CHANGES IN THE PHYTOHORMONE LEVELS DURING DROUGHT STRESS IN SOYBEAN (*GLYCINE MAX* .L) MERRIL.

A Thesis submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy

In

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PLANT PHYSIOLOGY

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CERTIFICATE

This thesis by ***NOUREEN FATIMA*** is accepted in its present form by the department of biological sciences, Quaid-i-Azam University, Islamabad as fulfilling the thesis requirements for the degree of Masters of philosophy in Biology (plant physiology).

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CONTENTS

TITLE.	Page#
Acknowledgements	i
List of Abbreviations	ii
List of Tables	iii
Abstract	iv
Introduction	1
Review of literature	7
Materials and Methods	13
Results	17
Discussion	33
References	38

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LIST OF ABBRIVIATIONS

ABA	Abscisic acid
ANOVA	Analysis of variance
BHT	Butylated Hydroxytoulene
BNF	Biological Nitrogen Fixation
DAS	Days after sowing
DMRT	Duncon s multiple range test
GA	Gibberellic acid
h	Hour
HPLC	High performance Liquid Chromatography
IAA	Indole-3-acetic acid
LSD	Least significant difference
NARC	National Agricultural Research Center
Prob	Probability
REF	Rotary film evaporator
RWC	Relative water contents

LIST OF TABLES

Title	Page #
Table 1: Effect of drought and rewatering on soil moisture content (%).	23
Table 2: Effect of drought and rewatering on the RWC(%)of the leaves of soybean.	24
Table 3a: ANOVA of IAA contents(*u*g/g)of leaves of soybean.	25
Table 3b: Effect of inoculation drought and rewatering on IAAcontents (*u*g/g)of the leaves of soybean.	25
Table 4a: ANOVA of GA contents (ug/g) of leaves of soybean.	26
Table 4b: Effect of inoculation, drought and rewatering on GA contents(ug/g)of the leaves of soybean.	26
Table 5a: ANOVA of ABA contents(ug/g) of leaves of soybean	27
Table 5b: Effect of inoculation, drought and rewatering on the ABA contents (ug/g) of the leaves of soybean.	27
Table 5c: DMRT of the means of total ABA contents of the treatments at T1,T2 and T3	27
Table 6: Effect of inoculation, drought and rewatering on the GA/IAA ratio of the leaves of soybean.	28
Table 7: Effect of inoculation, drought and rewatering on the ABA/IAA ratio of the leaves of soybean.	29
Table 8: Effect of inoculation, drought and rewatering on the ABA/GA ratio of the leaves of soybean.	30
Table 9a: ANOVA of number of nodules plant of soybean.	31
Table 9b: Effect of inoculation and drought on number of nodules plant of soybean.	31
Table 10a: ANOVA of diameter of pink bacteroid tissue of the nodules of soybean.	32
Table 10b: Effect of inoculation and drought on diameter of pink bacteroid tissue of the nodules of soybean.	32

ABSTRACT

The present study was carried out to study the effect of inoculation with *Rhizobium* strain, drought and re-watering on the endogenous phytohormone levels and root nodulation of two cultivars of soybean (*Glycine max* L.) i.e.NARC 2000 (cv_1) and NARC 2001 cv_2. The seeds of cv1 and cv2 were inoculated with carrier based inoculum of *Rhizobium leguminosarum*, strain TAL 377. Treatments made for both the cultivars include inoculated and un-inoculated stressed and their respective controls. Plants were grown under natural growing conditions. After 10 days of sowing, drought stress was imposed for 5 days by with-holding water supply. The stressed treatments were re-watered after 5 days and the plants were harvested after 48 hours of rewatering. Measurements for phytohormones were made at 10 days after sowing, 15 days after sowing and 19 days after sowing.

The results have shown a marked effect of inoculation, drought and re-watering on the different parameters studied. As a result of drought all the stressed treatments have shown a decrease in the relative water contents and the Indole-3-acetic acid (IAA) and Gibberellic acid (GA) levels of the leaves, whereas the abscisic acid (ABA) contents increased. Inoculation has resulted in increased nodulation whereas; drought has shown decrease in the number of nodules. Drought has also reduced the volume of pink bacteroid tissue of the nodules. The results also showed that after 48h of re-watering, the relative water contents, the indole acetic acid (IAA) and Gibberellic acid (GA) contents of the leaves increased while the abscisic acid (ABA) contents decreased. The GA/IAA ratio increased in the stressed treatments. The ABA/IAA and ABA/GA ratio also increased in the stressed treatments but decreased after rewatering. The level of recovery was more in cv_1 as compared to cv_2. The inoculated plants were more drought tolerant and showed early recovery following rewatering.

Introduction

INTRODUCTION.

SOYBEAN.

Soybean combines in one crop both the dominant world supply of edible vegetable oil and the dominant supply of high protein feed supplement for livestock. Other factors and derivatives of the seed have substantial importance in a wide range of industrial, food pharmaceutical and agricultural products (Herman 1992).

The soybean is a papilionoid legume family Fabacea, sub family faboidea, and belongs to genus *Glycine*. The cultivated form is *Glycine max* .L. Merril. It is an annual, erect, hairy plant which is 0.6 1.5 m in height, with large leaves and small white or purple flowers. The soybean flower is a typical papilionaceous flower. The pod contains two to four seeds. The nodulated root system is intermediate between a taproot type and a diffuse type. There are over 2500 varieties of soybean in cultivation, producing high protein beans of many sizes, shapes and colors (Carlson and Lersten 1987).

Seeds of soybean contain 43.6% to 45.1% of proteins and 18.5to 24% of oil giving the best solution for the worlds protein and oil hunger. Soybean protein is moderately well balanced in the essential amino acids including isolucine, leucine, lysine, methionine, phenyl amine, threonine, tryptophene, and valine. Soybean protein is higher in lysine and tryptophene and low in cystine than that of the common cereals. The average oil contents of soybean are 20% .The distribution of saturated and un- saturated fat is 15% and 85% respectively. It contains vitamin A, C and B complex. It is low in carbohydrates thus useful for diabetic patients as well as for weight reducing purposes. It also contains 5%minerals, 3% crude fibers and 9% moisture (Ghandi *et al*., 1985).

Soybean is used in many forms e.g. foliage is used as green manure, for forage, soiling and hay. Soybean is used primarily as human food, in the form of flour, pastry, cake, bread and infant food, and as feed for livestock and fertilizers. Beans are used in the form of soysouce, soymilk, and bean curd and as a substitute of coffee. Soybean oil is also valuable for its use as margarine, shortening, mayonnaise and other edible products It is also used in the manufacture of other products like glycerin, soap and plastics.

Soybean, a non traditional crop, was introduced in early 1960s in Pakistan. It has suffered a set back and has not been able to attain a respectable position among the oil seed crops.

Its cultivation remained limited to a very small acreage and showed a declining trend whenever efforts were not made for its promotion. Because of late maturing varieties, it faces difficulties in fitting well in rice-soybean-rice and cotton-soybean-cotton relations.

The seed looses its viability quickly. Its further expansion poses serious problems of seed production, shortage and transportation. Its variety requirements also change with the change in latitude from south to north. Hence Pakistan needs different varieties for different provinces. Soybean inoculums needs special care for keeping its viability intact while transported over long distances. Non-viability of threshers and considerable yield losses due to shattering is also a limitation in its further promotion.

Soybean can obtain its fifty percent of nitrogen requirements from the air when the nitrogen fixing bacteria rhizobia are present in the soil. Biological nitrogen fixation (BNF) plays an important role in the management of this crop. Nitrogen fixation is a result of the symbiotic, beneficial to both, relationship of *Rhizobia* and plant is unique to legumes. When infected by *Rhizobia* the plant forms special type of structure called nodules that harbor or encapsulate *Rhizobium* bacteria, inside where they fix nitrogen utilized by the host plant and in return they get food in the form of sugars from the host plant.

