sowing, life saving irrigation was given to all the treatments. Later on the moisture stress treatments were imposed as detailed below.

M_1 Treatment : Irrigation was given throughout crop duration
M_2 Treatment : Irrigation was not given during flowering
M_3 Treatment : Irrigation was not given during pod filling

M_1 treatment received total of six irrigations whereas the other 3 treatments received only four irrigations.

3.11 HARVESTING AND THRESHING

Harvesting was done from each net plot separately when the crop attained physiological maturity i.e. when more than 75 per cent of the leaves turned yellow and pods ripened completely. The pods were collected, sun dried and threshed. Then the seeds were cleaned. The seeds were further sun dried till a constant weight was obtained. Similarly the weight of the straw from each plot was also recorded after drying in sun.

3.12 OBSERVATIONS RECORDED

Plant samples were collected at 15 days intervals until harvest and several physiological parameters were recorded to understand the responses of soybean genotypes to induced water stress.

3.12.1 Morpho-physiological parameters

3.12.1.1 Plant height (cm)

Five plants were selected and labeled in each treatment for non-destructive sampling. The plant height was measured from the main base to the tip or terminal bud and expressed in cm per plant.

3.12.1.2 Number of branches

The number of branches for all labeled plants were counted and its mean was expressed as number of branches/plant.

3.12.1.3 Number of leaves

Green and fully expanded leaves were counted in all the labeled plants and its mean was expressed as number of leaves/plant.

3.12.1.4 Leaf area index (LAI)

Five plants were sampled at random treatment wise and replication wise and then leaves, stems and pods were separated. The leaf area was measured with LI-3100 area meter (LI-COR Inc. Nebrasca, USA). The leaf area index (LAI) was calculated by using the formula:

$$LAI = \frac{\text{Leaf area}}{\text{Unit ground area}}$$

3.12.1.5 Dry weight of plant parts (above ground parts only)

Five plants were sampled at random treatment wise and replication wise and then leaves, stems and pods were separated. The weights were recorded after oven drying of stems, leaves and pods at 80 °C for 48 hours when reached constant weight, computed for a square meter area.

3.12.1.6 Total dry weight

The total dry weights of sampled plants (excluding roots) were recorded by adding dry weights of leaves, stems and pods and computed for square meter area.

3.12.2 Yield and yield components

3.12.2.1 Seed yield (kg/ha)

Seeds were threshed from the plants harvested from a marked area of one m^2 and computed to hectare and expressed in kg/ha.

3.12.2.2 Number of pods/plant

The total number of pods from five tagged plants in each net plot were counted and mean of five plants was expressed as number of pods/plant.

3.12.2.3 Number of seeds/pod

The number of seeds/pod was calculated by averaging the number of seeds obtained from all the pods from five tagged plants and expressed as number of seeds/pod.

3.12.2.4 100-seed weight (g)

One hundred seeds were collected on unbiased basis and the weight of 100-seed was recorded treatment wise and replication wise and expressed in grams.

3.12.3 Phenology:

Parameters *viz.*, Days to emergence, flowering initiation, days to 50 percent flowering and days to harvest were recorded following criteria.

3.12.3.1 Days to emergence and flowering initiation

The number of days taken by the seedling to emergence from soil after sowing were recorded and expressed in days.

3.12.3.2 Days to 50 per cent flowering

The number of days taken from sowing date to that date, when 50 per cent of the plants showed at least on flower was considered as 50 per cent flowering

3.12.3.3 Days to harvest

The number of days taken from sowing date to the date of harvest were calculated and recorded.

3.12.4 Bio-chemical studies

Electrophoretic profiles

Native PAGE analysis was carried out for isozyme profile of trehalase, super oxide dismutase (SOD) and peroxidase (POX) adopting standard procedures. Isozyme studies are regarded as dependable biological tools used to detect variation in biological systems.

Electrophoretic principles and procedure

Electrophoretic technique is used for separation of proteins and isozymes in the electric field. Enzymes are charged molecules capable of mobility in an electric field. Their rate of mobility largely depends on the net charge carried out by the molecule. The large sized protein molecule is the function of its pH and hence, protein behave as cations moving towards the cathode in acidic pH and as anions moving towards the anions in basic pH. A isoelectic pH, the proteins show null mobility, as their net positive charge equals to the net negative charge. This property of isozymes in solutions of different pH is mainly taken advantage in separating enzymes by electrophoresis. The mobility of proteins/enzymes on supporting medium depends not only on their net charge but also on their molecular weight, molecular size and also the pore size of gel which serves as the molecular sieve. In case of polyacrylamide gel, the pore size can be regulated by changing the concentration of acrylamide monomer. Therefore, the proteins separated on the gels by electrophoresis reflect the differences in their molecular form.

Preparation of stock acrylamide solutions: The procedure as suggested by Hart and bhatia (1967) was followed:

i) Preparation of stock acrylamide solution as detailed below:

Acrylamide	:	30.0 g
N'N-methylene bis-acrylamide	:	0.8 g
Distilled water	:	100 ml

ii) Preparation of resolving/Separating gel buffer:

1.875 M Tris HCl	:	22.7 g
Distilled water	:	100 ml
pH adjusted to	:	8.0

iii) Stacking gel buffer is required for tracking diffusion enzymes or protein molecules, and the buffer is prepared by dissolving the following reagents as detailed below:

0.6 M Tris HCl	:	7.26 gm
Distilled water	:	100 ml
pH adjusted to	:	6.8

The pH of the buffer is then adjusted to 6.8 before it is used.

iv) Electrode buffer : Tris – Glycine

Tris HCl	:	6.0 g
Glycine	:	28.4 g

v) Sample buffer

Tris 0.5 M (pH 6.8) : 1.0 ml

Glycerol : 1.0 ml

β-2-mercaptoethanol : 0.1 ml

0.05% Bromophenol blue : 0.2 ml

vi) Polymerizing agents

a) Ammonium persulphate (APS) 10% (w/v) (freshly prepared)

b) TEMED (N, N, N',N'-tetramethylene diamine)(Fresh from refrigerator)

Freshly prepared polymerizing agents were used

Preparation of working solutions

Working solution was prepared freshly by mixing the stock solutions and distilled water as shown below:

Composition	**Separating / Resolving gel**		**Stacking gel (4%)**
	(7.5%)	**(10%)**	
Acrylamide Bis-acrylamide	2.5 ml	3.33 ml	0.675 ml
Tris HCl (pH 8.8)	2.5 ml	2.5 ml	-
Tris HCl (pH 6.8)	-	-	0.5 ml
Water	4.83 ml	4.0 ml	3.75 ml
APS (10%)	75 µl	75 µl	50 µl
TEMED	10 µl	10 µl	5 µl

Note:

1. The above procedure is same for all the three isozymes which staining solutions and incubation mixture for each isozyme is specific.

2. Ammonium persulphate (APS) and TEMED were added to the working solution just before the preparation of gel columns.

3. Low temperature (4°C) should be maintained both at the enzyme extraction and also all the while running the gels, in order to safeguard the specific activity of enzymes.

4. Resolving/separating gel of 7.5% was used for analysis of trehalase while 10% for SOD and POX.

Enzyme extraction

About 0.5 gm of leaf tissue was weighed and gently ground in a prechilled pestle and mortar with 1.0 ml of Tris buffer (pH 8.0). Care was taken to maintain the low temperature (4°C) by keeping the pestle and mortar on ice bath while grinding. The samples were then centrifuged at 18,000 rpm for 20 minutes at 4°C in a REMI refrigerated centrifuge. The supernatants were collected and stored frozen for further studies.

Electrophoretic apparatus

BIOTECH Electrophoresis system is a slab gel system of rectangular model (Plate-1) and was used in the present study which was provided with two electrode vessels, an upper and lower chamber. The 50 µl of the sample (crude enzyme extract) was loaded into each well of the gel and a drop of bromophenol blue was added which served as an indicator dye.

The upper and lower tanks were filled with the electrode buffer (pH 8.3). The upper tank was so arranged over the lower tank that all the gel tubes were dipped in the buffer of the lower tank. In the upper tank running buffer was poured until all the gel tubes and upper electrode were completely immersed.

The two terminals were then connected to the respective terminals of power source. Electrophoresis was conducted at low temperature (about 4°C) by applying 3 mA current to gel and the voltage was stabilized at about 100-110 volts. The samples were allowed to run until the bromophenol blue dye front had reached the bottom of the gel. Then the gel were removed.

Staining procedure for identifying isozyme profiles

3.12.4.1 Identification of Trehalase

Trehalase was identified by staining the gels with incubation mixture (Manchenko, 1994).

Staining solution:

a) 0.5 M citrate buffer, pH 5.6

b) 30 mM citrate buffer, pH 5.6

c) 0.1 M α, α-trehalose

d) 0.1 M iodoacetamide

e) 0.2%, 2, 3, 5-Triphenyl tetrazolium chloride (TTC)

Procedure

1. Electrophorized gel was soaked in solution 'a' for 5 min.

2. Gel was incubated in solution 'b' at 30°C for 20 min

3. Gel was washed with distilled water and immerse in solution 'c' for 5min

4. Rinsed the gel again with water, immerse in solution 'd' (in the dark) and place in a boiling water bath for 4 min.

5. Violet bands appeared in the gel where trehalase activity is localized.

6. The stained gels were fixed in 7.5% acetic acid

3.12.4.2 Superoxide dismutase (SOD)

The gels were incubated in dark for half an hour at room temperature in the solution prepared as follows:

Potassium phosphate buffer (250 mM)	:	20 ml
EDTA (10 mM)	:	1 ml
TEMED	:	200 µl
Riboflavin (300 mM)	:	1 ml
NBT (10 mM)	:	2.5 ml

After incubation the gels were kept under light with slight shaking till bands appeared. The reaction was stopped by adding 7% acetic acid. The gels were photographed immediately.

3.12.4.3 Peroxidase (POX)

Gel was incubated in the following mixture for half an hour:

Ammonium chloride	: 6%
Benzidine	: 100 mg

After incubation 1 ml of H_2O_2 (30 %) was added in drops till the blue coloured bands appeared. The enzyme reaction was stopped by using 7% acetic acid. The gels were photographed immediately.

The following observations were made from electrophoretic profiles of isozymes:

Calculation of (Rm) values

The relative migration (Rm) value for each band on the gel was calculated as follows:

$$(Rm) = \frac{\text{Distance traveled by the band from the top of the gel (cm)}}{\text{Distance traveled by the dye from the top of the gel (cm)}}$$

Classification of zymograms

The banding profiles were classified based on the intensity of their reaction and electric mobility.

Based on electrophoretic mobility (Rm values)

Low mobility	Rm values	→	0.0 – 0.3
Medium mobility	Rm values	→	0.3 – 0.6
High mobility	Rm values	→	0.6 – 1.0

3.12.4.4 Ascorbic acid

Ascorbic acid was determined by 2,6-dichlorophend indophenol visual titration method (Ranganna, 1986).

Preparation of reagents

1. 2,6-dichlorophenol indephenol dye solution

In a beaker 50 mg of sodium salt of 2,6-dichlorophenol indophenol dye and 42 mg of sodium bicarbonate were taken and dissolved in 150 ml (hot) distilled water. The volume was made up to 200 ml with distilled water.

2. Metaphosphoric acid (3%)

Three grams of metaphosphoric acid was dissolved in a small quantity of distilled water and the volume made up to 100 ml.

3. Standard ascorbic acid

Hundred milligrams of L-ascorbic acid was dissolved in a small quantity of 3% metaphosphoric acid in 50 ml and made it to 100 ml volumetric flask and from this 10 ml was taken in another 100 ml volumetric flask and volume was made up with 3% metaphosphoric acid.

1 ml = 0.1 mg of ascorbic acid

Standardization of dye

In a 100 ml conical flask, 5 ml of standard ascorbic acid solution was taken and 5 ml of 3% metaphosphoric acid was added. The dye solution was filled in a micro pippette and standard ascorbic acid solution was titrated. The end point was pink color which persisted for 15 seconds. This was done in triplicates.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

Preparation of sample

In a 100 ml volumetric flask, 10 ml of sample was taken and the volume was made up with 3% metaphosphoric acid.

Procedure

5 ml of 3% metaphosphoric acid extract of the sample was taken in a conical flask and titrated with standard dye. The end point was pink colour which existed for 15 seconds. The titre value was noted:

$$\text{Mg ascorbic acid/100 ml} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made} \times 100}{\text{Aliquat taken} \times \text{Volume of sample taken}}$$

3.12.4.5 Catalase

Catalase (EC. 1.11.1.6) is an enzyme present in nearly all animal and plant cells, which catalyzes the breakdown of H_2O_2 to water and molecular oxygen.

Principle

The enzyme activity is assayed by estimating the residual H_2O_2 in the reaction mixture, which is then determined by oxidation with $KMnO_4$ titrimetrically.

Reagents

1. 0.1 M Potassium phosphate buffer (pH 7.0)
2. 0.005 M H_2O_2 : Prepare fresh
3. 0.7 N H_2SO_4
4. 0.01 N $KMnO_4$

Enzyme extraction

1. The sample was grind (1.0 g) with 0.1M phosphate buffer, pH 7.0 in a pre chilled mortar and pestle.
2. Centrifuged at 15,000 g for 30 min at 4°C.
3. Used the supernatant as enzyme source.

Enzyme assay (modified Braber, 1980)

1. Pipette out 3 ml of phosphate buffer, 2 ml of H_2O_2 and 1 ml of enzyme extract into a test tube.

2. Incubated at 20°C for 1 min.

3. After 1 min stopped the reaction by adding 10 ml of 0.7 N H_2SO_4.

4. Titrated the reaction mixture against 0.01N $KMnO_4$ to find out the residual H_2O_2 until a faint purple colour persists for a least 15 sec.

5. Prepared the blank by adding the enzyme extract to an acidified solution of reaction mixture at zero time.

Calculation

Expressed the enzyme activity as units/min and specific activity as units/min/mg protein or per g weight of sample. One unit of catalase is defined as that amount of enzyme which breaks down 1 micro mole of H_2O_2 under the assay conditions.

Note

1. Calculated the concentration of H_2O_2 using the extinction coefficient 0.036/micro mole/ml

2. The activity of the enzyme may be also expressed as n moles of H_2O_2 used/min/g weight of sample.

3.12.4.6 Glutathione-S-transferase (GST)

The GST enzyme is estimated by following the method of Rick (1982).

Principle

The spectrophotometer method of estimation of GST enzyme activity is based on the formation of S-(2-chloro-4-nitrophenyl) glutathione by the reaction of 3,5-dichloronitrabenzene and GST enzyme. The change in the ultraviolet absorption spectrum that occurs when the substrate 3,4-dichloronitrobenzene converted to S-(2-chloro-4-nitrophenyl) glutathione is measured at absorption maxima of A_{344}.

Reagents

A quantity of 0.1 M potassium phosphate buffer : 1.49 g of potassium chloride and 13.625 g of potassium dihydro orthophosphate were dissolved in 90 ml of distilled water. The pH was adjusted to 8.0 with 1N KOH or HCl and final volume was made upto 100 ml.

Procedure

15 day old seedlings were ground in prechilled pestle and mortar with 3 ml of 0.1 mg potassium phosphate buffer. The homogenate was filtered through three layers of cheese cloth and centrifuged at 20,000 rpm for 20 min. The supernatant was added to 5 mM reduced GST in a test tube. The enzyme was incubated fro 26 min to

facilitate conversion of maximum substrate to S-(2-chloro-4-nitro phenyl) glutathione at 37°C, the constituents are brought to this temperature before the determination. After stirring the absorbance maxima at 344 nm in UV range was recorded against the blank (consisting of all the reaction mixture constituents except 3,4-dichloro nitrobenzene) on a UV-VIS 119 (systronics) with constant temperature quartz cell housing.

3.12.4.7 Estimation of proline content

The proline content in the leaves of 21 days old seedlings of test plants was estimated following the method of Bates *et al*. (1973).

Principle: This spectrophotometric method is based on the formation of chromophore (red colour) by the reaction of ninhydrin and compounds having free amino groups.

Preparation of reagents

a) 3% aqueous sulfosalicylic acid : 3 g of sulfosalicylic acid was dissolved in 100 ml distilled water till the formation of clear solution.

b) Acid ninhydrin: 1.24 g of ninhydrin was dissolved in a solvent prepared by mixing 30 ml glacial acetic acid and 20 ml of 6 M phosphoric acid till a clear solution was obtained.

c) Toluene: Guaranteed reagent (merk) was used.

Procedure

Fresh-tissue weighing 100 mg was ground with 2 ml of 3% aqueous sulfosalicylic acid in a clean pestle and mortar. The homogenate was centrifuged for clear supernatant. Two ml of the extract was added to 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube and kept in hot water bath at 100°C for one hour. However, the reaction was terminated by keeping in an ice both. The reaction mixture was extracted with 4 ml of toluene after mixing vigorously in a separating funnel for 20 seconds. The reactant chromophore, containing toluene was aspirated from aqueous phase and was brought to room temperature and the absorbency was red at 520 nm wavelength against toluene blank in Spectrophotometer (Spectronic-20). Proline concentration in the samples was determined from the standard curve developed with different concentrations of praline. The concentration of praline in the tissue was expressed as mg of praline/g fresh weight.

3.12.4.8 Estimation of glycine betaine

Total betaine content was measured colorimetrically according to the method of Grieve and Grattan (1983).

The tissue was homogenized in 10 ml of methanol/chloroform/water (12:5:3) extraction media in a large glass centrifuge tube. The tube was kept in an ice bath during extraction to counteract heat generation by the ultraturrax, since excessive heat can cause break down of the chloroform with the production of HCl. After extraction, 10 ml of distilled water was placed in a glass centrifuge tube and used to wash the grinding head. The resulting emulsion was added to the first homogenate. The

homogenate was centrifuged in a refrigerated centrifuge (Remi C 40) at 12,000 g/10'/20°C. The supernatant was removed and stored for the analysis of betaine by non-specific periodide method in which quarternary ammonium compound (QACs) and betaines are precipitated at different pHs. The acid potassium-triodide solution (for total QAC) was prepared by dissolving 7.5 g iodine and 10 g potassium iodide in IM HCl and filtered while the same reagents were dissolved in a 0.4 M KH_2PO_4 – NaOH buffer pH (8.0) provided the alkaline reagent to determine betaine. Precisely 0.2 ml of either acid or alkaline potassium triodide reagent was added to the sample. The mixture was shaken and left for at least 90 minutes in an ice bath with intermittent shaking. Two ml ice-cooled H_2O was added rapidly to the mixture to reduce the absorbency of the blank. This was quickly followed by 20 ml of 1, 2-dichloroethane at -10°C and the two layers were mixed by a constant stream of air bubbles for 5 minutes while the temperature was maintained at 4°C. The absorbency of the lower organic layer was measured at 365 nm in Spectrophotometer (Spectronic-20). Glycine betaine concentration in the samples was determined from the standard curve developed with different concentrations of glycinebetaine chemical.

3.12.4.9 S-adenoryl L-methionine synthetase (SAMS)

The enzyme SAMS is estimated by the procedure given by Shannon (1968).

Procedure

The enzyme activity is determined by the disappearance of S-adenosyl methionine in the assay mixdture of total volume of 0.5 ml.

Assay mixture

- 60 μ moles of potassium acetate pH 5.6
- 20 μ moles of potassium phosphate pH 5.6
- 1.5 μ moles of S-Adenosyl methionine

Procedure

The assay mixture incubated for 2 hrs at 37°C. The mixture after cooling is deproteinized with 0.05 ml of 40 per cent per chloric acid, and the protein is removed by centrifugation. An aliquat of supernatant solution is brought to a pH of 6.7-7.0 by adding an ice-cold mixture of potassium ydroxide and potassium phosphate. The precipitated potassium per chlorate is removed by centrifugation. A suitable aliquot of the supernatant solution is absorbed on Xe-64 column as follows:

The column is prepared (6 x 1 cm) by treating with 50 ml of 0.25 M potassium phosphate pH 7.0 followed by 15 ml of a 1:25 dilution of the same buffer. After absorption, the column is washed with 10-20 ml of 0.01 m potassium phosphate, pH 7.0 to wash the methyl thioadenosine down. S-adenosyl methionine is eluted from the column by 10-20 ml of 4 ml acetic acid and determined spectrophotometrically by using the molar absorbancy index of 15, 100 at 257 mμ (UV range pH 2.0).

3.13.5 Drought indices

Radford (1967) have defined some parameters to measure drought tolerance. The response of cultivars to drought was assessed by drought index and drought tolerance efficiency, based on yield under both fully irrigated and stressed conditions.

$$\text{Drought index} = \frac{\text{Yield under drought}}{\text{Yield under no stress}}$$

$$\text{Drought tolerance efficiency} = \frac{\text{Yield under stress}}{\text{Yield under moisture}}$$

STATISTICAL ANALYSIS

The data were analysed statistically as split plot design with OCM spectra 31 : KO 560 computer following the method of Panse and Sukhatme (1978). Wherever the results were significant, critical difference (CD) was calculated at 5 per cent level (P=0.05) and the biochemical analysis done statistically as two way analysis.

Table 1 Meteorological data during crop growth period (November, 2005 to March, 2006)

Standard week	Date and Month	Temperature		RH (%)		Rainfall (mm)	Sunshine hrs	Wind speed (km/hr)	Evaporation (mm)
		Max.	Min.	I	II				
46	12-18 NOV	29.3	11.4	80	28	0.0	9.9	2.0	3.5
47	19-25	29.2	14.1	85	35	0.0	8.4	2.1	3.4
48	26-02 DEC	28.4	13.5	87	38	0.0	8.0	2.3	2.8
49	03-09	29.1	15.0	79	30	0.0	7.5	2.5	3.4
50	10-16	28.8	11.3	81	28	0.0	9.3	1.8	3.0
51	17-23	28.4	14.7	92	44	0.0	8.3	2.1	2.0
52	24-31	27.3	11.3	87	30	0.0	8.8	2.2	2.1
1	01-07 JAN	27.2	11.1	89	37	0.0	9.3	2.4	2.4
2	08-14	29.4	14.6	92	41	0.0	9.1	2.1	2.4
3	15-21	32.0	13.7	86	26	0.0	9.7	2.2	2.8
4	22-28	29.8	10.6	80	22	0.0	10.1	2.2	2.9
5	29-04 FEB	29.8	11.9	81	29	0.0	10.0	2.9	3.1
6	05-11	30.7	12.2	80	22	0.0	10.1	3.0	3.2
7	12-18	33.1	14.3	80	21	0.0	10.2	2.4	3.5
8	19-25	35.5	15.4	76	17	0.0	10.3	3.2	4.6
9	26-04 MAR	34.9	18.3	80	31	58.0	9.6	4.6	5.7
10	05-11	31.7	20.9	93	49	30.0	7.6	4.3	3.6
11	12-18	31.7	20.1	87	35	0.0	8.9	3.0	3.8
	Average	**30.35**	**14.13**	**84.16**	**31.27**	**4.88**	**8.61**	**2.62**	**3.23**

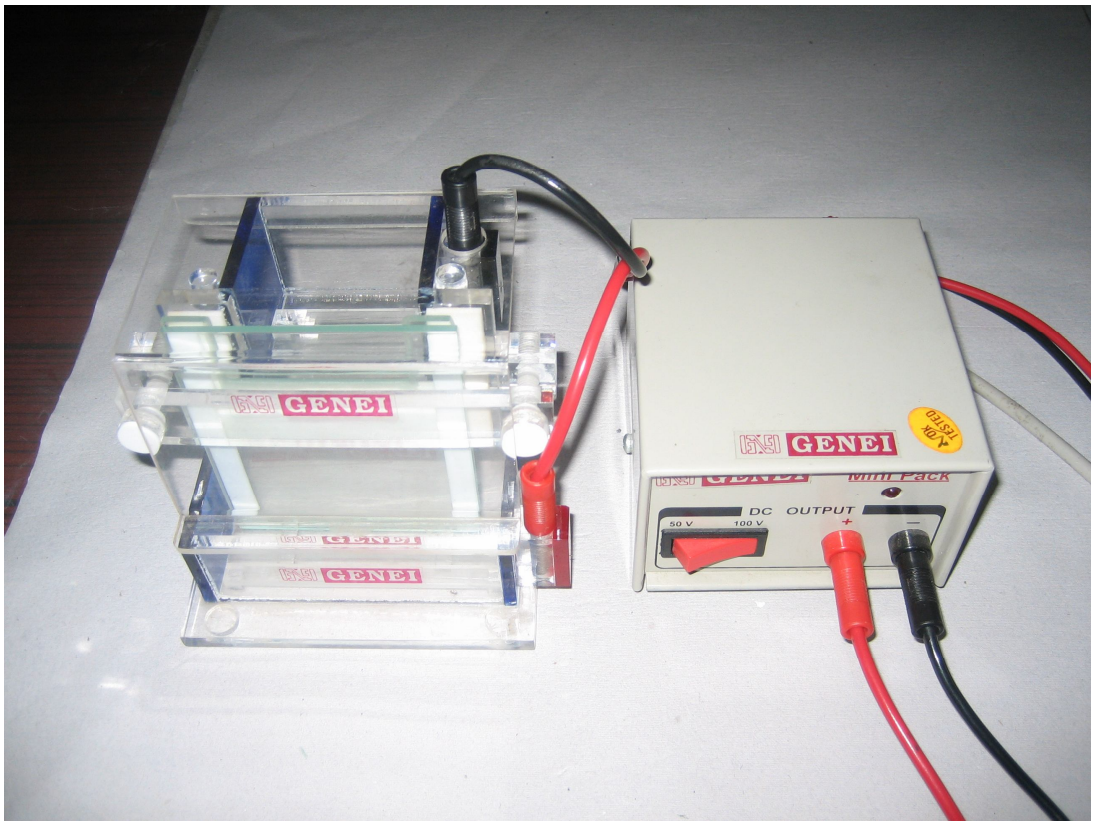
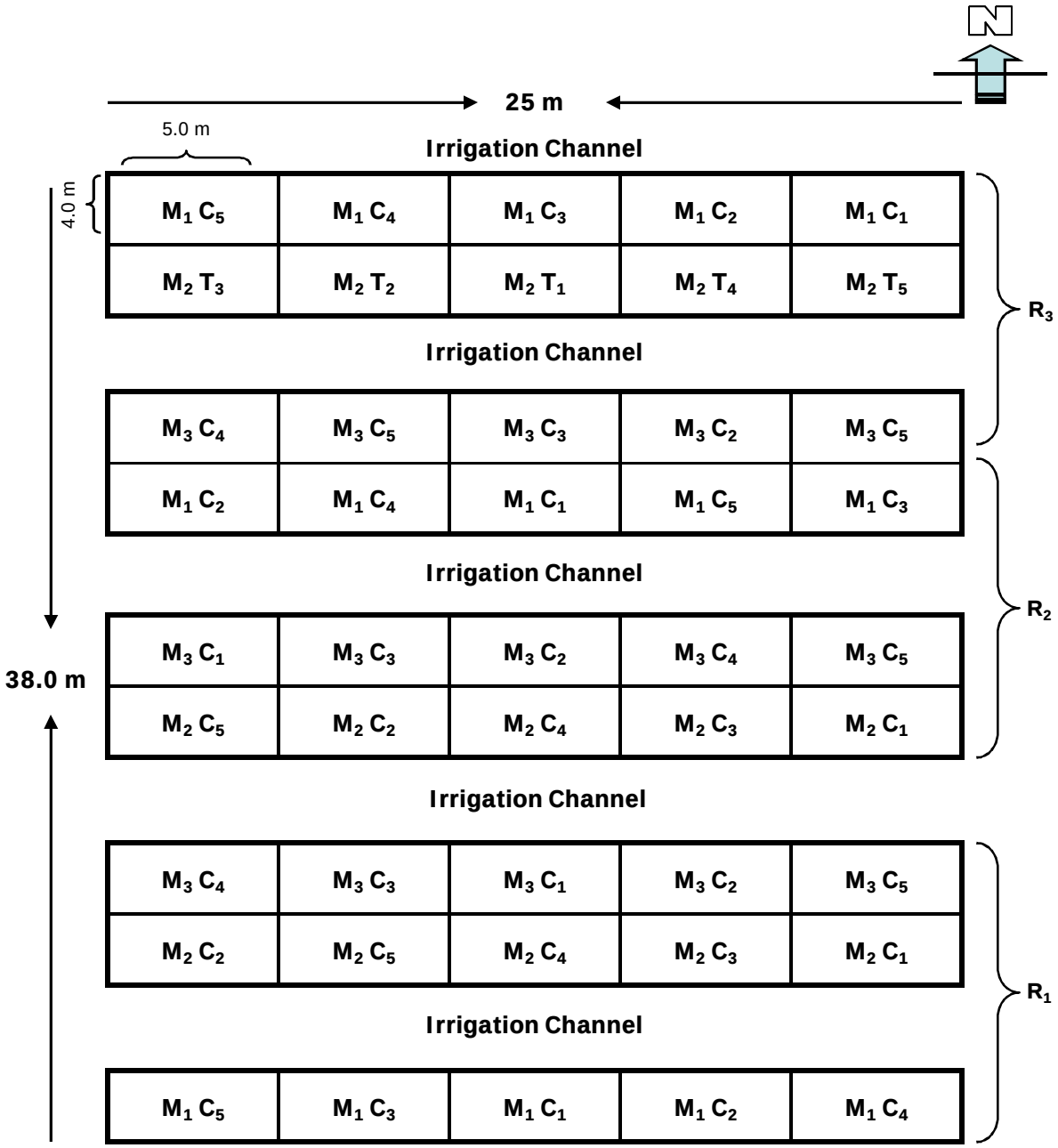


Plate 1 PAGE Equipment



Design : Split Plot Replications : 3 Plot size : 4 x 5 m

Main Treatments

M_1 = Control (no moisture stress)
M_2 = Moisture stress during flowering
M_3 = Moisture stress during pod filling

Sub-Treatments

C_1 = MAUS-47
C_2 = JS-335
C_3 = JS 93-05
C_4 = PK 1029
C_5 = NRC 37

Fig. 1 Layout Plan of the Experimental Field

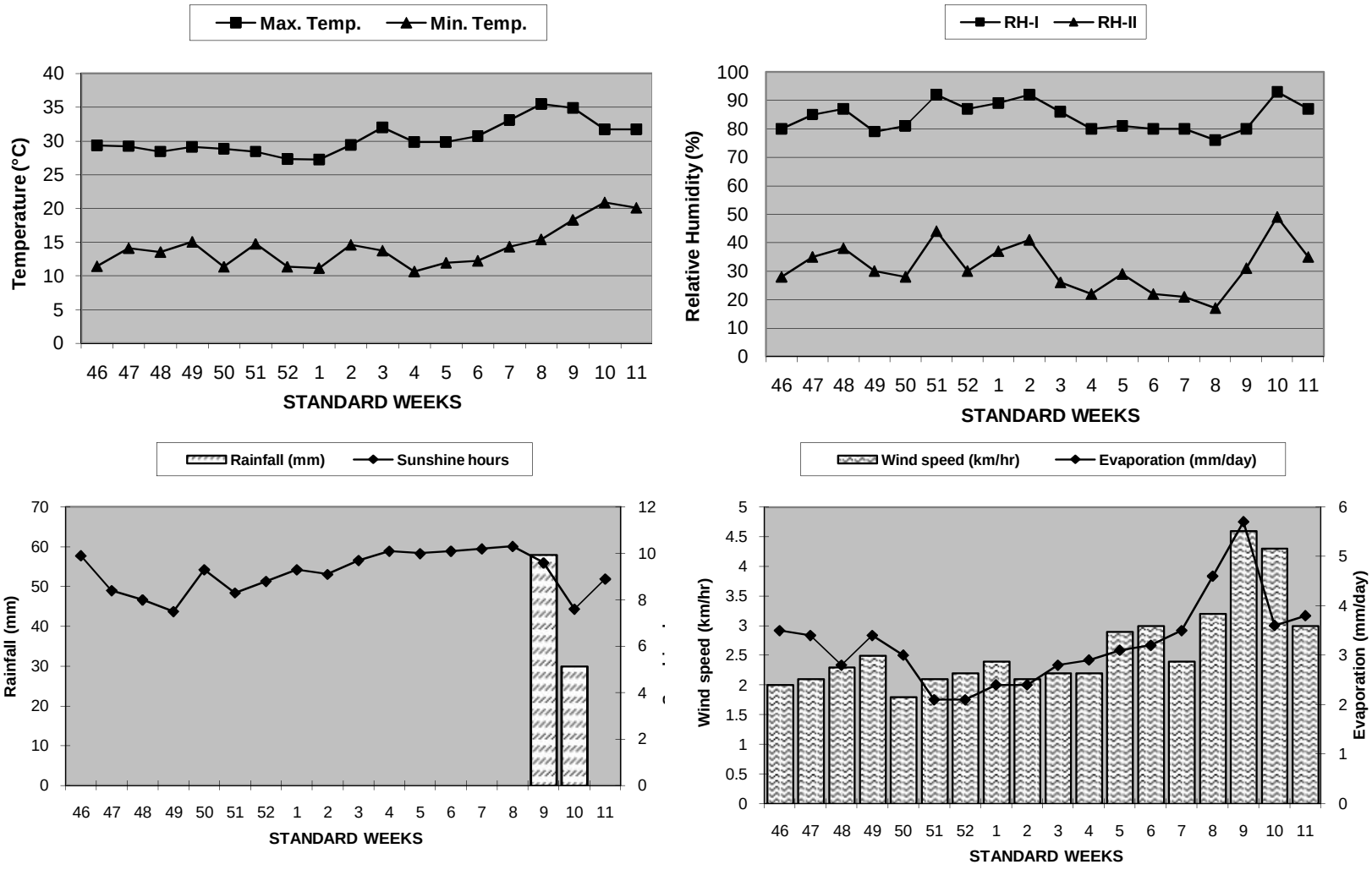


Fig 1. Weekly mean meteorological data during the crop growth period

Fig 1 Weekly mean meteorological data during the crop growth period

CHAPTER IV

RESULTS

4.1 PLANT HEIGHT

The data on plant height (cm) recorded at 15 days interval from 20 DAS till harvest are presented in Table 2. Plant height continuously increased throughout the crop growth with maximum increase between 50 to 65 DAS. The increase in plant height was evident even up to harvest though such growth was only marginal.