The special type of *Rhizobium* responsible for symbiosis with soybean is known as *Bradyrhizobium*. Most soils where this legume is grown show a very high population of *Bradyrhizobium* (Andrade and Hungria, 2001).

BNF represents the major source of N_2 input in the agricultural soils including those in arid regions. The major N_2 fixing systems are the symbiotic systems, which can play a significant role in improving the fertility and productivity of low nitrogen soils.

The *rhizobium*-legume symbioses have received most attention and have been examined extensively. The behavior of some nitrogen fixing systems has been studied under severe environmental conditions such as drought stress. Major stress factors suppress the growth and symbiotic characteristic of most rhizobia; however, several strains, distributed among various species of rhizobia, are tolerant to stress effects. Some strains of *Rhizobia* form effective nitrogen fixing symbiosis with their host legume under stress. The *Rhizobium*-legume symbiosis is suggested to be the ideal solution to the improvement of soil fertility and the rehabilitation of arid lands and is an important direction of further research (Zahran 1999).

DROUGHT.

On a global basis drought, assumed to be soil and/ or atmospheric water deficit, in conjunction with coincident high temperature and radiations; poses the most important environmental constraint to plant survival and to crop productivity. Agriculture is a major user of water resources in many regions of the world. With increasing aridity and a growing population, water will become an even scarce commodity in the near future. Even though in viable agriculture severe water deficit should be a rare event, a better understanding of the effect of drought on plants is vital for improved management practices and breeding efforts in agriculture and for predicting the fate of natural vegetation (Chaves *et al.*, 2003).

Water is required for all aspects of plant growth and development. The control of rate of water loss and water uptake is of vital importance in this regard. When there is any reduction in the availability of water below the amount that is required for maximum growth, then that condition is termed as drought.

It can be defined as, a period of abnormally dry weather which persists for long enough to produce serious hydrological imbalances e.g. crop damage; water supply shortage etc.The severity of drought depends upon the degree of moisture supply deficiency, its duration and the size of affected area.

Drought is a meteorological condition that is tolerated by all plants that survive it and avoided by none (Taiz and Zeiger 1998) .There are three main categories of plants i-e. desiccation postponers that are the water savers, drought escapers, which complete their life cycle during wet season before the onset of drought and the drought tolerant plants which posses the ability to function while dehydrated.

Drought can also be defined as an absence of rain fall for a period of time long enough to cause depletion of soil moisture and damage to plants. The length of time that is necessary to cause injury depends on the kind of plant, the water holding capacity of the soil in which it is growing and the atmospheric conditions that affects the rate of evaporation and transpiration.

Drought maybe permanent as in desert areas, seasonal as in areas of well defined wet and dry seasons or un predicted as in many humid climates. Most of the world's agriculture is subjected to drought problems. Agricultural drought refers to a situation when the amount of moisture in the soil can no longer meet the needs of a particular crop. (Bahrun *et al.*, 2002).

Although plant water stress always accompanies drought it may occur in the absence of drought, because of excessive transpiration or because water absorption is inhibited by cold soil or excess of salt in the soil solution, deficient aeration or injury to root system. Over the last two decades there has been much discussion on the different factors regulating growth and physiological processes in plants subjected to drought. Variations in soil moisture can induce various physical and physiological changes in the plants and thereby enable them to sense water status and adapt to decreasing soil moisture contents. When exposed to water stress plants reduce the rate of water flow by decreasing hydraulic conductance of the organs in transpiration pathway and often as a response at the stomatal level from root to shoot chemical signals (Lovisolo *et al.*, 2002).

A chain of signal events is suggested eventually leading to stomatal closure and leaf surface reduction through interactive effects of reduce nitrogen supply and plant growth regulators under drought stress (Bahrun *et al.*, 2002).

The problem of testing for drought resistance is of great practical importance but is hampered in field tests by great fluctuations that occur from year to year and from location to location in drought incidence even in semi-arid regions. The development of a satisfactory laboratory test would be of great aid in selecting plants for drought tolerance (Salim *et al.*, 1965).

Research into the plant response to water stress is becoming increasingly important, as most climate change scenarios suggest an increase in aridity in many areas of the globe.

PHYTOHORMONES.

A plant growth hormone (phytohormone) is an organic substance synthesized in one part of a plant and translocated to another part where, in very low concentrations it causes a physiological response (Salisbury, 1994).

Plant hormones or regulators are essential for various steps in plant growth and development. They are divided into plant growth promoters (auxins, cytokinins, and gibberellins) and plant growth inhibitor (ABA and ethylene).

HORMONES COORDINATING PLANT RESPONSE TO STRESS.

The role of hormones in regulating the response to stress was initially proposed for the situation in which one organ e.g. root was exposed to stress and the hormone served as a messenger that transfers the signal to a remote or responding organ e.g. shoot (Itai *et al.* 1968). This specific role of root to shoot communication by phytohormones acquired considerable support in the last years (Tardien and Davies 1993).

Studies conducted in the last 30 years provided evidence for the wide spread involvement of phytohormones in stress responses in addition to their role as messengers. Broadly, these studies can be divided into two groups i.e. physiological and genetic or molecular.

The physiological approach is to correlate the endogenous levels of phytohormones with the physiological responses of the plant to stress. Endogenous hormonal changes were recorded in all phases of the response to stress (Itai, 1999).

Hormones are not only found as stress metabolites, but they are also part of signaling system particularly under stress. Plant hormones play an integral role in controlling the growth and development of plants, e.g. ABA a phytohormone found in all higher plants, activates inter-cellular responses to environmental stress (Davis *et al.*, 1987).

In previous attempts to bring stress responses under a common umbrella, the hormonal shifts were always considered as an important response to stress but hormones were not assigned the central role (Morghan 1990, Lerner *et al.*, 1994, and Bohnet *et al.*, 1995).

However a general hypothesis for the central role of phytohormones in the regulation of plant response to stress is now proposed, based on the following facts:

1. plants respond to various stresses in a similar manner
2. this response is the result of many coordinated processes
3. all phytohormones are involved in this coordination.

Hence it is suggested that the modified hormonal balance is the key determinant of the responses. Hormonal regulation is involved in the control of water potential and membrane permeability and thus in the control of plant water deficits.

Aims and objectives.

The present work was conducted mainly to study:

1. Changes in the phytohormone levels during drought stress and after rewatering
2. Effect of inoculation on the phytohormone levels under drought stress.
3. Drought tolerance of the plants in terms of endogenous phytohormone changes during and after drought stress.

Review of literature

REVIEW OF LITERATURE.

DROUGHT.

Drought is a condition when there is a reduction in the availability of water below the amount that is required for maximum growth. It can be defined as, a period of abnormally dry weather which persists for long enough to produce serious hydrological imbalances e.g. crop damage. (Taiz and Zeiger, 1998).

On a global basis, drought (soil and/or atmospheric water deficits), poses the most important environmental constrains to plant survival and to crop productivity (Chaves *et al.*, 2003).

According to the findings of Hsiao (1973) water deficit is known to affect many physiological and developmental process including cell division, cell expansion and primordial development.

Wang *et al.*, (1995) concluded that water stress affects al the growth parameters. It results in an increase in the growth period and decreased plant dry weight, crop growth rate leaf area index, net assimilation rate and pod growth rate.

EFFECT OF DROUGHT ON RELATIVE WATER CONTENTS (RWC %) OF LEAVES.