The plant height decreased with the waterstress treatments imposed at flowering and pod filling phase. The decrease in height was more when stress was imposed at flowering phase than that at pod filling phase. The same trend continued up to harvest. The differences between the main treatments i.e., water stress were statistically significant.

Among the cultivar means no significant differences were recorded in respect of plant height during entire crop growth period, While the interactions between main and sub-treatments were statistically significant after imposing waterstress from 50 days onwards.

Among the cultivar interactions JS-93-05 recorded maximum plant height (32.79 m) in control. The same variety recorded highest values when stress was imposed at flowering and pod filling. However, the decrease in plant

height due to waterstress at flowering was 17.6 per cent but it was reduced to 9.4 per cent at pod filling in this cultivar, While the lowest plant height was recorded by MAUS-47 throughout the crop growth period. The decrease in plant height due to stress at flowering was 17.2 per cent and it was 4.9 per cent at pod filling when compared to control.

4.2 NUMBER OF BRANCHES

The data on the number of branches were recorded at 15 days interval from 20 DAS till harvest were presented in Table 3.

Number of branches per plant were significantly different from 65 DAS in main treatments. The number of branches decreased with the waterstress treatments imposed at flowering and pod filling phase. The decrease in number of branches was more when stress was imposed at flowering compared to stress imposed during pod filling. The same trend continued up to harvest. At harvest the number of branches per plant in control was 5.13, while in water stress imposed at flowering was 4.34 and 4.72 at pod filling phase.

Among cultivar means, maximum number of branches were observed in JS-93-05 (5.33) followed by NRC-37 (4.94), JS-335 (4.70), PK-1029 (4.65) and MAUS-47 (4.03).

Among the interactions number of branches per plant were non-significant throughout the crop growth period.

4.3 NUMBER OF LEAVES PER PLANT

Number of leaves per plant recorded at 15 days interval starting from 20 DAS till harvest are presented in Table 4. Number of leaves per plant gradually increased with the age of the crop reaching the peak values at 65 DAS in all the varieties but declined.

Increase in leaf number was significant among main treatments from 65 DAS onwards. Among the main treatments, the number of leaves recorded in water stress at flowering phase was 18.35, while it was 22.53 at pod filling, However maximum leaf number per plant was recorded in no stress treatment i.e., 25.54 at 65 DAS.

The differences between the cultivars were statistically evident from 50 DAS onwards. Among varieties, IS-93-05 recorded significantly maximum number of leaves per plant (28.55) followed by NRC-37 (22.05), JS-335 (20.83), PK-1029 (20.75) and MAUS-47 (18.51).

The interactions between main and sub-treatments were statistically different from 50 DAS onwards. Cultivar JS-93-05 recorded highest leaf number (35.70) per plant in control at 65 DAS, but decreased to 21.3 when water stress was imposed at flowering phase and to 28.64 when water stress was imposed at pod filling phase.

Lower number of leaves per plant were recorded in MAUS-47 throughout the crop growth period. The maximum number of leaves per plant was recorded as 20.63 at 65 DAS in the culture. Water stress imposed at flowering and pod filling further decreased the leaf number to 16.68 and 22.4 respectively.

4.4 LEAF AREA INDEX (LAI)

The data on LAI (Table 5) indicated a gradual increase up to 65 DAS but decreased. A steep increase in LAI was observed between 35 – 65 DAS in all cultivars and all treatments.

Significant differences were observed 50 DAS onwards between main treatments. Maximum LAI was recorded in no waterstress treatment (4.68), but found decreased with waterstress at flowering phase (4.25) and at pod filling phase (4.52).

Significant differences with respect to LAI were observed between the cultivars. Among the cultivars JS-93-05 recorded maximum LAI (4.62) followed by NRC-37 (4.54), JS-335 (4.49), PK-1029 (4.44) while it was least in MAUS-47 (4.34).

4.5 LEAF DRY WEIGHT

The data on leaf dry weight (g/m2) recorded at 15 days interval from 20 DAS till harvest are presented in Table 6. Leaf dry weight gradually increased

with the age of the crop, reaching the peak at 65 DAS in all the varieties and decreased.

Leaf dry weight was decreased with the water stress treatments imposed at flowering and pod filling phase. The decrease in leaf dry weight was more when stress was imposed at flowering phase. The same trend continued up to harvest. The differences between the main treatments were statistically significant.

Significant differences were recorded among the cultivar means in respect of leaf dry weight from 50 DAS onwards. After 65 DAS, JS-93-05 recorded highest leaf dry weight (135.87) followed by NRC-37 (129.64), JS-335 (128.97), PK-1029 (127-50) and MAUS-47 (124.18).

The cultivar interactions showed that greater leaf dry weight (165.46) was recorded by JS-93-05 in no waterstress condition (control), even under stress imposed at flowering and pod filling. However, the decrease in leaf dry weight due to waterstress was 35.60 per cent at flowering and 18.08 per cent at pod filling in the cultivar. The lowest leaf dry weight was recorded by MAUS-47 throughout the crop growth period. The decrease in leaf dry weight due to stress at flowering (35.2 per cent) and (27.9 per cent) at pod filling when compare to control.

4.6 STEM DRY WEIGHT

The data on stem dry weight (g/m^2) recorded at 15 days interval from 20 DAS till harvest are presented in Table 7.In general stem dry weight gradually increased with the age of the crop up to 80 DAS in all varieties. In control, stem dry weight gradually increased reaching the peak at 80 DAS and decreased thereafter in all varieties.

Stem dry weight was decreased more with the waterstress treatments imposed at flowering than that at pod filling phase.

Among the cultivar means, significant differences were recorded in respect of stem dry weight in most of the growth stages. JS-93-05 recorded highest stem dry weight (148.77) followed by MAUS-47 (146.53) NRC-37 (146.23), JS-335 (140.98) and PK-1029 (137.60).

Among the cultivars MAUS-47 recorded greater value (199.79) in no stress condition, while the least value recorded by same cultivar (126.03) when stress was imposed at flowering phase.

4.7 POD DRY WEIGHT

The data on pod dry weight (g/m^2) recorded at 15 days interval from 65 DAS till harvest are presented in Table 8. Pod dry weight gradually increased with the age of the crop, reaching the peak values at harvest in all the varieties.

Pod dry weight decreased with the waterstress treatments imposed at flowering and pod filling phase. The decrease in pod dry weight was more when stress was imposed at flowering phase. The differences between the main treatments were statistically significant.

Among the cultivar means significant differences were recorded in respect of pod dry weight. JS-93-05 recorded highest pod dry weight (414.35) followed by NRC-37 (396.25) JS-335 (382.53), PK-1029 (370.76) and MAUS-47 (349.20).

Among the cultivars greater pod dry weight (494.33) was recorded by JS-93-05 in no water stress condition (control) as well as when stress was imposed at flowering and pod filling. However, the decrease in pod dry weight due to water stress at flowering was 26.4 per cent and pod filling it was 22.5 per cent. The lowest pod dry weight was recorded by MAUS-47 throughout the crop growth period. The decrease in pod dry weight due to stress at flowering was 28.6 per cent and 24.8 per cent at pod filling compare to control in this cultivar.

4.8 TOTAL DRY MATTER PRODUCTION (TDMP) (g/m^2)

The data on total dry matter production (g/m^2) recorded at 15 days interval from 20 DAS till harvest are presented in Table 9 and Fig 3&4. The increase in total dry matter production (TDMP) was evident even up to 80 DAS and decreased thereafter.

Total dry matter production decreased with the water stress treatments imposed at flowering and pod filling phase. The decrease was more when stress was imposed at flowering phase than pod filling phase. The same trend continued up to harvest. The differences between the main treatments i.e., waterstress treatments were statistically significant.

Among the cultivar means, JS-93-05 recorded highest TDMP (528.96) at harvest followed by NRC-37 (509.69), JS-335 (412.96), PK-1029 (478.33) MAUS-47 (464.35) at harvest.

Among the cultivar interactions, JS-93-05 recorded maximum TDMP (636.90) in no waterstress condition (control)as well as when stress was imposed at flowering and pod filling. However, the decrease in TDMP due to water stress at flowering and at pod filling 26.98 percent and 24.1 percent respectively. The lowest TDMP was recorded by MAUS-47 throughout the crop growth period. The decrease in TDMP due to stress at flowering was 30.35 percent and at pod filling as 27.22 percent when compared to control in this cultivar.

4.9 YIELD AND YIELD COMPONENTS

4.9.1 Seed Yield

The data on seed yield (kg/ha) are presented in Table 10 and Fig 5. The differences between main treatments and cultivars were significant.

Control (2672 kg/ha) registered significantly higher seed yield followed by water stress at pod filling phase (2198) and water stress at flowering phase (2064).

The data on mean yield of varieties, irrespective of main treatments i.e. control, water stress at flowering and at pod filling respectively indicated that JS-93-05 recorded more yields (2950, 2270 and 2420) followed by NRC-37 (2800, 2150 and 2210), JS-335 (2620, 1990 and 2280), PK-1029 (25230, 2050 and 2150) and MAUS-47 (2460, 1860 and 2030) .

4.9.2 Number of Pods Per Plant

The data on number of pods per plant recorded at the time of harvest was presented in Table 10. The differences between main treatments and cultivars were significant.

Main treatment differences were significant in respect of number of pods per plant. Maximum pod number was observed in control followed by waterstress at pod filling phase and at flowering phase.

The mean values of varieties irrespective of main treatments indicated that significant differences were observed in the number of pods per plant with maximum values in JS-93-05 followed by JS-335, MAUS-47, PK-1029 and NRC-37.

4.9.3 Number of Seeds Per Pod

The data on the number of seeds per pod were presented in Table 10 and Fig 6. The differences between main treatments and cultivars were

significant. Maximum seed number was observed in control and in waterstress at flowering phase than in water stress at pod filling phase.

The mean values of varieties irrespective of main treatments indicated that significant differences were observed in the number of seeds per pod. The maximum number of seeds per pod was observed in JS-93-05 followed by JS-335, MAUS-47, NRC-37 and PK-1029.

4.9.4 100 Seed Weight (g)

The data on 100 seed weight (g) are presented in Table 10 and Fig 7. The differences between main treatments and cultivars were significant.

Significantly higher test weight was recorded in control followed by in water stress at pod filling phase and waterstress at flowering phase in all varieties.

The mean seed weight of varieties irrespective of main treatments indicated that significant differences were observed between the varieties. Among them JS-93-05 (12.94) recorded highest value followed by PK-1029(12.76), MAUS-47 (12.75), NRC-37(12.61) and JS-335 (12.52).In the treatment where water stress was imposed at flowering phase gave maximum 100 seed weight in JS-93-05 and PK-1029 (11.80) recorded highest values followed by MAUS-47 (11.66), NRC-37 (11.33) and JS-335(11.30), while that at waterstress at pod

filling phase the maximum 100 seed weight was observed in JS-335 and JS-93-05 (12.76) , PK-1029 and NRC-37 (12.70) and MAUS-47 (12.50).

4.10 PHENOLOGY

In the present study the variations in crop growth and development was due to the differences in availability of water. The recorded phenological events of Cvs., MAUS-47, JS-335, JS-93-05, PK-1029 and NRC-37 under water stress at different growth phases were furnished in Table 11.

4.10.1 Emergence and Flower Initiation

It was evident from phenological chart (Table 11) that emergence and flower initiation did not show any significant difference among cultivars as water stress did not imposed till that time.

4.10.2 Days to 50 Per cent Flowering

The 50 percent of flowering occurred within 36 to 38 days after sowing in control, while it was delayed to 42 to 44 days when water stress was imposed at flowering phase and 37 – 39 days when waterstress was imposed at pod filling phase. Among all varieties JS-93-05 took less time compared to cultivars for attaining 50 per cent of flowering.

4.10.3 Harvest Maturity

Harvest maturity occurred at 105 days for PK-1029, 106 days for MAUS-47, 109 days for JS-335, NRC-37 and 111 days for JS-93-05 when waterstress given at flowering phase. Waterstress given at pod filling phase advanced by + or – 2-3 days where as it was delayed by 8 days in JS-335; 11 days in PK-1029 and NRC-37 and 12 days in MAUS-47.

BIOCHEMICAL STUDIES

4.11 PATTERN OF ISOZYMES OF TREHALASE

Water stress induced and control leaves of soybean genotypes (MAUS-47, JS-335, JS-93-05, PK-1029 and NRC-37) were subjected to native PAGE analysis for pattern of trehalase activity. Observations from Plate 2&3, evendently revealed that trehalase activity was completely absent in control (non-stressed leaves) while its was found in all stressed cultivars.

The band of Rm value 0.24 was found in all stressed genotypes. However the band in JS-93-05 was found at higher intensity**.**

4.12 Pattern of isozymes of superoxide dismutase (SOD)

Stress induced and control leaves of soybean genotypes (MAUS-47, JS-335, JS-93-05, PK-1029 and NRC-37) were subjected to native PAGE analysis for detection of pattern of isozymes of superoxide dismutase (SOD).

Observations from Plate 4&5, evidently revealed that isozymes of superoxide dismutase (SOD) was completely absent in control (non-stressed genotypes i.e. MAUS-47, JS-335, JS-93-05, PK-1029 and NRC-37). A band with Rm value of 0.46 was commonly found in all genotypes. However the band intensity in JS-93-05 was found higher than other genotypes tested.

4.13 Pattern of isozymes of peroxidase

Observations from Plate 6&7, evidently revealed that peroxidase activity was completely absent in control. Relative migration (Rm) values of isozymes of peroxidase in stress induced leaves of soybean cultivars showed 0.34.

The band was found common for all stress induced cultivars but the band found in cultivars MAUS-47 and JS-93-05, was of higher intensity.

4.14 Catalase activity in leaves of soybean genotypes under water stress

The changes in catalase activity were significant among the treatments and control were presented in Table 13. An increase in the catalase activity was found in all water deficit stress genotypes. The highest mean catalase activity was recorded in JS-93-05 (0.330) followed by NRC-37 (0.295), JS-335 (0.270), MAUS-47 and PK-1025 (0.205).

The interactions showed that higher values were recorded in stress condition in all genotypes over control. Under stress NRC-37 showed higher per

cent increase (291.6) in catalase followed by JS-93-05 (166.6), JS-335 (117.6), PK-1029 (115.3) and MAUS-47 (73.3).

4.15 Ascorbic acid content in leaves of soybean genotypes under water stress

There were significant differences among the treatments, control and there interactions and presented in Table 12.

The highest mean ascorbic acid content was recorded in JS-93-05 (9.755) followed by NRC-37 (9.430), JS-335 (8.425), MAUS-47 (7.870) and PK-1029 (7.860).

The interactions showed that higher values were recorded in control in all genotypes over stress conditions under control JS-335 showed higher per cent increase (86.5) in ascorbic acid followed by NRC-37 (74.52), JS-93-05 (62.23), PK-1029 (53.95) and MAUS-47 (44.4).

4.16 Glutathione-s-transferase (GST)

The GST activity differed significantly among control, treatments and interactions (Table 14.). The highest mean glutathione-S-transferase (GST) activity was recorded in JS-93-05 (0.223) followed by NRC-37 (0.173), JS-335 (0.158), MAUS-47 (0.063) and PK-1029 (0.057).

The interactions showed that higher values were recorded in stress condition in all genotypes over control. Under stress JS-335 showed higher per

cent increase (1463) in GST activity followed by PK-1029 (1428), MAUS-47 (1200), NRC-37 (735) and JS-93-05 (708).

4.17 Proline accumulation in leaves of soybean genotypes under water stress

The proline content in leaf tissues differed significantly among genotypes, treatments and their interactions (Table 15). The highest mean proline content was recorded in JS-93-05 (2.950) followed by JS-335 (2.545), NRC-37 (2.430), PK-1029 (2.380) and MAUS-47 (1.990).

The interactions showed that higher values were recorded in stress condition in all genotypes over control. Under stress NRC-37 showed highest per cent increase (77.7) in proline followed by JS-335 (73.6), MAUS-47 (52.8), JS-93-05 (50.0) and PK-1029 (4).

4.18 Glycine betaine (µ mol g^{-1} d.w) of soybean genotypes

The glycine betaine activity differed significantly among control, treatments and interactions (Table 16). The highest mean glycine betaine activity was recorded in JS-93-05 (3.06) followed by NRC-37 (2.995). JS-335 (2.985), MAUS-47 and PK-1029 (2.44).

The interactions showed that higher values were recorded in stress condition in all genotypes over control. Under stress JS-93-05 showed higher per cent increase (430) in glycine betaine activity followed by JS-335 (415), NRC-37 (405) MAUS-47 and PK-1029 (297).

4.19 S-adenosyl L-methionine synthetase (SAMS)

The SAMS activity in leaf tissues differed significantly among genotypes, treatments and their interactions (Table 17). The highest mean SAMS activity was recorded in JS-93-05 (11.164) followed by PK-1029 (10.031) NRC-37 (9.477), JS-335 (9.1385) and MAUS-47 (7.454).

The interactions showed that higher SAMS activity recorded in stress condition in all genotypes over control. Under stress PK-1029 showered higher percentage activity (77.48) in SAMS followed by JS-93-05 (67.17), NRC-37 (64.83), JS-335 (64.00) and MAUS-47 (51.952).

4.20. DROUGHT INDICES

The data on drought indices revealed that there was a remarkable difference in drought index and drought tolerance efficiency among the cultivars.

Maximum drought index and drought tolerance efficiency were recorded by JS-93-05 when waterstress imposed at flowering phase followed by PK-1029, NRC-37, JS-335 and MAUS-47, whereas waterstress at pod filling phase exhibited maximum drought index and drought tolerance efficiency in JS-93-05 followed by JS-335, NRC-37, PK-1029 and MAUS-47.

The data on drought indices also revealed that higher drought index and drought tolerance efficiency occurred in waterstress at pod filling phase compared to waterstress at flowering phase.

Plate 6 Native PAGE of peroxidase (POX) isozyme in soybean genotypes under control

Plate 7 Native PAGE of peroxidase (POX) isozyme in soybean genotypes under water stress conditions

1 : MAUS-47; 2 = JS-335; 3 : JS-93-05; 4 : PK-1029; 5 : NRC-37

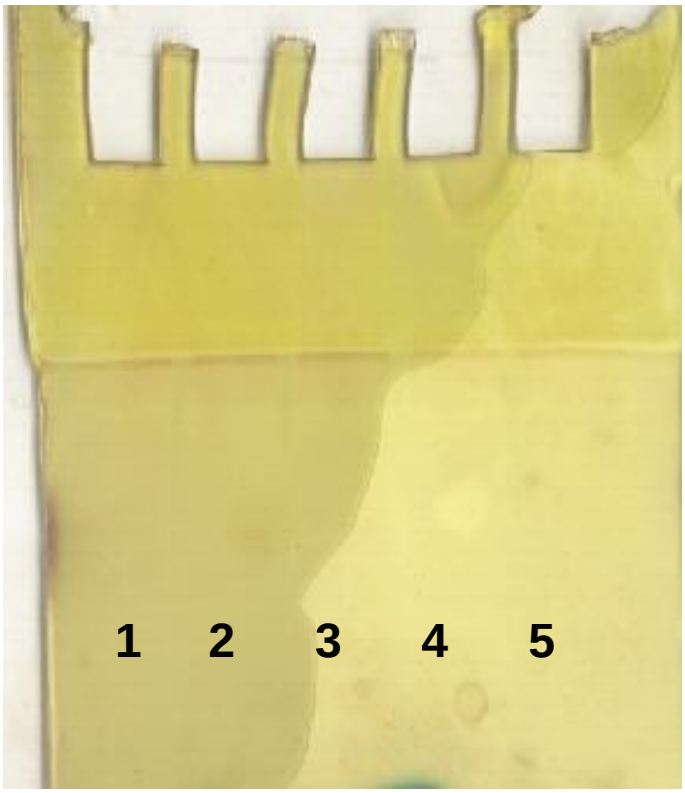


Plate 4 Native PAGE of super oxide dismutase (SOD) in soybean genotypes under control

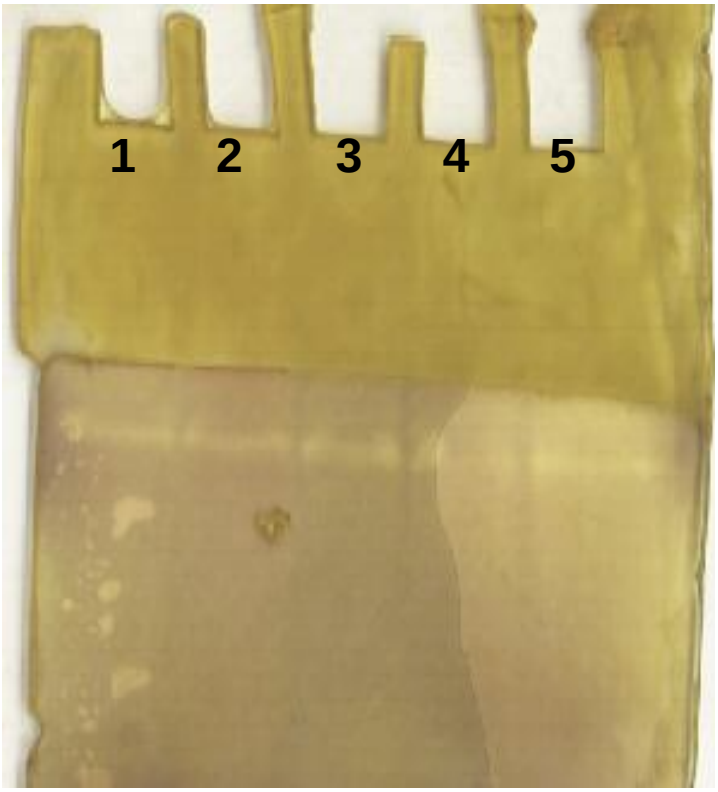


Plate 5 Native PAGE of super oxide dismutase (SOD) in soybean genotypes under water stress conditions

1 : MAUS-47; 2 = JS-335; 3 : JS-93-05; 4 : PK-1029; 5 : NRC-37

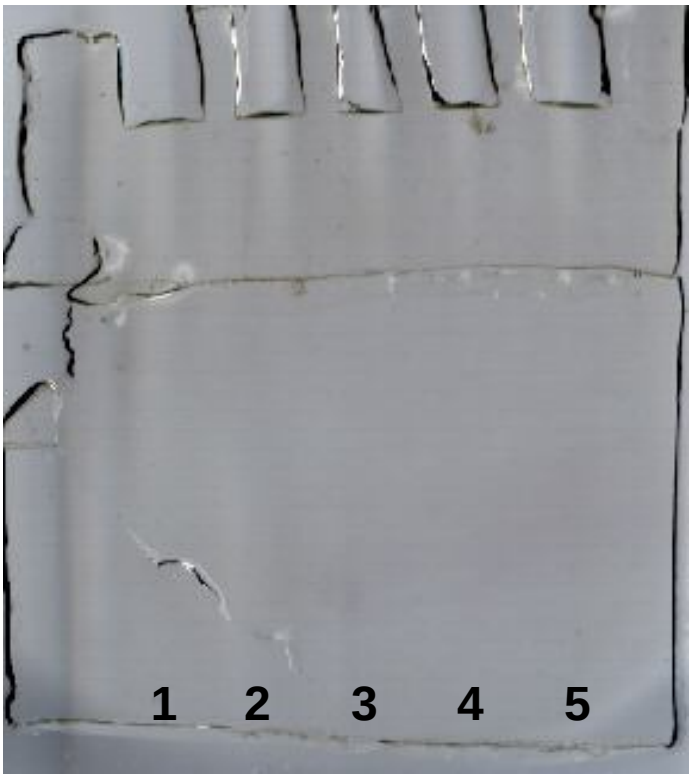
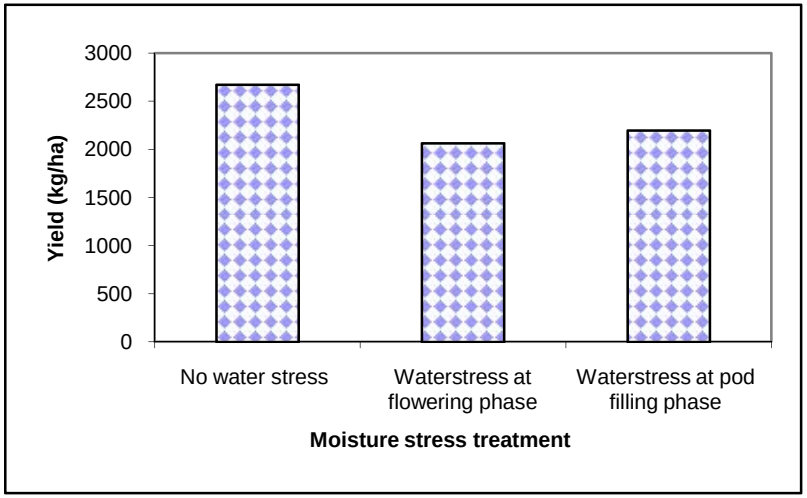
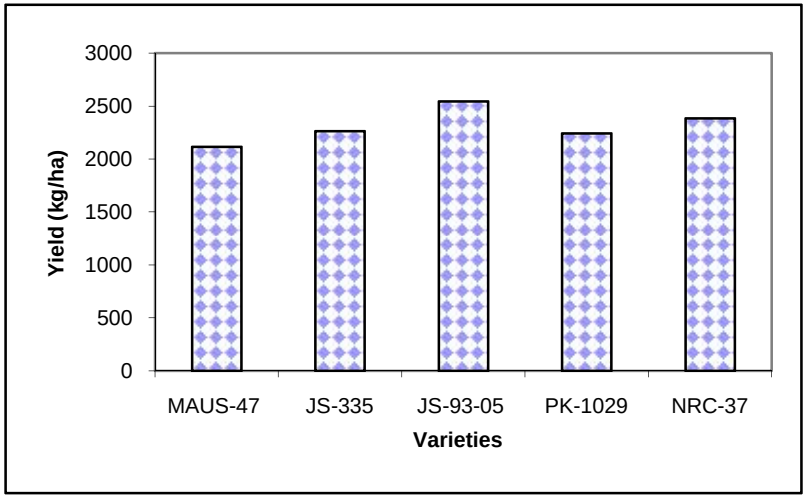


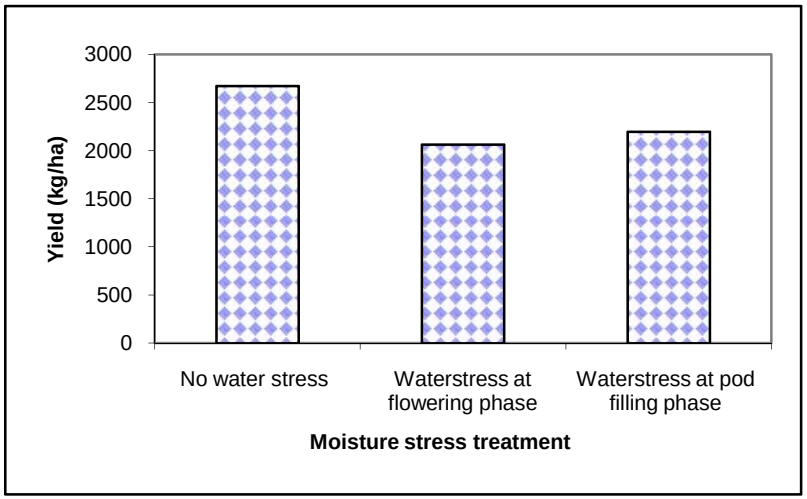
Plate 2 Native PAGE of Trehalase in soybean genotypes under control

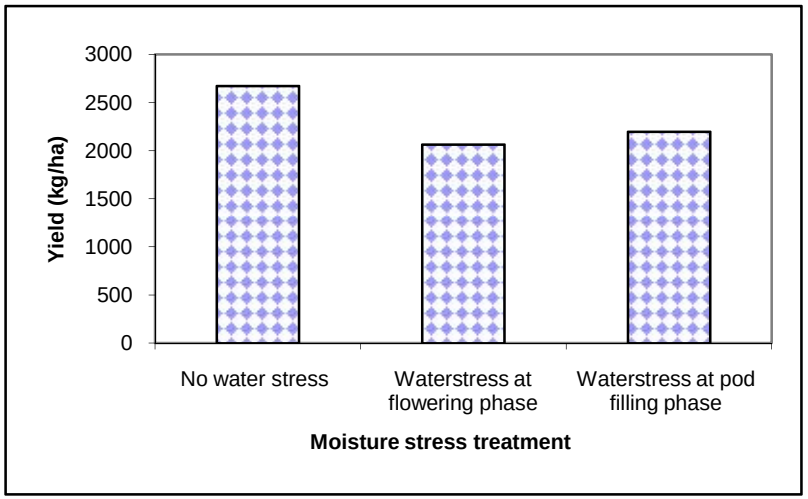
Plate 3 Native PAGE of Trehalase in soybean genotypes under water stress conditions

1 : MAUS-47; 2 = JS-335; 3 : JS-93-05; 4 : PK-1029; 5 : NRC-37


3000
2500
2000
1500
1000
500
0
Yield (kg/ha)
No water stress
Waterstress at flowering phase
Waterstress at pod filling phase
Moisture stress treatment


3000
2500
2000
1500
1000
500
0
Yield (kg/ha)
MAUS-47
JS-335
JS-93-05
PK-1029
NRC-37
Varieties


3000
2500
2000
1500
1000
500
0
Yield (kg/ha)
No water stress
Waterstress at flowering phase
Waterstress at pod filling phase
Moisture stress treatment


3000
2500
2000
1500
1000
500
0
Yield (kg/ha)
No water stress
Waterstress at flowering phase
Waterstress at pod filling phase
Moisture stress treatment