RWC is an important physiological trait which confers some sort of drought tolerance to the plants. (Rehman *el al.*,2000)

It has been proposed by several workers that water retention of excised leaves or whole plant and relative water contents of leaves can be used as a test for drought resistance. As a result of water stress RWC of the leaves decreases, however more resistant varieties have higher RWC% as compared to control. (Towenly-Smith and Hurd 1979).

In a study conducted by Thomas (1991) it was found that relative water contents declined significantly under water stress in *Lolium perene*. Same were the findings of Aspirall and Hussain (1970) that Water stressed oat plants had a relative water content which was 23% less than control.

Javanovic and Quarrie (1990) found that during a cycle of drought stress, leave growth was reduced and leave water potential declined steadily but largely recovered in young leaves after rehydration in maize.

Zou *et al*., (1994) reported that soil moisture contents decreased in soybean subjected to water stress with subsequent decrease in saturated osmotic potential and RWC% of the stressed leaves.

EFFECT OF DROUGHT ON NODULATION.

Root nodules are the site of beneficial symbiotic association between legume plants and soil bacteria of the genera *Rhizobium* and *Bradyrhizobium*.

Water stress induced significant reduction in nitrogen fixation and nodulation in soybean (Kirda *et al*., 1989).

Masyhudi and Patterson (1991) reported that total nitrogen accumulation, nitrogen fixation and number and size of nodules were reduced due to water stress.

Water stress decreased nodulation but on watering it was recovered completely in plants stressed at vegetative stage but not in plants stressed at reproductive stage in *Phaseolus vulgaris* (Pena-Cabriales and Castellanos, 1993).

Salama and Sinclair (1994) reported that water stress decreased nodulation, nitrogen fixation and plant growth in soybean.

In a field study related to amount of rainfall and soil moisture contents during the growing season, it was found by Buttreu *et al*.,(1998), that soil compaction and soil moisture deficit conditions resulting due to drought conditions, have adverse effects on nodulation and plant growth.

Pink bacteroid tissue is the active site of nitrogen fixation. As a result of drought diameter of pink bacteroid tissue is also reduced (Subba-Rao1990).

EFFECT OF DROUGHT ON PHYTOHORMONES.

A plant growth hormone (phytohormone) is an organic substance synthesized in one part of a plant and translocated to another part where, in very low concentrations it causes a physiological response. Plant hormones are essential for various steps in plant growth and development (Salisbury, 1994).

Plant hormones are divided into plant growth promoter (auxins, gibberellins and cytokinins) and plant growth inhibitors (ABA, ethylene). In ideal conditions plant growth is regulated by a delicate balance between various hormones(Salisbury and Ross,1992). Plants respond to stress like drought by modifying their hormones by an interaction between hormones and various environmental factors (Bradford and Trawavas 1994).

Phytohormones are widely involved in stress responses in addition to their role as messenger. Endogenous phytohormone changes were recorded in all phases of the response to stress condition (Chaves *et al* 2003).

As a result of drought stress, endogenous phytohormone level changes. It results in an increase in ABA and ethylene levels and decrease in levels of cytokinins, gibberellins and auxins (Morgan, 1990).

It has been estimated by several workers over the last thirty years that alterations in the endogenous phytohormone level is a result of various environmental stresses including drought. Under conditions of progressive drought, leaf ABA contents increased, as tested in maize. (Itai, 1999).

Changes in water relations and phytohormone content in diploid and tertraploid plum trees (*Prunus salicina* Lind L.) was studied by Pustovoitova *et al.*, (1996) and it was suggested that ABA was directly involved in the induction of protective responses under water stress conditions.

Water potential in the leaves of the plants is composed of two main components turgor and osmotic potential; both are directly affected by ABA, cytokinins and auxins (Westgate *et al.*, 1996).

Osmotic potential is of great importance under water stress and is regulated by auxins and ABA (Ishitani *et al.,* 1995).

Abscisic acid (ABA) concentration can affect plant responses to drought and has been suggested as a selection criterion to improve drought tolerance (Landi *et al.*, 2001).

Quarrie (1984) suggested that all crop plants accumulate ABA in response to water stress. For a given soil moisture content different cultivars produce different amounts of ABA. Abscisic acid (ABA) concentration in wheat (*Triticum aestivum* L.) plants increased during heat and drought stress and is associated with stomatal closure, low photosynthetic rate and senescence; this research was conducted by Lu *et al*., (1990).

Plants subjected to drought commonly produce higher levels of abscisic acid in the guard cells of the leaves, in order to close stomata and reduce the loss of water by transpiration (Harborne, 1997). ABA accumulation and synthesis increases in the leaves of plants suffering from soil moisture deficit conditions (Taiz and Zeiger, 1998).

Leung and Giraudate (1998) reported that endogenous ABA concentration increases in response to water stress during vegetative growth.

Drought responses in leaves of lupine (*Lupinus angustifolia*) was investigated by Jensen *et al*.,(1998).During soil drying,mid-day leaf ABA contents increased relative to that in fully irrigated plants before any appreciable decrease occurred in water potential.

Postovojtova *et al*., (2000) studied that growth inhibition of cucumber leaves (*Cucumis sativus* L.) is preceded by a significant decrease in IAA and cytokinins under drought. Drought adaptation generally occurs while growth is inhibited. IAA and cytokinins can significantly rise during adaptation period. ABA levels rose in the beginning and in the final step of adaptation process. All three hormones are involved in drought adaptation.

Plant dehydration caused an increase in endogenous ABA.Under optimal water supply exogenous ABA inhibited protein synthesis and under drought stress it not only restored the suppressed protein synthesis but also stimulated protein formation in comparison with the control variants (Victorova *et al*., 2000).

The effects of drought on European beech (*Fagus sylvatical*) were assessed in a pot experiment under controlled conditions. Plants, which differed in annual precipitation, were exposed to a 3 wk drought period in a glasshouse after the first of shoot growth had been completed. Drought reduced water content of 97% of the leaves as compared to control. Leaf concentrations of abscisic acid (ABA) increased in the drought treated plants compared with the controls. (Peuke *et al.*,2002).

ROLE OF *RHIZOBIUM* INOCULATION ON NODULATION AND PHYTOHORMONES.

Rhizobium can improve plant growth by fixing atmospheric nitrogen that is used by plant and by production of plant growth regulators as auxins and GA which directly effect plant growth. (Cooper,*et al.,* 1988).

Okon *et al.,* (1996) reported that apart from enhancing growth parameters including nodulation, the *Rhizobium* has the ability to produce phytohormones. Two phytohormones i.e. IAA and GA have been detected in cell free culture media. Some bacterial strains produce both IAA and GA, while other produces only IAA.

To assess the competitiveness of Bradyrhizobium in infecting soybean cultivars, in a study, seeds were inoculated with different strains of Bradyrhizobium. Results show that nodule number dry weight, total nitrogen and seed yield were significantly increased because of effective and competitive strains (Sharma and Suneja, 1994).

Elshek and Osman (1995) concluded that number and dry weight of nodules, yield and nitrogen fixation significantly increased by inoculation of Rhizobium legumnosarum in *Vicia faba*

The inoculation with effective strains of Rhizobium increased growth and yield and number of nodules in *Pisium sativum* (Hoeflic and Rupple, 1994).

Di-bonito *et al.,* (1989) reported that Rhizobium inoculation increased number of nodules and yield in inoculated plants of chickpea (*Cicer arutinum* L.)

Yanni *et al.,* (1991) reported that inoculation greatly enhanced nodulation in terms of nodule number, size and weight.

A direct growth promoting effect of *Rhizobium* inoculation is production of IAA and cytokinins non-leguminous plants also (Noel *et al.*, 1995).