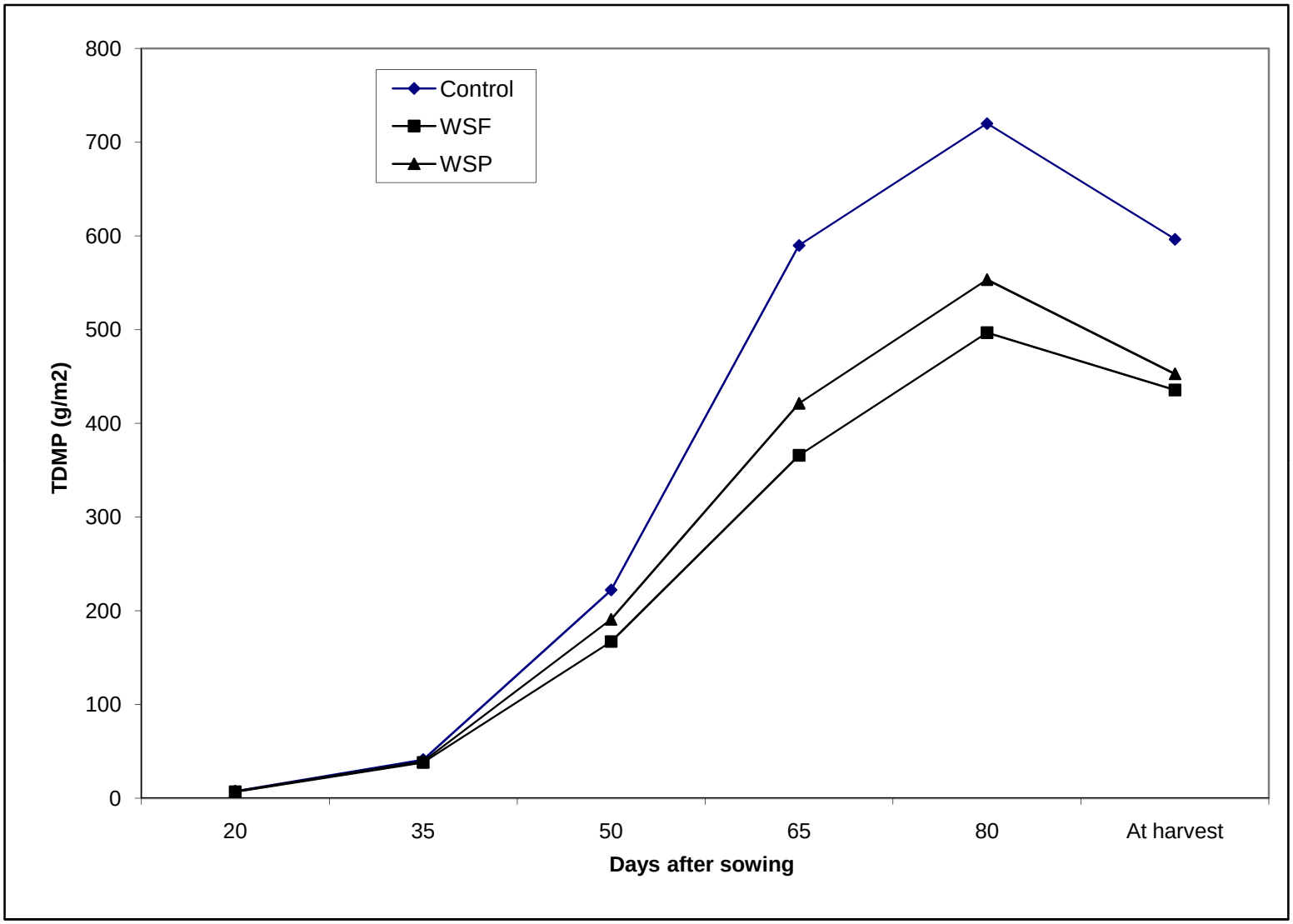


Fig. : 3 **Total dry matter production of soybean genotypes as influenced by waterstress**

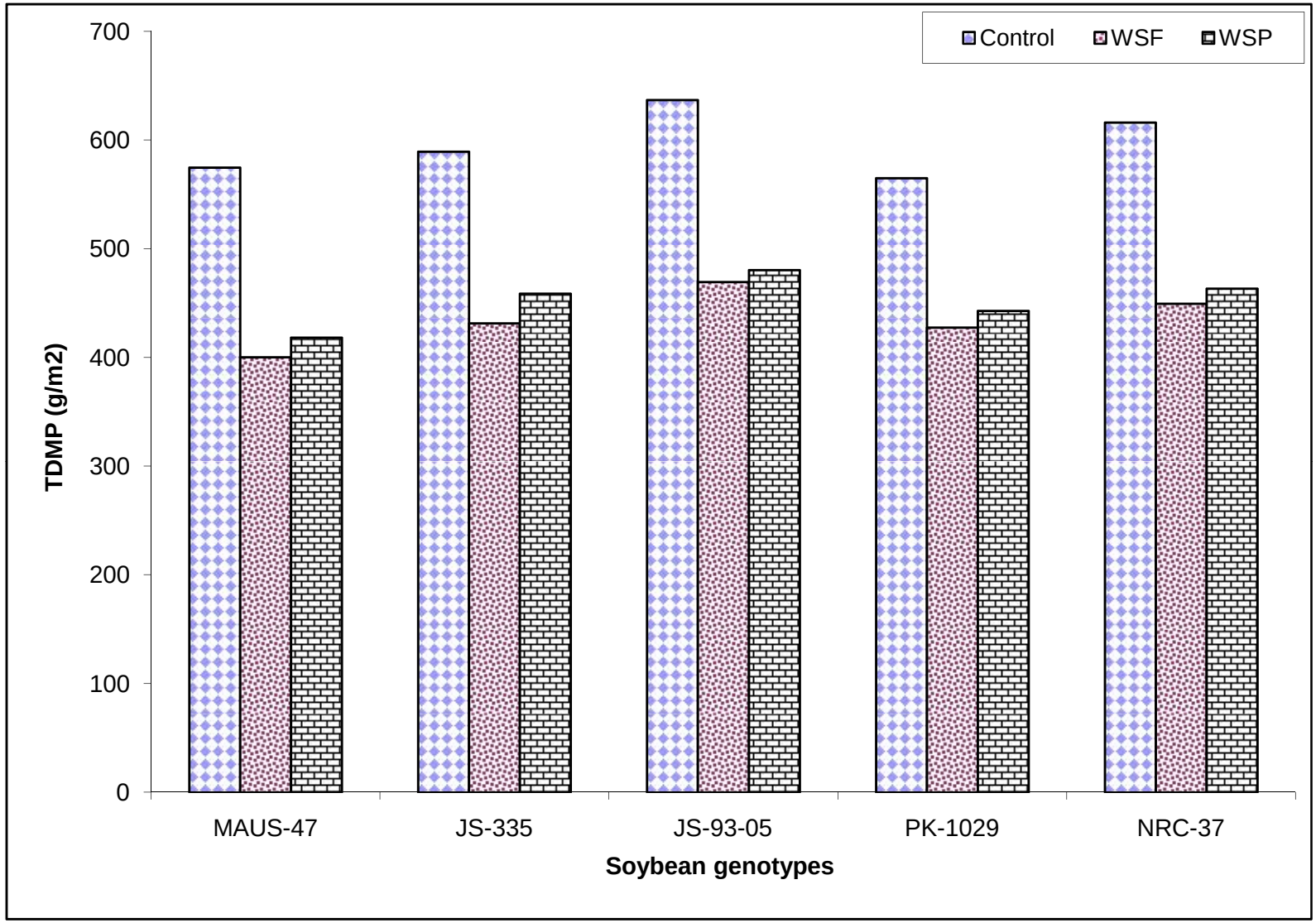


Fig. : 4 Total drymatter production of soybean genotypes as influenced by waterstress

Fig. :

Table 2 : Plant height (cm) of soybean genotypes

Treatment	Days after sowing					
	20	35	50	65	80	At harvest
Control(No water stress)						
MAUS-47	8.50	13.01	23.23	27.36	28.00	28.10
JS-335	9.51	14.15	26.20	30.48	31.28	31.73
JS-93-05	10.49	15.75	31.51	31.05	33.01	32.79
PK-1029	8.80	14.01	24.08	29.78	31.25	32.70
NRC-37	10.27	15.51	29.27	30.64	32.50	32.50
Water stress at flowering phase						
MAUS-47	8.08	13.06	15.44	22.74	23.27	23.27
JS-335	9.57	14.24	17.51	24.42	25.78	25.92
JS-93-05	11.02	16.81	18.83	25.43	27.18	27.03
PK-1029	8.85	14.06	16.89	23.06	24.50	25.83
NRC-37	10.35	15.58	18.53	24.85	26.70	26.90
Water stress at pod filling phase						
MAUS-47	8.24	13.17	18.37	24.57	26.73	26.73
JS-335	9.62	14.27	22.22	27.03	28.31	28.81
JS-93-05	10.67	16.88	26.20	27.45	30.42	29.71
PK-1029	8.91	14.24	21.54	26.10	27.40	27.74
NRC-37	10.43	15.50	24.14	27.42	29.14	29.53
CD a (P=0.05)	NS	NS	1.56	7.73	9.88	5.19
CD b (P=0.05)	NS	NS	1.50	6.90	8.80	5.01
Main treatment means						
Control	9.71	16.69	29.04	33.80	36.64	37.12
Water stress at flowering phase	9.69	16.75	18.92	25.48	26.68	26.99
Water stress at pod filling phase	9.77	16.81	24.49	29.71	26.40	31.70
CD (P=0.05)	NS	NS	1.50	3.76	4.79	2.77
Sub-treatment means						
MAUS-47	8.27	13.09	19.01	24.09	28.00	26.03
JS-335	9.57	14.22	21.98	27.31	28.48	28.82
JS-93-05	10.72	16.48	25.54	27.97	30.20	29.84
PK-1029	8.82	13.65	20.50	26.10	27.23	27.43
NRC-37	9.14	13.84	20.00	26.50	27.64	27.80
CD (P=0.05)	NS	NS	NS	NS	NS	NS

Table 3 : Number of branches of soybean genotypes

Treatment	Days after sowing					
	20	35	50	65	80	At harvest
Control(No water stress)						
MAUS-47	3.00	3.67	3.90	4.20	4.20	4.20
JS-335	3.00	4.25	4.72	4.88	5.10	5.08
JS-93-05	2.99	4.75	5.35	5.80	6.03	6.80
PK-1029	3.00	4.11	4.22	4.75	5.06	5.02
NRC-37	2.99	4.47	4.86	5.01	5.35	5.35
Water stress at flowering phase						
MAUS-47	3.10	3.53	3.42	3.60	3.75	3.75
JS-335	2.99	4.05	3.79	3.87	4.34	4.36
JS-93-05	3.05	4.44	4.02	4.30	4.55	4.75
PK-1029	3.00	3.93	3.73	3.84	4.6	4.34
NRC-37	3.07	4.17	3.85	3.96	4.44	4.52
Water stress at pod filling phase						
MAUS-47	2.98	3.72	3.72	3.96	4.13	4.14
JS-335	3.07	4.35	4.22	4.38	4.62	4.68
JS-93-05	3.02	4.63	4.45	4.78	4.97	5.24
PK-1029	3.00	4.08	4.19	4.29	4.53	4.60
NRC-37	3.04	4.51	4.32	4.53	4.85	4.95
CD a (P=0.05)	NS	NS	NS	NS	NS	NS
CD b (P=0.05)	NS	NS	NS	NS	NS	NS
Main treatment means						
Control	2.99	4.25	4.61	4.92	5.15	5.13
Water stress at flowering phase	3.04	4.02	3.76	3.91	4.27	4.34
Water stress at pod filling phase	3.02	4.26	4.18	4.38	4.62	4.72
CD (P=0.05)	NS	NS	NS	0.21	0.47	0.11
Sub-treatment means						
MAUS-47	3.02	3.64	3.68	3.92	4.2	4.03
JS-335	3.02	4.21	4.24	4.37	4.68	4.70
JS-93-05	3.02	4.60	4.60	4.96	5.19	5.33
PK-1029	3.00	4.04	4.04	4.29	4.61	4.65
NRC-37	3.03	4.38	4.34	4.50	4.88	4.94
CD (P=0.05)	NS	NS	NS	0.56	0.66	0.68

Table 4 : Number of leaves of soybean genotypes

Treatment	Days after sowing					
	20	35	50	65	80	At harvest
Control(No water stress)						
MAUS-47	3.84	9.78	19.30	20.63	18.43	8.42
JS-335	4.16	12.13	19.82	22.82	22.53	11.07
JS-93-05	5.04	15.62	25.15	35.70	33.21	21.10
PK-1029	4.12	11.95	19.75	23.23	21.96	10.73
NRC-37	4.72	13.43	21.33	25.25	24.46	14.28
Water stress at flowering phase						
MAUS-47	3.66	9.07	15.95	16.41	16.68	4.51
JS-335	4.08	11.17	17.43	18.12	17.54	5.44
JS-93-05	4.77	14.08	19.46	21.32	19.44	6.32
PK-1029	4.02	10.89	16.80	17.52	17.66	4.69
NRC-37	4.48	12.04	18.72	18.39	18.41	5.77
Water stress at pod filling phase						
MAUS-47	3.79	9.48	16.43	18.50	16.54	5.03
JS-335	4.18	12.25	18.87	21.56	18.42	5.73
JS-93-05	4.94	14.69	22.56	28.64	22.42	6.82
PK-1029	4.06	11.59	18.69	21.40	18.28	5.38
NRC-37	4.53	13.14	19.69	22.53	19.45	5.34
CD a (P=0.05)	NS	NS	0.53	1.03	1.35	0.44
CD b (P=0.05)	NS	NS	0.74	0.83	2.08	0.64
Main treatment means						
Control	4.37	12.58	21.07	25.54	24.12	13.12
Water stress at flowering phase	4.20	11.45	17.69	18.35	17.94	5.34
Water stress at pod filling phase	4.30	12.23	19.25	22.53	19.02	5.66
CD (P=0.05)	NS	NS	NS	0.44	1.18	0.36
Sub-treatment means						
MAUS-47	3.76	9.44	17.22	18.51	17.21	5.98
JS-335	4.14	11.85	18.70	20.83	19.49	7.41
JS-93-05	4.91	14.79	22.39	28.55	25.02	11.41
PK-1029	4.06	11.47	18.41	20.75	19.30	6.93
NRC-37	4.58	12.87	19.91	22.05	20.77	8.46
CD (P=0.05)	NS	NS	0.30	0.59	0.78	0.25

Table 5 : Leaf area index (LAI) of soybean genotypes

Treatment	Days after sowing					
	20	35	50	65	80	At harvest
Control(No water stress)						
MAUS-47	0.16	0.71	2.43	4.55	3.57	0.28
JS-335	0.15	0.75	2.57	4.70	3.74	0.28
JS-93-05	0.16	0.82	2.72	4.80	3.82	0.31
PK-1029	0.14	0.74	2.54	4.64	3.67	0.28
NRC-37	0.15	0.77	2.64	4.74	3.78	0.30
Water stress at flowering phase						
MAUS-47	0.16	0.66	2.13	4.10	3.29	0.23
JS-335	0.15	0.74	2.28	4.25	3.44	0.24
JS-93-05	0.16	0.80	2.40	4.42	3.58	0.25
PK-1029	0.15	0.72	2.25	4.19	3.43	0.23
NRC-37	0.15	0.75	2.32	4.33	3.52	0.28
Water stress at pod filling phase						
MAUS-47	0.16	0.65	2.30	4.39	3.42	0.27
JS-335	0.18	0.74	2.42	4.54	3.49	0.28
JS-93-05	0.18	0.82	2.55	4.65	3.73	0.30
PK-1029	0.17	0.73	2.42	4.48	3.55	0.27
NRC-37	0.14	0.79	2.46	4.56	3.66	0.28
CD a (P=0.05)	NS	NS	NS	NS	NS	NS
CD b (P=0.05)	NS	NS	NS	NS	NS	NS
Main treatment means						
Control	0.15	0.75	2.58	4.68	3.71	0.29
Water stress at flowering phase	0.15	0.73	2.27	4.25	3.45	0.24
Water stress at pod filling phase	0.17	0.74	2.43	4.52	3.57	0.28
CD (P=0.05)	NS	NS	0.03	0.05	0.03	0.05
Sub-treatment means						
MAUS-47	0.16	0.67	2.29	4.34	3.42	0.26
JS-335	0.16	0.74	2.42	4.49	3.55	0.26
JS-93-05	0.17	0.81	2.55	4.62	3.71	0.28
PK-1029	0.15	0.73	2.40	4.44	3.55	0.26
NRC-37	0.14	0.77	2.47	4.54	3.65	0.28
CD (P=0.05)	NS	0.05	0.06	0.06	0.06	NS