Datta and Basu (2000) reported about a *Rhizobium* spp isolated from root nodules of a leguminous pulse *Cajanus cajan* that was found to produce high amounts of IAA as compared to control.

Nehru and Rangaish (1998) found that *Rhizobium* strains are effective in initiating nodulation in forty-nine genotypes of soybean (*Glycine max*).

Chebotra *et al.*, (2001) studied the effect of inoculation of different strains of *Bradynhizobium japonicum* on soybean it was suggested that the inoculation resulted in an enhanced effect on nodulation which could be due to production of growth promoting substances that stimulate the growth of *B.japonicum*.

Inoculation primarily stimulated the growth of soybean plant. This includes increased nodulation, probably as a result of the stimulation of phytohormone production. (Shabaev *et al.*, 1995).

The study of rhizobial root of the monocotyledonous *Roystenea regia* revealed that the rhizobium sp. Isolated from the root nodules produce high amount of indole acetic acid (IAA), hormone production is shown to be the beneficial aspect of this symbioses (Basu and Ghosh, 2001).

EFFECT OF DROUGHT ON RHIZOBIUM INOCULATION.

Moisture or water stress results in the decreased levels of soil moisture contents which limits not only the survival of rhizobia but also their symbiotic association with the legumes (Vekateswark and Rao, 1987).

Taneja et al., (1980) reported that water stress (-2 to-4bars), apart from affecting other growth parameters of the host plant also resulted in decreased growth of rhizobia.

Similar reports were provided by Kulkarni et al.,(1988) that water stress affects Rhizobium legume symbiosis and results in decreased growth of Rhizobium sp.

Materials and Methods

MATERIALS AND METHODS.

Plant material and growing conditions.

Seeds of soybean *Glycine max* L. cv. NARC 2000 and NARC 2001 were used for the experiment. The seeds were surface sterilized with methanol for one minute followed by shaking in 10% clorax for1-2 minutes, then successively washed with sterilized water. Seeds were then inoculated with a strain of *Rhizobium japonicum* i.e. TAL 377. The inoculated seeds were then sown in earthen pots 24X30 cm^3 filled with soil and sand in 1:1.Four plants per pot were allowed to grow. Plants were grown in natural growing conditions. Field capacity of the soil was calculated.

Treatments made.

For NARC 2000

1. Uninoculated control	disserted as	V_1C
2. Inoculated control	disserted as	V_1CI
3. Uninoculated stressed	disserted as	V_1D
4. Inoculated stressed	disserted as	V_1DI

For NARC 2001

1. Uninoculated control	disserted as	V_2C
2. Inoculated control	disserted as	V_2CI
3. Uninoculated stressed	disserted as	V_2D
4. Inoculated stressed	disserted as	V_2DI

Induction of drought.

Drought was imposed at 10 DAS by withholding water supply of the respective pots i.e. drought stressed treatments.

Parameters studied.

1. Soil moisture contents.
2. Relative water contents of leaves.
3. Number of nodules per plant.
4. Volume of pink bacteroid tissue of nodules.
5. Phytohormones level of the leaves at 10DAS, after a drought stress of 5 days (15 DAS) and after 48h of re-watering stressed plants (19DAS).

For relative water contents and phytohormones, all the measurements were made at the vegetative stage, before flowering.

Soil moisture contents.

Soil (20 g) was taken from uniform depth i.e. 6 inches from the surface of pots. Dry weight was determined after drying the soil in the oven for 72 h at 70C. After recording the dry weight, the percentage of soil moisture contents was calculated.

Relative water contents of leaves.

Relative water contents of leaves were determined following the method given by Gupta (1995). The leaves of plants were harvested from the pots and weighed. The leaves were then soaked in distilled water in beakers for 24h, and then fully turgid leaves were again weighed. Thereafter leaves were dried in oven for 72h at70C, until constant weight of leaves was obtained.

Relative water contents of leaves was calculated by applying following formula

$$RWC\% = \frac{FW - DW \ X \ 100}{FTW - DW}$$

Where:

RWC= relative water contents

DW= dry weight

FW= Fresh weight

FTW= fully turgid weight

RWC was calculated in control and drought stressed plants before and after the induction of drought and after re-watering

Measurement of diameter of pink bacteroid tissue of nodules.

The nodules were taken from the roots of the plants at the time of final harvesting i.e. after 48 hrs of re-watering. Thin sections of nodules were made and the volume of pink bacteroid tissue was measured under light microscope (Beck, 1938).

Each reading of ocular meter was multiplied with 2.5 to get exact value. The ocular meter (O.M) was calibrated against a stage micrometer (S.M) at X 40 magnification and multiplication factor (2.5) was derived as follow:

One div of S.M is equivalent to 10um. 20 divisions of O.M coincide with 5 divisions of S.M. Therefore 20 div of O.M were equivalent to 50um and one div of O.M was equal to 2.5.

1div of S.M= 10um

20 div of O.M= 5 div of S.M

20 div of O.M = 50um

1 div of O.M = 2.5um

Extraction and Identification of phytohormones i.e. IAA, GA and ABA.

Leaves were ground in 80 % methanol with butylated hydroxy toluene (BHT) (10*u*g/l)used as anti-oxidant and extracted for 72 h with subsequent changes in solvent after every 24h. The extracted sample was centrifuged and the supernatant was taken and reduced to aqueous phase using rotary film evaporator (RFE). The pH of aqueous phase was adjusted to 8 before it was partitioned three times with one- third volume of ethyl acetate. The pH of aqueous phase was readjusted to 2.5-3.0 and it was partitioned three times with one- third volume of ethyl acetate. The organic ethyl acetate phase was dried down completely using RFE. The sample was redissolved in 1ml of methanol (100%) and analyzed for the presence of phytohormones (IAA, GA and ABA) using HPLC.

The data was subjected to statistical analysis. ANOVA and DMRT was applied.

Results

RESULTS.

EFFECT OF DROUGHT AND REWATERING ON THE SOIL MOISTURE CONTENT (%).

Table 1. showed that at the time of sowing, the moisture contents of the soil in the pots of all the treatments of both the varieties i.e. cv $_1$&cv $_2$ was same.

Measurements taken at 10DAS, when all the treatments were growing under similar water supply conditions. At this time also, no significant difference was observed among the values of all the treatments. However, as compared to the time of sowing the values for soil moisture contents were lower.

After 10 days, the water supply of the treatments V_1D, V_1DI, V_2D&V_2DI was withheld for 5 days. So the readings at 15 DAS showed the effect of drought on these treatments. Hence the data at this time has shown a reduction in the soil moisture contents as compared to control treatments i.e. V_1C, V_1CI, V_2C, &V_2CI.

After a drought period of 5 days, the stressed treatments were re-watered, and after 48h of re-watering data were taken at 19DAS. The stressed treatment V_1D has shown a little increase in the value as compared to the measurements at 15DAS, but the value of its respective control i.e. V_1C is still higher at T3(19DAS).

Other stressed treatments i.e. V_1DI, V_2D& V_2DI have shown no significant recovery. The values of these treatments are very less as compared to their respective control treatments V_1CI, V_2C, &V_2CI.

EFFECT OF DROUGHT AND RE-WATERING ON THE RELATIVE WATER CONTENT (RWC%) OF THE LEAVES.

Table 2 showed that at 10 DAS, when plants were growing under similar moisture conditions, the RWC of all the treatments of cv$_1$ were higher than the RWC of cv$_2$.

For measurements made at 15DAS, showed the effect of drought on the RWC of the stressed treatmentsV_1D, V_1DI, V_2D&V_2DI. All the stressed treatments have shown a marked reduction in the values as compared to control treatments V_1C, V_1CI, V_2C& V_2CI respectively.