Table 6 : Leaf dry weight (gm^{-2}) of soybean genotypes

Treatment	Days after sowing					
	20	35	50	65	80	At harvest
Control(No water stress)						
MAUS-47	4..04	20.70	98.50	157.20	142.00	7.10
JS-335	4.14	23.03	121.30	163.80	137.90	7.38
JS-93-05	4.79	24.70	125.70	165.46	143.23	7.41
PK-1029	4.11	23.03	122.33	162.70	139.10	7.24
NRC-37	4.13	23.50	123.50	163.06	142.40	7.40
Water stress at flowering phase						
MAUS-47	3.65	19.37	77.23	102.00	77.56	4.97
JS-335	3.68	22.33	84.10	103.46	78.90	5.06
JS-93-05	4.19	23.76	90.66	106.60	90.46	5.37
PK-1029	3.69	21.80	82.56	102.50	78.83	5.02
NRC-37	3.77	22.13	90.10	105.23	84.70	5.11
Water stress at pod filling phase						
MAUS-47	3.84	20.36	92.50	113.36	78.83	5.44
JS-335	4.08	22.56	97.50	119.66	82.96	5.46
JS-93-05	4.44	24.36	99.66	135.56	84.06	5.57
PK-1029	4.09	22.26	96.33	117.30	80.50	5.51
NRC-37	4.16	22.56	98.06	120.63	82.50	5.49
CD a (P=0.05)	NS	6.70	1.57	6.43	2.33	NS
CD b (P=0.05)	NS	3.50	2.40	6.81	2.39	NS
Main treatment means						
Control	4.24	22.99	118.26	162.44	140.92	7.31
Water stress at flowering phase	3.79	21.88	84.93	103.96	82.09	5.11
Water stress at pod filling phase	4.12	22.42	96.81	121.30	81.77	5.49
CD (P=0.05)	0.53	1.20	1.36	3.83	1.34	0.05
Sub-treatment means						
MAUS-47	3.84	20.14	89.41	124.18	99.46	5.83
JS-335	3.96	22.64	100.96	128.97	99.92	5.97
JS-93-05	4.47	24.27	105.34	135.87	105.92	6.12
PK-1029	3.96	22.36	100.41	127.50	99.47	5.92
NRC-37	4.02	22.73	103.88	129.64	103.20	6.600
CD (P=0.05)	NS	NS	0.90	3.71	1.35	0.09

Table7 : Stem dry weight (gm^{-2}) of soybean genotypes

Treatment	Days after sowing					
	20	35	50	65	80	At harvest
Control(No water stress)						
MAUS-47	2.45	16.40	93.60	153.63	199.79	142.93
JS-335	3.94	17.80	105.43	138.80	181.11	127.18
JS-93-05	3.41	20.20	109.76	146.36	197.06	135.16
PK-1029	2.84	17.30	105.50	136.60	176.72	121.56
NRC-37	3.10	18.20	104.46	141.70	195.05	132.57
Water stress at flowering phase						
MAUS-47	2.45	14.76	74.20	126.03	113.23	91.75
JS-335	3.21	15.93	84.73	130.16	116.54	94.56
JS-93-05	3.01	18.06	86.50	134.70	125.16	99.26
PK-1029	2.59	15.94	81.43	128.66	111.79	95.28
NRC-37	2.82	16.12	83.50	131.83	118.71	96.34
Water stress at pod filling phase						
MAUS-47	2.31	15.14	79.46	138.00	126.59	93.29
JS-335	3.37	16.76	93.53	137.20	125.31	92.56
JS-93-05	3.15	19.10	106.86	135.63	124.29	91.05
PK-1029	2.63	16.36	95.13	135.90	124.11	88.59
NRC-37	2.89	16.50	94.80	139.80	127.51	93.41
CD a (P=0.05)	NS	NS	2.91	8.96	3.21	1.03
CD b (P=0.05)	NS	NS	3.09	8.13	1.01	0.09
Main treatment means						
Control	3.04	17.98	103.75	141.27	189.94	131.88
Water stress at flowering phase	2.81	16.16	82.07	132.67	117.08	95.43
Water stress at pod filling phase	2.87	16.77	93.96	137.06	125.56	91.78
CD (P=0.05)	NS	1.16	3.00	3.45	5.91	5.98
Sub-treatment means						
MAUS-47	2.40	15.43	82.42	139.22	146.53	109.32
JS-335	3.51	16.83	94.56	135.38	140.98	104.76
JS-93-05	3.19	19.12	101.04	138.90	148.77	108.49
PK-1029	2.68	16.53	94.02	133.72	137.60	101.81
NRC-37	2.94	16.97	94.25	137.77	146.23	107.44
CD (P=0.05)	NS	NS	3.29	2.37	1.71	1.13

Table 8 : Pod dry weight (gm^{-2}) of soybean genotypes

Treatments	Days after sowing		
	65	80	At harvest
Control(No water stress)			
MAUS-47	259.60	343.16	424.60
JS-335	289.00	384.73	454.96
JS-93-05	300.53	434.80	494.33
PK-1029	291.40	379.90	436.30
NRC-37	290.60	403.23	476.30
Water stress at flowering phase			
MAUS-47	96.36	234.70	303.56
JS-335	127.93	305.60	331.93
JS-93-05	155.43	333.66	364.80
PK-1029	131.53	302.20	327.20
NRC-37	135.03	312.36	347.93
Water stress at pod filling phase			
MAUS-47	104.10	306.50	319.50
JS-335	164.33	355.20	360.70
JS-93-05	199.43	373.80	383.90
PK-1029	16860	336.00	348.80
NRC-37	178.53	358.10	364.53
CD a (P=0.05)	3.54	302	5.50
CD b (P=0.05)	4.59	1.52	6.83
Main treatment means			
Control	286.22	389.16	457.30
Water stress at flowering phase	129.25	297.70	335.07
Water stress at pod filling phase	163.00	346.04	355.48
CD (P=0.05)	2.61	2.45	3.88
Sub-treatment means			
MAUS-47	153.35	294.78	349.20
JS-335	193.75	348.51	382.53
JS-93-05	218.46	380.75	414.35
PK-1029	197.17	339.56	370.76
NRC-37	201.38	357.90	396.25
CD (P=0.05)	2.04	1.74	3.18

Table 9 : Total dry matter production (gm^{-2}) of soybean genotypes

Treatment	Days after sowing					
	20	35	50	65	80	At harvest
Control(No water stress)						
MAUS-47	6.49	37.10	192.10	570.43	684.95	574.63
JS-335	8.08	40.83	226.73	591.60	703.74	589.38
JS-93-05	8.21	44.90	235.46	612.35	775.09	636.90
PK-1029	6.95	40.33	227.83	590.70	695.72	565.10
NRC-37	7.23	41.70	227.16	595.36	740.68	616.27
Water stress at flowering phase						
MAUS-47	6.10	34.13	151.43	324.39	425.49	400.28
JS-335	6.89	38.26	168.82	361.55	501.04	431.55
JS-93-05	7.20	41.82	177.16	396.73	549.28	469.43
PK-1029	6.28	37.75	163.99	362.69	492.82	427.50
NRC-37	6.57	38.25	173.60	372.09	515.77	449.38
Water stress at pod filling phase						
MAUS-47	6.15	35.50	171.96	355.46	511.92	418.23
JS-335	7.45	39.32	191.03	421.19	562.47	458.72
JS-93-05	7.59	43.40	206.52	470.62	581.95	480.52
PK-1029	6.72	48.62	191.46	421.80	540.81	442.90
NRC-37	7.05	39.06	192.86	438.96	568.11	463.43
CD a (P=0.05)	NS	1.01	3.43	7.51	2.01	2.13
CD b (P=0.05)	NS	1.96	3.95	6.82	1.91	1.67
Main treatment means						
Control	7.38	40.97	222.01	589.93	720.02	596.49
Water stress at flowering phase	6.60	38.04	167.00	365.88	496.79	435.61
Water stress at pod filling phase	6.99	39.19	190.77	421.36	553.37	452.75
CD (P=0.05)	NS	0.09	4.01	6.93	3.33	2.96
Sub-treatment means						
MAUS-47	6.24	35.57	171.83	416.75	540.77	464.35
JS-335	7.47	39.47	195.52	458.10	589.41	492.96
JS-93-05	7.66	43.39	206.38	493.20	635.44	528.96
PK-1029	6.65	38.89	194.43	458.39	576.63	478.33
NRC-37	6.96	39.70	198.13	468.79	607.33	509.69
CD (P=0.05)	NS	1.57	3.31	2.14	4.14	2.19

Table10 : Yield and yield attributes in soybean genotypes

Treatments	Seed yield (kg ha^{-1})	Pods per plant	Seeds per pod	100 seed weight (g)
Control(No water stress)				
MAUS-47	2460	21.80	2.89	14.08
JS-335	2620	20.90	2.82	13.50
JS-93-05	2950	24.80	2.88	14.27
PK-1029	2530	18.20	2.63	13.80
NRC-37	2800	18.40	2.52	13.80
Water stress at flowering phase				
MAUS-47	1860	19.40	2.06	11.66
JS-335	1990	20.05	2.21	11.30
JS-93-05	2270	21.80	2.93	11.80
PK-1029	2050	21.15	2.79	11.80
NRC-37	2150	17.28	2.80	11.33
Water stress at pod filling phase				
MAUS-47	2030	19.50	2.80	12.50
JS-335	2280	22.50	2.76	12.76
JS-93-05	2420	21.60	2.87	12.76
PK-1029	2150	23.40	2.49	12.70
NRC-37	2210	21.03	2.70	12.70
CD a (P=0.05)	NS	1.78	0.02	NS
CD b (P=0.05)	NS	1.73	0.02	NS
Main treatment means				
Control	2672	21.82	2.75	13.89
Water stress at flowering phase	2064	20.42	2.71	11.58
Water stress at pod filling phase	2198	20.53	2.75	12.68
CD (P=0.05)	211.36	0.96	0.01	0.63
Sub-treatment means				
MAUS-47	2116	20.59	2.74	12.75
JS-335	2263	21.15	2.75	12.52
JS-93-05	2546	22.73	2.89	12.94
PK-1029	2243	20.58	2.63	12.76
NRC-37	2386	19.57	2.67	12.61
CD (P=0.05)	179.82	1.03	0.01	0.45

Table18: Drought index of soybean genotypes at different stress periods

Genotype	Drought index for water stress at flowering phase	Drought index for water stress at pod filling phase
MAUS-47	0.756	0.789
JS-335	0.759	0.832
JS-93-05	0.810	0.849
PK-1029	0.769	0.820
NRC-37	0.767	0.825

Table 19 : Drought tolerance efficiency of soybean genotypes at different stress periods

Genotype	Drought tolerance efficiency for water stress at flowering phase (%)	Drought tolerance efficiency for water stress at pod filling phase (%)
MAUS-47	75.60	78.90
JS-335	75.90	83.20
JS-93-05	81.00	84.90
PK-1029	76.90	82.00
NRC-37	76.70	82.50

Table11 : Phenology of soybean genotypes (days)

Treatments	Days after sowing			
	Emergence	Flowering initiation	50% Flowering	Harvest maturity
Control				
MAUS-47	5.00	24	37	118
JS-335	5.00	24	38	117
JS-93-05	4.66	24	36	123
PK-1029	5.33	24	38	116
NRC-37	5.00	24	37	120
Water stress at flowering phase				
MAUS-47	4.66	24	42	106
JS-335	5.33	24	43	109
JS-93-05	5.00	24	42	111
PK-1029	5.00	24	44	105
NRC-37	5.00	24	42	109
Water stress at pod filling phase				
MAUS-47	5.00	25	38	105
JS-335	5.00	23	39	107
JS-93-05	5.00	24	37	113
PK-1029	5.00	24	38	107
NRC-37	5.00	24	39	108

Table 14 : Glutathione-S-transferase (GST) activity (ODA_{344}) of soybean cultivars

S. No.	Genotypes	Control	Treatment	Mean	% increase in GST
1	MAUS-47	0.009	0.117	0.063	1200
2	JS-335	0.019	0.297	0.158	1463
3	JS-93-05	0.049	0.396	0.223	708
4	PK-1029	0.07	0.107	0.057	1428
5	NRC-37	0.037	0.309	0.173	735
	Mean	0.024	0.245	0.135	920

	Control	Treatment	Interaction
CD (0.05)	0.0049	0.0078	0.0110
SEm	0.0017	0.0026	0.0037

Table 15 : Proline content (µ moles g^{-1} fresh weight) of soybean genotypes

S. No.	Genotypes	Control	Treatment	Mean	% increase in proline
1	MAUS-47	1.840	2.140	1.990	52.80
2	JS-335	1.860	3.230	2.545	73.60
3	JS-93-05	2.360	3.540	2.950	50.00
4	PK-1029	2.330	2.430	2.380	4.00
5	NRC-37	1.750	3.110	2.430	77.70
	Mean	2.028	2.890	2.459	42.50

	Control	Treatment	Interaction
CD (0.05)	0.0576	0.0911	0.1289
SEm	0.0195	0.0309	0.0437

Table 12: Ascorbic acid content (mg per 100 gm) of soybean genotypes

S. No.	Genotypes	Control	Treatment	Mean	% decrease in ascorbic acid
1	MAUS-47	9.300	6.440	7.870	44.40
2	JS-335	10.970	5.880	8.425	86.50
3	JS-93-05	12.070	7.440	9.755	62.23
4	PK-1029	9.530	6.190	7.860	53.95
5	NRC-37	11.990	6.870	9.430	74.52
	Mean	10.772	6.564	8.668	64.17

	Control	Treatment	Interaction
CD (0.05)	0.3415	0.2160	0.4829
SEm	0.1157	0.0732	0.1637

Table 13: Catalase activity (µg mg^{-1}) of soybean genotypes

S. No.	Genotypes	Control	Treatment	Mean	% increase in catalase
1	MAUS-47	0.150	0.260	0.205	73.30
2	JS-335	0.170	0.370	0.270	117.60
3	JS-93-05	0.180	0.480	0.330	166.60
4	PK-1029	0.130	0.280	0.205	115.30
5	NRC-37	0.120	0.470	0.295	291.60
	Mean	0.150	0.372	0.261	148.00

	Control	Treatment	Interaction
CD (0.05)	0.0070	0.0110	0.0155
SEm	0.0024	0.0037	0.0053

Table 16: Glycine betaine (µ mol g^{-1} d.w) of soybean cultivars

S. No.	Genotypes	Control	Treatment	Mean	% increase in Glycine betaine
1	MAUS-47	0.98	3.90	2.44	297
2	JS-335	0.97	5.00	2.985	415
3	JS-93-05	0.97	5.15	3.06	430
4	PK-1029	0.98	3.90	2.44	297
5	NRC-37	0.99	5.00	2.995	405
	Mean	0.978	4.59	2.784	369

	Control	Treatment	Interaction
CD (0.05)	0.475	0.0899	0.1157
SEm	0.0189	0.0302	0.417

Table 17: S-adenosyl L-methionine synthetase (SAMS) activity (µ moles / 100g) of soybean cultivars

S. No.	Genotypes	Control	Treatment	Mean	% increase in SAMS
1	MAUS-47	5.917	8.991	7.454	51.952
2	JS-335	6.923	11.354	9.1385	64.004
3	JS-93-05	8.357	13.971	11.164	67.177
4	PK-1029	7.230	12.832	10.031	77.482
5	NRC-37	7.157	11.797	9.477	64.831
	Mean	7.1168	11.789	9.452	65.089

	Control	Treatment	Interaction
CD (0.05)	0.2570	0.3984	0.4926
SEm	0.0783	0.1346	0.1391

CHAPTER V

DISCUSSION

In the present study, an attempt was made to study the physiological and biochemical responses of soybean genotypes to induced waterstress during post-rainy season of 2005-2006.