Measurements taken at taken at 19DAS have shown that after 48h if re-watering, the RWC of the stressed treatments have increased but the values are still less than control treatments.

The comparison between the stressed treatments of both the varieties cv_1& cv_2 have shown that the level of recovery was also higher in the stressed treatments of cv_1.

EFFECT OF INOCULATION, DROUGHT AND RE-WATERING ON IAA CONTENTS OF LEAVES OF SOYBEAN.

Data presented in Table 3b. showed a comparison of IAA contents of the leaves, at three different times. All the measurements were made at the vegetative stage, before flowering.

Measurements taken 10 DAS have shown that in the treatments of cv_1, the IAA contents were insignificantly high in the inoculated treatments i.e. V_1CI&V_1DI as compared to the un-inoculated treatments i.e. V_1C&V_1D.

Similarly in cv_2, the IAA contents were insignificantly high in the inoculated treatments V_2CI&V_2DI as compared to the un-inoculated treatments V_1C&V_1D.

The IAA contents of all the treatments of cv_2 were significantly higher than all the treatments of cv_1.The inoculated treatments of cv_2 i.e. V_2CI&V_2DI, have higher IAA contents as compared to the inoculated treatments of cv_1 i.e. V_1CI&V_1DI. Similarly the un-inoculated treatments of cv_2 i.e. V_2C&V_2D have higher IAA contents as compared to the un-inoculated treatments of cv_1 i.e. V_1C&V_1D.

After 5 days of drought i.e. 15DAS the Table 3b. showed that drought stress has significantly reduced IAA contents of the stressed treatments as compared to the control treatments i.e. V_1C, V_1CI, and V_2C&V_2CI respectively. The decrease in values of stressed treatments of cv_2 i.e. V_2D&V_2DI respective to their control treatments V_2C&V2CI is more as compared to the decrease in the values of stressed treatments of cv_1.

At 19DAS (T3), data showed the effect of re-watering on the stressed treatments. The stressed treatments (inoculated and un-inoculated) of both the varieties, have shown an insignificant increase in the value at 19DAS as compared to drought condition i.e.15DAS, but the values were still less than the control treatments. The effect of re-watering on the recovery of IAA contents in the stressed treatments of both the varieties was almost same.

EFFECT OF INOCULATION, DROUGHT AND RE-WATERING ON THE GA CONTENTS OF THE LEAVES OF SOYBEAN.

Data presented in the table 4b. showed a comparison of GA contents of the leaves of the treatments. The GA contents of both the varieties were higher than the IAA contents. The values were also higher in the treatments of cv $_2$ as compared to cv $_1$. the inoculated treatments of both cv $_1$&cv $_2$. i.e. V_1CI, V_1DI and V_2CI, V_2DI have shown significantly higher values than the un-inoculated treatments V_1C, V_1D and V_2C, V_2D respectively.

Measurements taken at 15DAS showed an effect of 5 days drought on the following treatments i.e. V_1D, V_1DI, V_2D&V_2DI. All the stressed treatments have shown significant decrease in the GA contents as compared to T1 (10DAS) as well as the values of control treatments. The decrease is more in the stressed treatments of cv $_2$ i.e. V_2D&V_2DI, as compared to the decrease in the stressed treatments of cv $_1$ i.e. V_1D&V_1DI. Moreover at this time, in both the treatments decrease in GA contents of the un-inoculated stressed treatments (with respect to their control) is more than the decrease in inoculated treatments.

T3 (19DAS) showed the values of GA contents after 48h of re-watering of the stressed treatments. All the stressed treatments have shown an insignificant increase in the values as compared to the drought condition (T2). The values of stressed treatments at19DAS is still less than their respective control treatments. The level of recovery is more in case of inoculated stressed treatments of both the varieties i.e. V_1DI&V_2DI, as compared to the un-inoculated stressed treatments i.e. V1D&V_2D.

The comparison between the two varieties at 19DAS has shown that the recovery of GA contents of the stressed varieties of cv $_1$ is more as compared to the stressed varieties of cv $_2$.

EFFECT OF INOCULATION, DROUGHT AND RE-WATERING ON THE ABA CONTENTS OF THE LEAVES OF SOYBEAN.

Table 5b. showed a comparison between the ABA contents of the treatments.

For measurements made at10DAS, there is no significant difference among the ABA contents of all the treatments.

After 5days of drought, imposed on V_1D, V_1DI, V_2D&V_2DI, the measurements made at 15DAS the data showed an effect of drought on the stressed treatments. All the stressed treatments have shown an increase in the ABA contents as compared to T1 (10DAS), as well as an increase over control treatments (V_1C, V_1CI, V_2C&V_2CI) at T2. The increase in ABA contents of the inoculated stressed treatments of both the varieties cv $_1$&cv $_2$ i.e. V_1DI&V_2DI, was more as compared to the un-inoculated stressed treatments i.e. V_1D&V_2D, with respect to the values of their control treatments.

The increase in the ABA contents of the stressed treatments of cv $_1$ (both inoculated and un-inoculated) is more as compared to the stressed treatments of cv $_2$ (both inoculated and un-inoculated).

Data at 19DAS is showing the effect of re-watering on the ABA contents of the stressed treatments. All the stressed treatments have shown decrease in the values, as compared to 15DAS. The decrease in the values of ABA or the level of recovery is more in the stressed treatments (both inoculated and un-inoculated) of cv $_1$, as compared to cv $_2$.

Table 5c. Showed DMRT of means of total ABA contents of the treatments at three times i.e. 10DAS, 15DAS &19DAS. The Table6. Showed that in the treatments of cv $_1$ the total ABA contents of all the three times, T1, T2and T3, were significantly higher in the stressed treatments i.e. V_1D&V_1DI, as compared to control i.e. V_1C&V_1CI respectively. Similar pattern was exhibited in cv $_2$; the values were significantly higher in stressed treatments V_2D&V_2DI, as compared to the control V_2C &V_2CI respectively.

GA/IAA RATIO.

Data presented in Table 6. showed a comparison between the GA/IAA ratio of the treatments at 10DAS, at15DAS, and at 19DAS.

Table 7.showed that at 10DAS, the values of GA/IAA is almost same in all the treatments. At 15DASafter drought stress of 5 days the stressed treatments of cv $_1$ i.e. V_1D&V_1DI showed an increase in the value as compared to control treatments V_1C&V_1CI respectively. The increase was more in case of un-inoculated stressed treatment (V_1D). Similarly in case of treatments of cv $_2$ the stressed treatments V_2D&V_2DI have shown an increase in the values over control treatments V_2C&V_2CI, but the increase is more in inoculated stressed treatment i.e. V_2DI.

At 19DAS i.e. after 48h of re-watering the values of IAA/GA of stressed treatments of cv $_1$ both inoculated and un-inoculate, are still higher than the control. Whereas, in case of cv $_2$ the value of un-inoculated stressed treatment V_2D is less than the control i.e. V_2C and the value of inoculated stressed treatment V_2DI was equal to that of control i.e. V_2CI.

ABA/IAA

The ABA/IAA ratio presented in the Table7. has shown that at 15DAS i.e. after a drought stress of 5 days, the stressed treatments of both the varieties have shown an increase in the value as compared to control treatments respectively. The increase is more in the un-inoculated stressed treatments of both the varieties i.e. V_1D&V_2D as compared to inoculated stressed treatments I.e. V_1DI&V2DI.

At 19DAS, i.e. after re- watering, there is a decrease in the ABA/IAA of the treatments as compared to the measurements made at 10 DAS. The decrease was more in cv $_1$ as compared to the stressed treatments of cv $_2$.

ABA/GA

The ABA/IAA ratio presented in the Table 8. has shown that at 10DAS the values of all the treatments were almost similar. At 15DAS i.e.under drought stress, the stressed treatments of both cv 1&cv 2 have shown an increase in the values as compared to their respective control.

After re-watering i.e. 19DAS the stressed treatments have shown a decrease in the values as compared to that of stressed condition; however the value were still higher than the control. The decrease in the ABA/GA of stressed treatments of cv 1i.e. was higher than that of cv 2 i.e. V2D&V_2DI.

EFFECT OF INOCULATION AND DROUGHT ON THE NUMBER OF NODULES PER PLANT.

Table 9b. has presented the mean value of number of nodules per plant of all the treatments. The Table 9b. indicated a marked effect of inoculation as well as drought on the number of nodules.

In case of un-inoculated treatments of both cv 1 and cv 2, the numbers of nodules were significantly higher in the control treatment (V_1C&V_2C) as compared to the stressed treatments (V_1D&V2D). Similar was the case in inoculated treatments of cv 1&cv 2 that the numbers of nodules were significantly high in the control treatments (V_1CI&V_2DI) as compared to the stressed treatments (V_1DI&V2DI).

In both control and stressed conditions, the numbers of nodules were higher in the inoculated treatments of both the varieties i.e. V1CI, V_1DI, V2CI& V2DI, as compared to the un-inoculated treatments i.e. V_1C, V_1D, V_2C&V_2D respectively.

EFFECT OF INOCULATION AND DROUGHT ON THE DIAMETER OF PINK BACTEROID TISSUE OF THE NODULES.

Data presented in Table 10b. has shown that the diameter of pink bacteroid tissue of the nodules of the un-inoculated treatments of both the varieties i.e.V_1D&V_2D was significantly less than all other treatments.

The value of un-inoculated stressed treatments of both cv 1and cv 2 i.e. V_1D&V_2D, is significantly less than the control i.e. V_1C&V_2C

Table 1. Effect of drought and rewatering on soil moisture content (%)

Treatments	Sowing	T 1 (10 DAS)	T 2 (15 DAS)	T3(19DAS)
V 1 C	23.44	18	13.05	12
V 1 CI	28.01	18.21	15.91	12
V 1 D	27.34	18.01	8.35	9.11
V 1 DI	27.38	19.89	8.1	7.38
V 2 C	22.87	19.01	10.59	11.2
V 2 CI	26.87	19.91	12.25	11.52
V 2 D	25.21	18.95	7.99	6.25
V 2 DI	20.51	19.77	7.92	6.1

T 1 =Measurements made at 10 DAS
T 1 =Measurements made at 15DAS (after 5 days of drought)
T 3 = Measurements made at 19DAS (after 48h of rewatering)

Table 2. Effect of drought and rewatering on the RWC(%) of the leaves of soybean.

Treatments	T 1	T 2	T 3
V 1 C	96	97	97
V 1 CI	95	96	92
V 1 D	95	62.2	58.9
V 1 DI	96	55.2	59.8
V 2 C	91.3	89	90.5
V 2 CI	88	82.4	86
V 2 D	83	59.3	67
V 2 DI	89	62.06	65

T 1 =Measurements made at 10 DAS
T 1 =Measurements made at 15DAS (after 5 days of drought)
T 3 = Measurements made at 19DAS (after 48h of rewatering)

Table 3a. ANOVA of IAA contents (ug/g) of leaves of soybean

K- value	Source	Degree of freedom	Sum of square	Mean square	F-value	Prob.
2	Factor A	2	23610.528	11805.264	8.2539	0.008
4	Factor B	7	226908.431	32415.49	22.664	0
6	AB	14	120911.028	8636.502	6.0384	0
-7	Error	48	68652.667	1430.264		
	Total	71	440082.653			

Table 3b. DMRT Effect of inoculation, drought and re-watering on IAA contents (ug/g) of the leaves of soybean.

treatments	T 1	T 2	T 3
V 1 C	127DEFGH	144 DEFGHI	155. DEFGHI
V 1 CI	185 CDEFGH	224 BCD	283. AB
V 1 D	117 EFGH	70. I	81. I
V 1 DI	166 DEFGH	120 EFGHI	132 FGHI
V 2 C	210 BCDE	202 BCDEF	138.DEFGHI
V 2 CI	2730 BC	366 A	186. CDEFG
V 2 D	198 BCDEF	89 HI	97.GHI
V 2 DI	280 AB	145. DEFGHI	150.3 DEFGHI

L.S.D. value(0.01)=82.82

T 1 =Measurements made at 10 DAS
T 2 =Measurements made at 15 DAS (after 5 days of drought stress)
T 3= Measurements made at 19 DAS (after 48h of rewatering)

All such means sharing acommon English letter differ insignificantly from each other, otherwise they differ significantly at P<0.01.

Table 4a. A NOVA of GA contents (ug/g) ofleaves of soybean

K-value	Source	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
2	Factor A	2	708461.083	354230.542	58.7628	0
4	Factor B	7	3988673.556	569810.508	94.5251	0
6	AB	14	187076.694	13362.621	2.2167	0.0209
-7	Error	48	289350.667	6028.139		
	Total	71	5173562			

Table 4b. DMRT Effect of inoculation, drought and re-watering on GA conntents(ug/g) of the leaves of soybean.

treatments	T 1	T 2	T 3
V 1 C	417.3 GHI	381.0 HIJ	346.7 HIJK
V 1CI	692.7 CDE	512.3 FG	435.0 GH
V 1 D	403.8 GHI	221.0 K	240.7 JK
V 1 DI	664.0 CDE	349.3 HIJ	405.3 GHI
V 2 C	729.3 CDE	606.7 DEF	589.3 EF
V 2 CI	1102.0 A	991.0 AB	941.7 B
V 2 D	647.3 DEF	252.0 JK	252.0 JK
V 2 DI	1068 AB	738.3 CD	791.7 C

L.S.D. value (0.05) =127.5

T 1= Measurements made at 10DAS
T 2= Measurements made at 15DAS(after 5 days of drought stress)
T 3=Measurements made at 19 DAS(after 5 days of drought stress)

All such means sharing a common English letter differ insignificantly from each other, other wise they differ significantly at $P<0.05$.

Table 5a. ANOVA of ABA contents (ug/g) of leaves of soybean

K-value	Source	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
2	Factor A	2	11384.028	5692.014	6.3515	0.0036
4	Factor B	7	65233.986	9319.141	10.399	0
6	AB	14	13019.306	929.95	1.0377	0.4346
-7	Error	48	43016	896.167		
	Total	71	132653.319			

Table 5b. Effect of inoculation, drought and re-watering on ABA contents (ug/g) of the leaves of soybean.

treatments	T 1	T 2	T 3
V 1 CI	109	104	94
V 1 CI	117	111	101
V 1 D	114	175	123
V 1 DI	113	191	144
V 2 C	63	61	58
V 2 CI	71	70	66
V 2 D	74	132	82
V 2 DI	99	125	105

T 1= Measurements made at 10DAS
T 2=Measurements made at 15DAS (after 5days of drought stress)
T 3=Measurements made at 19DAS (after 48h of rewatering)

Table 5c. DMRT of the means of total ABA contents of the treatments at T1, T2 and T3.

Treatments	Means	Treatments	Means
V 1 C	102.7 CD	V 2 C	61.22 E
V 1 CI	109.7 CD	V 2 CI	69.33 DE
V 1 D	144.1 AB	V 2 D	114.3 BC
V 1 DI	153.3 A	V 2 DI	112.3 BC

L.S.D. value (0.01) =37.85

All such means sharing a common English letter diffre in significant achother otherwise they differ significantly from eachother at P<0.01.