5.1 EFFECT OF WATERSTRESS ON MORPHO-PHYSIOLOGICAL CHARACTERS.

5.1.1 Plant Height

Plant height continuously increased throughout the crop growth and maximum increase was observed between 50 to 65 DAS in all treatments. Since soybean is a short day plant, warm day and low night temperature, normal solar radiation, high RH coupled with critical day lengths increases plant height. The differences in plant height between moisture stress treatments were significant. Plant height decreased drastically when moisture stress was imposed during flowering. These results are in conformity with the results of Eck *et al.* (1987) who reported that early stress reduced plant height. Similar results were also reported by Patterson *et al.* (1977). In the present investigation among the cultivars no significant differences were recorded in plant height.

5.1.2 Number of Branches

Number of branches increased continuously throughout the crop growth up to harvest. In general, the maximum number of branches recorded in control followed by waterstress at pod filling and waterstress at flowering. These results were in conformity with those reported by Momen *et al*. (1979). In the current investigation in branch number were significant and maximum number of branches were recorded by JS-93-05 followed by NRC-37, JS-335, PK-1029 and MAUS-47 .

5.1.3 Number of Leaves

The photosynthetic potential, activity and duration are governed by the behaviour of leaf initiation, expansion, number of leaves and senescence. The loss of number of leaves in the stress treatments occurred by drying of leaf lamina. Similar results were reported by Boyer (1970) and Wenkert *et al* (1978). Increase in leaf area was the result of both cell division and cell enlargement and one of the consequences of water deficits was a reduction in leaf area.

Though the leaf number is varietal character, the varieties differed in their reaction to waterstress. Maximum leaf number was recorded in JS-93-05 followed by NRC-37, JS-335, PK-1029 and MAUS-47.

5.1.4 Leaf Area Index (LAI)

LAI increased up to 65 DAS and declined thereafter in all treatments. Treatment in which waterstress was given at flowering recorded less LAI than other treatments. Similar results recorded by Sivakumar and Shah (1978) and Neyshaboun and Hatfield (1986). Significant differences with respect to LAI were observed between the cultivars. Among the cultivars JS-93-05 recorded maximum LAI followed by NRC-37, JS-335, PK-1029 and least was recorded in MAUS-47.

5.1.5 Dry Weights of Plant Parts

Determination of phytomass production is the most accurate and precise method of expressing growth.

Whenever the waterstress was imposed leaf dry weight decreased. According to Fellows *et al*. (1987) during stress period, the rate of accumulation of drymatter in leaves declined. Similar results reported by constable and Heam (1978) and Fim and Brun (1980). The decrease in leaf dry weight during the pod growth period indicated increased translocation of photosynthesis to the sinks viz., growing pods and seeds. The leaf expansion helped in the subsequent interception and efficient utilization of light resulting in better accumulation and partitioning of dry matter to leaves and shoots. On the other hand, stem dry

weight increased untill the pods have reached physiological maturity. Dry matter production of stems gradually increased up to 80 DAS. Moisture levels significantly reduced the stem drymatter in all waterstress treatments. Similar results were recorded by Silvius *et al*. (1977), Cox and Jollifff (1986) and Kirda *et al*. (1989).

Water stress when imposed during pod filling and flowering phase reduced the pod dry weight. The differences in the dry weights of plant parts and total drymatter were significant and higher values were recorded in control followed by water stress at pod filling phase and waterstress at flowering phase. Among the cultivars, JS-93-05 recorded maximum values for leaf dry weight, stem dry weight, pod dry weight and total dry weight and the least values were recorded by MAUS-47.

Increase in total dry matter even up to last stage was largely due to increase in the dry weight of pods and total dry weight was also reduced by the stress treatments.

5.2 YIELD AND YIELD COMPONENTS

Yield in crop plants depends upon many contributing factors, the yield components. In the analysis of seed yield it was appropriate to consider the effect of waterstress on yield components *viz*., number of pods per plant, number of seeds per pod and 100 seed weight. Among the main treatments no waterstress registered higher seed yield (2672 kg/ha) followed by waterstress at pod filling

(2198) and waterstress at flowering (2064). Among varieties JS-93-05 recorded more yield followed by NRC-37, JS-335, PK-1029 and MAUS-47. These results shows that soybean yields were sensitive to waterstress during flowering and pod filling phase. These results are in confirmation with the findings of Ashley and Eturudge (1978), Korte *et al.* (1983), Albrecht *et al.* (1984) and Eck *et al*. (1987).

Pod number was significantly effected by waterstress. The number of pods per plant were maximum in JS-93-05 followed by JS-335, MAUS-47, PK-1029 and NRC-37. pod number was the yield component most sensitive to soil water deficits. Similar results reported by Shaw and Laima (1976), Fehr and Caimess (1980), Vidal *et al*. (1981), Core and Jolliff (1986) and Khodhambashi *et al*. (1988).

Moisture stress reduced the seed number per pod. Control treatment recorded maximum seed number. Similar results also recorded by Eck and his associates (1987) and Simiciklas *et al*. (1989). The maximum number of seeds per pod was observed in JS-93-05 followed by JS-335, MAUS-47, NRC-37 and PK-1029.

Significantly higher test weight was recorded in control, followed by water stress at pod filling and flowering phase. Among the genotypes, JS-93-05 recorded maximum 100 seed weight followed by MAUS-47, NRC-37, PK-1029 and JS-335. Primary influence of moisture stress is to reduce the production and translocation of drymatter into developing seeds, resulting in less seed weight. Similar results were reported by Constable and Heam (1978).

5.2.1 Phenology

The observations on the crop phenology indicated that events like 50 per cent flowering delayed in waterstress imposed at flowering phase.

These differences in the phenological events could be attributed to waterstress at different stages as well as maximum and also minimum temperature regimes, solar radiation, relative humidity and sunshine hours, that prevailed during particular phenological stage.

5.3 EFFECT OF MOISTURE STRESS ON BIOCHEMICAL PARAMETERS

5.3.1 Trehalase

Trehalose is a disaccharide sugar which occur in both animal and plant tissues as stress protectant with regard to desiccation. But, it has not yet been conclusively identified as an endogenous compound in vascular plants, except the two well documented cases in "resurrection plants" *Selaginella lepidophylla* and *Myrothammus flabellifolia* (Meller *et al*., 1995). The presence of functional genes encoding the enzymes of trehalose synthesis indicates the ability of higher plants to synthesize trehalose (Blazquez *et al.*, 1998; Goddijn and Smeekens, 1998; Goddiyn and Vandum, 1999; Muller *et al*., 1999). However, trehalase, The

trehalose hydrolyzing enzyme, which is ubiquitous was reported in higher plant tissues (Muller *et al*., 2001).

In our investigation, trehalase isomorph was expressed with mobility and various intensity with waterstress, while it was totally absent in control. Hence, the increasing intensity of trehalase isomorph indicates its role in stress protection.

5.3.2 Superoxide dismutase (SOD) isozymes

Superoxide dismutase (SOD) isozymes resolved by polyacrylamide gel electrophoresis conducted in non-denaturing conditions on extract from soybean genotypes revealed that the expression / activity of SOD isozymes increased with stress. Band with higher intensity found in cultivars JS-93-05 and MAUS-47. Similar results were reported under stress conditions by Fambrins *et al*. (1999) in sunflower. SODs are covering with other enzymes of anti-oxidative scavenging system (AOSS). The enzymes of AOSS catalyze hydrogen peroxide-detoxification systems and play an essential role in protecting living cells against the indirect deliterious effects of superoxide free-radicals species (Scandalios, 1993; Bowler *et al.*, 1994) prevalent during oxidative stress. Maintenance of optimum levels of endogenous SOD isozymes in the tissues with a balanced and coordinated expression of all essential antioxidant enzymes help the cells in effectively combating stress damages.

5.3.3 Peroxidase (POX)

The isozymic pattern of POX activity in soybean genotypes revealed significant increase in peroxidase activity, although isozymes were absent in no water stress. This isoenzyme with (Rm 0.34) could be presumably a molecular marker for stress tolerance in high stress adapted leaves of JS-93-05 and MAUS-47. Increased activity of peroxidase in the stress tolerant genotypes suggests its enhanced catalytic function in scavenging free radicals that are the usual products during oxidative stress. Similar observations were also made by Strongonov (1964); Sheoran and Grag (1979) under moisture stress. As it is evident, peroxidase plays an important role in ethylene biosynthesis, IAA oxidation, proline hydroxidation, and in lignification of injured cells. Hence, higher POX activity under high stress conditions appears to be a safe mechanism that plant cells adopt to oxidise or degrade the resultant phenolic compounds that are generally accumulated during stress (Venkateshwar Rao, 1992).

5.3.4 Catalase

Changes in catalase activity were significant among the treatments and control. Increase in catalase activity was found in all stressed genotypes. The highest mean catalase activity was recorded in JS-93-05 (0.330) followed by NRC-37 (0.295), JS-335 (0.270), MAUS-47 and PK-1029 (0.205). This may be due to the protective activity of catalase (CAT) in maintaining the cell integrity by mopping up the excessive free radicals and lowering active oxygen / hydroxyl

radical formation (Kumar and Knowles, 1993; Scandalios, 1993) that triggered during stress periods.

5.3.5 Glutathione-s-transferase (GST)

In general all genotypes induced by stress recorded higher GST activity than control. This suggests that plant GSTs play a direct role in stress tolerance, a view supported by the demonstration of a dual GST and POX activity for waterstress (Cummins *et al.*, 1999; Roxas *et al.*, 2000). The highest mean glutathone-s-transferase (GST) activity was recorded in JS-93-05 (0.223) followed by NRC-37 (0.173), JS-335 (0.158), MAUS-47 and PK-1029 (0.057).

5.3.6 Proline

Accumulation of proline in waterstress plants was considered to be an important character responsible for drought tolerance. In severely stressed plants, proline is typically the major free amino acid, which accounts for up to 30 per cent of the total soluble nitrogen in leaves, due to conversion of precursor glutamic acid to proline ,which acts as an osmoticum i.e., for storage of N in a non-toxic form and / or for storage of energy during moisture stress and subsequent use upon relief from stress (Mukherjee, 1974).

In the present study also, more proline accumulation was noticed in water stress conditions compared to control. The highest mean proline content was recorded in JS-93-05 (2.950) followed by JS-335(2.545), NRC-37

(2.430),PK-1029 (2.380) and MAUS-47 (1.990). Such accumulation of proline during water stress condition was reported by Singh *et al*. (1973) and Fukutoku and Yamada (1981).

5.3.7 Glycine betaine (µ mol g-1 dry weight)

Highest glycine betaine content (5.15) was recorded by JS-93-05 followed by NRC-37 (2.995), JS-335 (2.985), MAUS-47 and PK-1029 (2.44) in stress imposed soybean genotypes. Glycine betaine is a major cytoplasmic osmoticum accumulated under stress in plants (Rhodes *et al*., 1993). Hence, increasing accumulation of glycine betaine with salt stress is an indication of salt stress tolerance. Superior performance of JS-93-05 to moisture stress may be due to accumulation of compatible solutes like glycine betaine.

5.3.8 S- adenosyle L- methionine synthetase (SAMS)

Changes in SAMS activity were significant among the treatments and control. Increase in SAMS activity was found in all stressed genotypes, while higher mean values recorded by JS-93-05 (11.164) followed by PK-1029 (10.031), NRC-37 (9.477), JS-335 (9.1385), and MAUS-47 (7.454). This shows that expression of SAMS activity in legumes occurs due to stress (Arnon Mukhopadhyay *et al*., 2001).

CHAPTER VI

SUMMARY

The experiment entitled "Studies on physiological and biochemical responses of soybean genotypes to induced waterstress" was conducted during post-rainy season (PRS) of 2005-2006 at College Farm, College of Agriculture, Rajendranagar, Hyderabad. The experiment was laid out in a split plot design replicated three times with three main treatments viz., Control, M_1 (No waterstress), M_2 (waterstress at flowering phase) and M_3 (waterstress at pod filling phase) with five soybean genotypes *viz*., C_1 (MAUS-47), C_2 (JS-335), C_3 (JS-93-05), C_4 (PK-209) and C_5 (NRC-37) as sub-plot treatments. And for biochemical experiments, above genotypes raised through sand culture. – 6 bar polyethelene glycol (PEG) was used after standardization to impose/induce stress, while control was maintained with out PEG application.

Waterstress at flowering and pod filling phase reduced plant height, number of branches, number of leaves, LAI of all soybean genotypes compared to control or no waterstress. Among genotypes the performance of JS-93-05 was high while MAUS-47 showed lower performance with respect to above parameters.

Dry weights of leaf, stem, pod and total dry weights were low in waterstress at flowering and pod filling phase as compare to no waterstress. Among genotypes JS-93-05 recorded higher values in dry weights of leaf, stem, pod and total dry weights while MAUS-47 recorded lower values.

Yield and yield components *viz.*, number of pods per plant, number of seeds per pod, 100 seed weight were more in control, while the lower values recorded in waterstress at flowering and pod filling phase. Performance of JS-93-05 is high among the genotypes in respect of seed yield and yield components.

Higher concentration of proline, glycine betaine, catalase, GST, SAMS activity, decreasing of ascorbic acid content were recorded in stress condition compare to no waterstress in all soybean genotypes.

Increasing activity of Trehalase was found in stress conditions, and isomorphs of SOD and POX representing the anti-oxidative scavenging system, AOSS were also highly expressed by JS-93-05. This demonstrates that JS-93-05 could tolerate stress by scavenging the toxic free radical species generating during stress.

LITERATURE CITED

Aeschbacher R, Muller J, Boller T and Wilmkin A 1999 Purification of the trehalase Gm TRE 1 from soybean nodules and cloning of its C-DNA; GM TREI is expressed low level in multiple tissue. Plant Physiology 119 : 489-496.

Agboma P C, Sinclair T R, Jokinen K, Peltonen Saino P and Pehu E 1997 An evaluation of the effect of exogenous glycine betain on the groth and yield of soybean timing of application, watering regime and cultivars. Field Crops Research 54 : 51-64.

Albrecht S L , Bennett J M and Boote K J 1984 Relationship of nitrogenase activity to plant waterstress in field grown soybeans. Field Crops Research 8 : 61-71.

Arnon Mukhopadhyay, Sulabha Sharma and Akilash K Tyagi 2001 Isolation and characterization of novel S-adenosyl-L-methionine synthetase cDNA from rice. Plant Biochemistry and Biotechnology 10 : 25-29.

Ashley D A and Ethridge W J 1978 Irrigation effects on vegetative and reproductive development of three soybean cultivars. Agronomy Journal 70 : 467-471.

Bates L S, Waldren R P and Teare I D 1973 Rapid determination of free proline for water stress studies. Plant and Soil 39 : 205-208.

Begg J F and Turner 1976 Crop water deficits. Advances in Agronomy 28 : 161-217.

Blazuez M A, Santos E, Flores C L, Martiner zapater J M. Salerias J and Gancedo C 1998 Isolation and molecular characterization of Arabidopsis TPSI gene, encoding Tre-6-Phosphate synthase. Plant Journal 13 : 685-690

Bowler C, Van Montagu M and Inze D 1992 Superoxide dismutage and stress tolerance. Annual Review of Plant Physiology 43 : 83-116.

Boyer J S 1970 Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. Plant physiology 46 : 236-239.

Boyer J S and Phearson H G 1975 Physiology of water deficits in cereal crops. Advances in Agronomy 27 : 1-23.

Boyer J S, Johnson R R and Saupe S G 1980 Afternoon water deficits and grain yields in old and new soybean cultivars. Agronomy Journal 72 : 981-986.

Brown E A, Caviness E G and Brown D A 1985 Response of selected soybean cultivars to soil moisture deficit. Agronomy Journal 77 : 274-278.