Table 6. Effect of inoculation, drought and rewatering on the GA/IAA ratio of the leaves of soybean.

Treatments	T 1	T 2	T 3
V 1 C	3.278	1.88	2.23
V 1 CI	3.73	1.4	1.535
V 1 D	3.44	3.14	2.959
V 1 DI	3.99	2.91	3.609
V 2 C	5.23	4.231	4.26
V 2 CI	4.03	4.418	5.05
V 2 D	3.26	3.18	2.589
V 2 DI	3.81	5.09	5.267

T 1= Measurements made at 10DAS
T 2=Measurements made at 15 DAS (after 5 days of drought stress)
T 3= Measurements made at 19DAS (after 48h of rewatering)

Table 7. Effect of inoculation, drought and rewatering on ABA / IAA ratio of the leaves of soybean.

Treatments	T 1	T 2	T 3
V 1 C	0.85	0.72	0.6
V 1 CI	0.63	0.49	0.35
V 1 D	0.96	2.48	1.51
V 1 DI	0.67	1.59	0.86
V 2 C	0.29	0.3	0.42
V 2 CI	0.26	0.19	0.35
V 2 D	0.37	1.48	0.84
V 2 DI	0.35	0.86	0.69

T 1 =Measurements made at 10DAS

T 2 = Measurements made at 15DAS (after 5 days ofdrought)

T 3 = Measurements made at 19DAS (after 48h of rewatering)

Table 8. Effect of inoculation, drought and rewatering on ABA / GA ratio of the leaves of soybean

Treatments	T 1	T 2	T 3
V 1 C	0.26	0.28	0.27
V 1 CI	0.168	0.216	0.32
V 1 D	0.28	0.79	0.51
V 1 DI	0.169	0.54	0.28
V 2 C	0.08	0.1	0.09
V 2 CI	0.06	0.07	0.07
V 2 D	0.114	0.46	0.32
V 2 DI	0.09	0.16	0.13

T 1=Measurements made at 10DAS
T 2=Measurements made at 15DAS (after 5 days of drought stres)
T 3=Measurements made at 19DAS (after 48h of rewatering)

Table 9a. ANOVA of number of nodules/plant of soybean

	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
Between	7	4554.625	650.661	43.377	0
Within	16	240	15		
Total	23	4794.625			

Table 9b. DMRT of Effect of inoculation and drought on number of nodules/plant

Treatments	Means	Treatments	Means
V 1 C	20 B	V 2 C	23 B
V 1 CI	40 A	V 2 CI	44 A
V 1 D	9 C	V 2 D	17 C
V 1 DI	34 B	V 2 DI	36 B

L.S.D value (0.05) = 105.3

T 1 =Measurements made at 10 DAS
T 2 =Measurements made at 15DAS (after 5 days of drought)
T 3 = Measurements made at 19DAS (after 48h of rewatering)

Table 10a. ANOVA of diameter of pink bacteroid tissue of nodules of soybean

	Degree of fre	Sum of squar	Mean squar	F-value	Prob.
Between	7	75807.292	10829.613	2.929	0.0356
Within	16	59166.667	3697.917		
Total	23	134973.958			

Table 10b. DMRT of Effect of inoculation and drought on diameter of pink bacteroid tissue of the nodules

Treatments	Mean	Treatments	Means
V 1 C	229.5 AB	V 2 C	300 A
V 1 CI	300 A	V 2 CI	229.2 AB
V 1 D	137.5 B	V 2 D	150 B
V 1 DI	241.7 AB	V 2 DI	204.2 AB

L.S.D value (0.01) =9.236

T 1 =Measurements made at 10 DAS
T 1 =Measurements made at 15DAS (after 5 days of drought)
T 3 = Measurements made at 19DAS (after 48h of recovery)

All such means sharing a common English letter diffre in achother otherwise they differ significantly from eachother at $P<0.01$.

Discussion

DISCUSSION.

SOIL MOISTURE

Table 1.showed that at sowing and at 10DAS the treatments have not shown much difference in the values. This may be because at these times, the water supply condition for all the treatments was same and no stress was imposed at any treatment.

At 15DAS the treatment V_1D, V_1DI, V_2D, V_2DI have shown a reduction in the value as compared to the treatments V_1C, V_1CI, V2C&V_2CI. This is because the water supply of the treatment V_1D V_1DI V_2D&V_2DI was withheld for 5 days and so at 15 DAS drought stress has decreased the soil moisture contents of these stressed treatments as compare to control. This is in accordance with the previous findings of Zou *et al*., (1994), Battreu *et al*., (1998) and Vekateswark and Rao (1987) that drought stress resulted in decreased soil moisture contents.

At 19DAS, i.e. after 48 hours of re-watering, the stressed treatments have shown an increase in the value as compare to the measurements made at 15DAS. This has indicated that the recovery of treatments from drought, but the comparison with control have shown that even after re-watering, the stressed treatments have not completely regained soil moisture.

EFFECT OF DROUGHT ON RWC%

RWC is an important physiological trait, which is decreased under drought conditions and may be involved in conferring some sort of drought tolerance in plants (Rehman *et al*.,.2000).

Table 2. showed a comparison between the RWC% of all the treatments at T1 (10DAS), T2 (15DAS) and T3 (19DAS).

The results show a clear effect of drought on the RWC% of the stressed treatments.

At T1 (10DAS) the RWC% of all the treatments is high and almost same. This is because till T1 all the treatments were growing under similar water supply conditions. After that measurements made at T2 (15 DAS) shows that the RWC of the stressed treatments has

Aspiral and Hussain (1970), Javanovic and Quarrie (1990) and Thomas (1991) that relative water contents of the plants decrease under drought conditions as compared to the control.

The Table2 also showed that at T3 (19 DAS), after re-watering the value of RWC% has increased in the treatments subjected to drought as found by Jovanovic and Quarrie (1990) that leaf water potential is largely recovered in young plants after re-hydration.

EFFECT OF DROUGH ON IAA& GA LEVELS

Table 3b and 4b show a comparison of IAA & GA levels in the leaves of the soybean at three different times i.e. under control conditions before the induction of drought (T1), after the induction of drought (T2) and after 48 h of re-watering (T3).

After 10 days, water supply of the treatments (V_1D, V_1DI, V_2D, and V_2DI) was with-held. So the measurements taken at T2 shows the effect of drought on stressed treatments. As a result of drought, the IAA and GA contents of the stressed treatments have significantly decreased. This is in accordance with the previous reports by Morgan (1990), Westgate *et al.*, (1996) and Postovojtova *et al.*, (2000), that as a result of drought stress endogenous phytohormone level changes and there is a significant decrease in IAA and GA levels. During drought stress growth is inhibited which results in the decreased levels of IAA&GA. The observed decrease in IAA&GA levels of stressed treatments may be attributed to the drought induced growth inhibition.

On re-watering the IAA&GA contents have significantly increased in stressed treatments over control. After 48h of re-watering when the plants received the signals of stress release, it has resulted in increase in the levels of hormones (IAA& GA).

At all the three times i.e. T1, T2, and T3 if we compare the IAA&GA contents of the inoculated treatments with un-inoculated ones of both the varieties, the values are significantly higher in inoculated treatments as compared to un-inoculated treatments. This shows that inoculation with an effective strain of *Rhizobium* may have a marked effect on increasing the levels of IAA&GA. *Rhizobium* has previously been reported to improve plant growth by producing plant growth regulators such as auxins and gibberellins (Cooper *et al.*,1988 and Okon *et al* 1996).This is also in accordance with the findings of Noel *et al.*, 1995, Datta and Basu, 2000 and Basu and Ghosh, 2001, that findings of Noel *et al.*, 1995, Datta and Basu, 2000 and Basu and Ghosh, 2001, that inoculation resulted in the production of growth promoting hormones such as IAA &GA. Same were the findings of Wang *et al.*,(1982) and Cooper *et al.*,(1988) that *Rhizobium* strains produce IAA and GA.