Caviness C E and Thomas J D 1980 Yield reduction from defoliation of irrigated and non-irrigated soybeans. Agronomy Journal 72 : 977-980.

Constable G R and Heason A B 1978 Agronomic and physiological responses of soybean and sorghum crops to water deficits I growth development and yield. Australian Journal of Plant Physiology 5 : 159-167.

Cox W J and Jolliff G D 1986 Growth and yield of sunflower and soybean under soil water deficits. Agronomy Journal 78 : 226-230.

Cox W J and Jolliff G D 1987 Crop water relations of sunflower and soybean under irrigated and dryland conditions. Crop Science 27 : 553-557.

Crowe J H, Carpenter J F and Crowe L M 1998 The Role of vitrification in anhydrobiosis. Annual Review Physiology 60 : 73-103.

Cummins I, Cole D J, Edwards R 1999 A role of glutathione transferases functioning as glutathione peroxidases in resistances to multiple herbicides in blackgram. Plant Journal 18 : 285-293.

Cushman J C and Bohnert H J 2000 Genomic approaches to plant stress tolerance. Current opinion in plant biology 3 : 117-124.

Datta S K 2002 Recent developments in transgenic for abiotic stress tolerance in rice. JIRCAS working Report 43-53.

Deniell M B and Scott H D 1991 Water use efficiency of double cropped wheat and soybean. Agronomy Journal 83 : 564-570.

Doss B D, Pearson R W and Rodgess H T 1974 Effect of Soil water stress at various growth stages on soybean yield. Agronomy Journal 66 : 297-299.

Eck H V, Mathers A C and Musick J T 1987 Plant water stress at various growth stages and growth and yield of soybean. Field Crops Research 10 : 6-16.

Elbein A D 1974 The metabolism of α , α -trehalose. Advances in carbohydrate chemistry 30 : 227-256.

Fambrini M, Rossi V D, Sebastiani L, Vernieri P and Pugliesi C 1999 Characterization of electrophoretic variants for Cu/Zn SOD in suflower. Response to oxidative stress. Helia 22 : 155-162.

Fehr W R and Caviness C E 1980. Stages of soybean development, Iowa Agricultural Experimental journal station SR – 80.

Fellows R J, Patterson R P, Raper C D and Harrics D 1987 Nodule activity and allocation of photosynthate of soybean during recovery from water stress. Plant Physiology 84 : 456-460.

Finn G A and Brun A 1980 Water stress effects on Co_2 assimilation photosynthetic partiationing stomatal resistance and nodule activity in soybean. Crop Science 20 : 431-434.

Fukutoku Y and Yamada Y 1981 sources of proline nitrogen in water stressed soybean *Glycine max* (L.) I Protein metabolism and proline accumulation. Plant and Cell physiology 22 (8) : 1397-1404.

Goddijn O J M and Pen J 1995 Plants as bioreactors trends Biotechnology 13 : 379-387.

Goddijn O J, Mand Van Dunk 1999 Trehalose metabolism in plants. Trends plant Science 4 : 315-319.

Goddijn O T, Vermwoerd T C, Broogd E, Krutwagen R W, de Graat PT, Van Dunk, Poels J. Ponstein A S, Damm B and Pen J 1997 Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiology 113 : 181-190.

Grattan S R and Grieve C M 1985 Betaine status in wheat in relation to nitrogen stress and to transient salinity stress. Plant and Soil 85 : 3-9.

Grieve C M and Grattan S R 1983 Rapid assay for determination of water soluble quarternary ammonium compounds. Plant and Soil 70 : 303-307.

Hanson A D, May A M, Grumet R, Bode J, Jamieson G C and Rhodes D 1985 Betaine synthesis in chenopods : localization in chloroplasts proceedings of National Academy of Science USA 82 : 3678-3682.

Harinasut P, Tsutsui K, Takabe T, Nomura M, Takebe T and Kishitani S 1996 Exogenous glycine betaine accumulation and increased Salt tolerance in rice seedlings. Bioscience Biotechnology 7 : 125-128.

Hart G E and Bhatia C R 1967 Acrylamide gel electrophorosis of soluble leaf proteins and enzymes from necotiana species. Canadian Journal of Genetics and Cytology 9 : 367-374.

Heatherly G, Larry W J and Hinckley T M 1977 Water relations and growth of soybeans in drying soil. Crop Science 17 : 381-386.

Hoogenboom G, Peterson C M and Huck M G 1987 shoot growth rate of soybean as affected by drought stress. Agronomy Journal 79 : 598-607.

Huck M G, Ishihara K, Peterson C M and Ushijima T 1983 Soybean adoptation to water stress at selected stages of growth. Plant physiology 73 : 422-427.

Imhoff J F and Rodriguez Valera F 1984 Betaine is the main compatible solute of halaphilu bacteria. Journal of Bacteriology 160 : 478-479.

Jackson M l 1967 Soil chemical analysis prentice Hall of India Pvt. Ltd, New Delhi Plant Physiology, 153-154.

Khodambashi M, Karmi M, Monsavi S I 1988 Effects of soil moisture stress on yield and yield components in soybean. Iranian Journal of Agricultural Science 18 (1,2) : 51-62.

Kinda C, Danso K A and Zapaata F 1989 Temporal water stress effects on nodulation nitrogen accumulation and growth of soybean. Plant and Soil 120 : 49-55.

Korte L L, Specht J E, Williams J H and Sorenson R C 1983a Irrigation of soybean genotypes during reproductive ontogeny I Agronomic responses. Crop Science 23 : 521-527.

Korte L L, Specht J E, Williams J M and Sorensen R C 1983 Irrigation of soybean genotype during reproductive ontogeny II Yield component responses. Crop Science 23 : 528-533.

Kumar G N M and Knowles N R 1993 Changes in lipid peroxidation and lipolytic and free radical scavenging enzyme activities during aging and sprouting of potato (*Solonum tuberosum*). Seed-tubers. Plant Physiolgy 102 : 115-124.

Levitt J 1972 Response of plants to environmental stress. Academic Press, New York and London 697.

Maliwal G L and Paliwal K V 1972 Salt tolerance studies on some varieties of maize at germination stage. Science and Culture 38 : 446-447.

Manchenko G P 1994 Electrophoretic analysis of Trehalase. Handbook of detection of enzymes on electrophoretic gels. CRC Press, London.

Marrs K 1996 The functions and regulation of glutathione S-transferase in plants. Annual Review of Plant Physiology Plant Molecular Biology 47 : 127-158.

Mc Gonigle B, Keeler S J, Lau S M, Koeppe M K, O'Keefe D P 2000 A genomics approach to the comprehensive analysis of the glutathione S-transferase gene family in soybean and maize. Plant Physiology 124 : 1105-1120.

Mc Kersie B D, Bowley S F, Harjanto E and Leprince O 1996 Water deficit tolerance and field performance of transgenic alfalfa over expressing superoxide dimutase. Plant physiology 111 : 1177-1181.

Ministry of Agriculture, Government of India, 2005. Data collected from Library, ANGRAU.

Mohammad F A A, Reed R H and Stewart W D P 1983 The halophilic cyanobacterium synechocystic DUN 5t2 and its osmotic responses. FEMS Microbiology letter 16 : 287-290.

Momen N N, Carlson R E, Shaw R H and Arjmed O 1979 Moisture stress effects on yield components of two soybean cultivars. Agronomy Journal 71 : 86-90.

Muchow R C, Sinclair T R, Bennett J M and Hammond L C 1986 Response of leaf growth, leaf nitrogen, stomatal conductance to water deficits during vegetative growth of field grown soybean. Crop Science 26 : 1190-1195.

Mukherjee I 1974 The effect of potassium on proline accumulation in maize during wilting. Physiologia Plantarum 31 : 288-291.

Muller J, Aeschbacher R A, Wingler A, Boller T and Wiemken 2001 Trehalose and trehalase in Arabidopsis. Plant Physiology 125 : 1086-1093.

Muller J. Boller T and Wiemke N A 1995. Trehalose and Trehalase in plants recent developments. Plant Science 112: 1-9.

Muller J. Wiemken A and Aeschbacher R A 1999. Trehalose metabolism in sugar sensing and plant development. Plant Science 147 : 37-47.

Neyshaboun M R and Hat field J L 1986 Soil water deficit effects on semi-determinate and indeterminate soybean growth and yield. Field Crops Research 5 : 73-84.

Olsen S R, Cole L V, Watanabe F S and Dean L A 1954 Estimation of available phosphorus in soils in extraction with sodium bicarbonate, USDA, circulation Plant Physiology, 939.

Palfi G, Nemeth T, Pinter R, Kadar K and Bolke W 1978 Rapid determination of drought resistance of new rye, maize and lupine varieties with the line wilting proline test. Acta Biological society 24 : 39-51.

Panse V G and Sukhatme P V 1978 Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi pp 167-174.

Patterson D T, Peet M M and Bunce J M 1977 Effect of photoperiod and size at flowering on vegetative growth and seed yield of soybean. Agronomy Journal 69 : 631-633.

Patterson R P, Raper C D and Gross H D 1979 Growth and specific nodule activity of soybean during application and recovery of a leaf moisture stress. Plant physiology 64 : 551-556.

Perl A R, Perl T , Galili S, Aviv D, Shalgi E, Malkin S and Galun E 1993 Enhanced oxidative stress defense in transgenic potato expressing tomato Cu, Zn superoxide. Theory of Applied Genetics 85 : 568-576.

Piper C S 1950 Soil and plant analysis. Hans Publishers, Bombay.

Prasad K V R 1988 Response of soybean cultivars to water stress M.Sc. (Ag) Thesis submitted to Andhra Pradesh Agricultural University, Hyderabad.

Qiu, Xiao, Hirji, Rozina, Kurylo, Eugen, Selvaraj and Gopalan 1998 A putative betaine aldehyde dehydrogenase gene in *Brassica napus.* An Abstract from proceedings of American Society of Plant Biologists (ASPB).

Radford P J 1967 Growth analysis formulae their use and abuse. Crop Science 7 : 171-175.

Rakov N M, Klyshev L K and Strongonov B P 1969 Effect of sodium chloride on the protein compostion of pea roots. Firol Rast 16 : 22-28.

Ramseur E L, Wallace S U and Quisenberry V L 1985 Growth of Braston soybean as influenced by irrigation and intra spacing. Agronomy Journal 77 : 163-168.

Ranganna S 1986 Hand book of analysis and quality control for fruits and vegetable products. Tata Mc Graw Hill Publishing Co. Ltd., New Delhi.

Rhodes D and Hanson A D 1993 Quarternary ammonium and tertiary sulphonium compounds in higher plants. Annual Review of Plant Physiology and Plant molecular Biology 44 : 357-384.

Rick C M 1982 Isozymes in plant Breeding. New concepts in whole plant genetics; innovations in plant breeding. California Agriculture p.28.

Rircosky D C and Deaton D E 1979 Soybean water extraction leaf water potential and evapotraspiration during drought. Agronomy Journal 71 : 45-50.

Robero M J S, Reinders A, Boller T, Weimken A and Virgilio D C 1997 Trehalose synthesis is important in the acquisition of thermotolerance in schizosaccharomyces pombe. Molecular Microbiology 25 : 571-581.

Rodriguer E V and Shibles R 1985 Response of determinate and indeterminate tropical soybean cultivars to water stress. Field Crops Research 10 : 269-281.

Romero C, Belles J M, Vaya J L, Serrano R and Culianez Macia F A 1997 Expression of the yeast Tri-6-phosphate synthase gene in transgenic tobacco plants pleiotropic phenotypes include drought tolerance. Planta 201. 292-297.

Roxas V P, Lodhi S A, Garrett D K, Mohan J R and Allen R D 2000 Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. Plant Cell Physiology 41 : 1229-1234.

Sammons D J, Peters D B and Hynowitz T 1987 Screening soybeans for tolerance to moisture stress. Field Crops Research 3 : 321-335.

Scandalios J G 1993 Oxygen stress and superoxide dismutase. Plant Physiology 101 7-12.

Schake S A, Wong Vega L and Allen R D 1995 Analysis tobacco plants that overexpress pea chloroplastic manganese superoxide dismutase. Plant physiology 108 : 5-62.

Sen Gupta Neinen A J L, Holaday A S, Burke J J and Allen R D 1993 Increased resistance to oxidative stress in transgenic plants that over express chloroplastic cuton superoxide dismutase proceedings of National Academy of Science 90 : 1629-1633.

Shannon L M 1968 Plant isozymes. Ibid 19 : 187-210.

Shaw R H and Loung D R 1976 Moisture stress and plant response. In Pierre W H, Kirkham D, Pesak J and Shaw R H Plant environment and efficient water use. American Society of Agronomy. Madison Wisconsin, 9 : 73-94

Sheoran T S and Garg O P 1979. Quantitative and qualitative changes in peroxidase during germination of mung bean under salt stress. Physiologia plantarium 46 : 147-150.

Silvius J E, Johnson R R and Petens D B 1977 Effect of waterstress on carbon assimilation and distribution in soybean plants at different stages of development. Crop Science 17 : 713-716.

Singh T N, Paleg L G and Aspinall D 1973 Stress metabolism I nitrogen metabolism and growth in the barley plant during water stress. Australian Journal of Biological Science 26 : 45-56.

Sivakumar M V K and Shaw R M 1978 Leaf response to water deficits in soybeans. Physiologia plantarum 42 : 134-138.

Smiciklas K D, Mullen R E, Carlson R F and Knapp A D 1989 Drought induced stress effect on soybean seed calcium and quality. Crop Science 29 : 1519-1523.

Specht J E, Williams J H and Weidenbenner 1986 Differential responses of soybean genotypes subjected to a seasonal soil water gradient. Crop Science 26 : 922-934.

Steward C R and Boggess S F 1977 The effect of wilting on the conversion of orginine, ornithine and glutamate to proline in bean leaves. Plant Science letter 8 : 147-153.

Strogonov B P 1964 Physiological basis of salt tolerance in plants Academic Science pp. 560-568.

Subbaiah B V and Asya G L 1956 A rapid procedure for estimation of available nitrogen in soils. Current science 5 : 656-659.

Takahashi Y and Nagata T 1992 Differential expression of an auxin regulated gene, par C and a novel related gene, C-7, from tobacco mesophyll protoplasts in response to external stimuli and in plant tissues. Plant Cell Physiology 33 : 779-787.

Veluthambi K, Mahadevan S and Maheswari R 1981 Trehalose toxicity in *cuscuta reflexa*. Plant Physiology 68 : 1369-1374.

Venkateswar Rao 1992 Studies on tissue culture for selection of salt and drought resistance cell lines in rice (*Oryzae sativa L.*). Ph.D. thesis submitted to Acharya N.G.Ranga Agricultural University, Hyderabad.

Vidal A, Arnado D and Amonx M 1981a Drought resistance of soybean I. Drought stress effects on growth and yield. Agronomy Journal (4) : 295-302.

Vidal A, Arnaudo D and Arnoux M 1981b Drought resistance of soybeans II varietal responses to a drought stress. Agronomy Journal (4) : 303-314.

Waldren R P, Tears I D and Ebler S W 1974 Changes in free proline concentration in sorghum and soybean plants under field conditions. Crop Science 14 : 447-450.

Walkley A and Black I A 1934 The examination of the method of determining soil organic matter and a proposed modification of the chronic acid titration method. Soil Science 37 : 29-28.

Weigel P, Weretilnyk E A and Hanson A D 1986 Betaine aldehyde oxidation by spinach chloroplasts. Plant Physiology 82 : 753-759.

Wenkert W Lemon E R and Sinclair T R 1978. Leaf elongation and turgor pressure in field grown soybean.Agronomy Journal 70 : 761-764.

Westgate M E and Grant D T 1989 Effect of water deficits on seed development in soybean. Plant physiology 91 : 975-979.

Wiemken A 1990 Trehalose in yeast stress protectant rather than reserve carbohydrate. Journal of General molecular microbiology 58 : 209-217.

---

* Original not seen.

The pattern of "Literature Cited" presented above is in accordance with the "Guidelines" for thesis presentation for Acharya N. G. Ranga Agricultural University, Hyderabad.