EFFECT OF DROUGHT ON ABA LEVELS

Table 4 showed the comparison of ABA contents of all the treatments at T1 (10DAS), T2 (15DAS) &T3 (19DAS). The measurements showed that ABA contents were significantly high in all the stressed treatments at 15DAS i.e. after a drought stress of 5days, as compared to control.

This is in accordance with the findings of Leung and Giraudate (1998) that as a result of osmotic stress there is an increase in the levels of endogenous ABA concentration in the leaves during vegetative growth. It has also been reported by

Itai, (1999) that under conditions of progressive drought leaf ABA contents significantly increased relative to control. ABA is known as the stress hormone, which accumulates in the leaves of the plants as a result of various environmental stresses including drought. According to the previous study done by many workers such as Quarrie, 1984, Morgan, 1990 and Taiz and Zeiger 1998, as a result of drought stress ABA synthesis and accumulation increases in the plants.

ABA is a growth-inhibiting hormone and drought adaptation generally occurs while growth is inhibited. This could be the reason of increased ABA concentration in the treatments under drought conditions.

Early reports identified that drought stressed plants may become more sensitive to internal ABA concentrations of the growing zone of the leaves (Carmer and Quarrie, 2002).

Accumulation of ABA under water deficit may result from enhanced biosynthesis, and/or a decrease in breakdown. ABA is synthesized in the shoot and root in response to various stresses including drought. ABA is involved in the regulation of stress response like stomatal closure (Chaves *et al.*, 2003).

It was explained by Leung and Giraudat (1998) that endogenous ABA concentrations increased, in response to water stress during vegetative stage. Increased ABA levels alleviate growth inhibition by water deficit via reducing stomatal aperture and limiting water loss through transpiration.

At 19 DAS after 48h of re-watering the stressed treatments have shown an increase in the ABA contents as compared to stressed condition. Release of drought has resulted in decreasing the levels of ABA.

It may be suggested that response of plant to drought stress is a coordinated process in which phytohormones are involved and the modified hormonal balance is the key determinant of the response.

EFFECT OF DROUGHT AND INOCULATION ON NUMBER OF NODULES PER PLANT AND DIAMETER OF PINK BACTEROID TISSUE.

Table 9b.shows that the number of nodules per plant is significantly high in the inoculated control treatments. The number of nodules per plant is significantly less in the un-inoculated stressed treatments than all other treatments. This has shown the effect of drought stress as well as un-inoculation on nodulation. The previous study done by Kinda *et al.*, (1989), Masyhudi and Patterson (1991) and Pena-Cabriales and Catellanon (1993), explains that water stress induces significant reduction in nodulation and nitrogen fixation. It is also evident from the previous study that Rhizobium inoculation plays an important role in increasing the number of nodules per plant, as found by Di-bonito *et al.*,(1989), Elshek and Osman (1995).

Hoeflic and Rupple (1994) Chebotra *et al.*, (2000), that Rhizobium inoculation has an enhanced effect on nodulation, which could be due to growth promoting substances that stimulates the growth of *Rhizobium*.

In case of un-inoculated treatments, the number of nodules per plant is higher in control treatments as compared to stressed ones. Similarly in case of inoculated treatments the number of nodules per plant is higher in control treatments as compared to stressed ones. So the results have shown the effect of drought on decreasing nodulation in stressed treatments.

The table also indicates that nodulation is higher in all the inoculated treatments showing the enhanced effect of inoculation on nodulation.

Among stressed treatments, nodulation is higher in inoculated treatments as compared to un-inoculated stressed ones .This difference is again because of inoculation, which has increases nodulation.

Table10b.showed that the diameter of pink bacteroid tissue of nodules of the un-inoculated stressed treatments (i.e. V_1D&V_2D) is significantly less than all other treatments. This indicates the effect of drought on diameter of pink bacteroid tissue. Pink bacteroid tissue is the active sight of nitrogen fixation. According to the findings of Kirda et al.,(1989) , Masyhudi and Patterson (1991), Salama and Sinclair (1994), as a result of drought stress there is a decrease in the total nitrogen fixation. Hence this could be the reason of decrease in the diameter of pink bacteroid tissue of the nodules of the stressed treatments.

However there is no significant difference between the values of inoculated treatments and un-inoculated control treatments. The results have shown that inoculation resulted in developing efficient *Rhizobium* legume symbiosis and increasing the number of nodules per plant but did not have a significant effect on increasing the diameter of pink bacteroid tissue. Moreover the difference may not be marked as the volumes of maximum sized nodules of every plant were studied.

CONCLUSION

It is inferred from the present study that inoculation has an enhanced effect on the IAA and GA contents of the leaves. As a result of drought stress the endogenous phytohormone levels change resulting in increased level of ABA and decrease in IAA&GA contents. It may be suggested that response of plant to drought stress is a coordinated process in which phytohormones are involved and the modified hormonal balance is the key determinant of the response. ABA/IAA and ABA/GA ratio appear to act as a marker for selection of varieties for drought stress.

Inoculation has a marked effect on enhancing nodulation and inferring drought resistance in the varieties. Among the two cultivars studied cv_1 i.e. NARC 2000 proved to be more resistant as compared to cv_2 i.e. NARC 2001.

References

REFERENCES

Andrade , D.S.and M.Hungria 2001. Maximizing the contribution of Biological Nitrogen fixation in Tropical legume crops.In: Nitrogen Fixation .T.M.Finan, M.R.O'Brian, D.B Layzell,J.K.Vessey and W.Newton(Eds).CABI Publishing.

Aspirall, D.and I. Hussain 1970. The influence of flowering by water stress. Aust. J. Biol. Sci. 23: 925-936.

Bahrun ,A,C.R.Jensen ,F.Asch and V.O Mognesen 2002 Drough induced changes in Xylem pH, ionic composition, ABA concentration act as early signals in field grown maiz(*Zea mays*) .Journal of Experimental Botony 53,367:251-263.

Basu,P.S.and A.C.Ghosh 2001.Production of Indole acetic acid in culture by a *Rhizobium* species from the rest nodules of Monocotyledonous Tree,*Roystonea regia*.Acta-Biotechnol.21,1:65-72.

Beck, C. 1938. The microscope. In Animal tissue techniques. G.L. Humason, (Eds). W.H. Ferman and Company, San Francisco, USA. 76-77.

Bradford, K.J. and A.J. Trawavas 1994. Sensitivity thresholds and variable time scale in plant hormone action. Plant Physiology. 105:1025-1036.

Buttreu,B.R., C.S.Tans,C.F.Drury, S.J. Park, R.J. Armstrong and K.Y. Park. The effect of soil compaction and soil type on growth and nodulation of soybean and common bean. Can. J.Plant Sci.Rev.Can.Phytotech. 78,4: 576-577.

Carlson, J.B., and Levsten 1987. Reproductive Morphology. In Soybean Improvement, Production and Uses. J.R.Wilcox(Eds). Agronomy Society of America. 2:131.
Chaves,M.M..,J.P.Maroco and T.S. Pereira 2003. Understanding plant responses to drought from gene to the whole plant. Functional Plant Biology . 30, 239-264.

Chebotar,V.K.,C.A Asis,and Jr.Akao 2001.Production of growth promotion substances and high colonization ability of *Rhizobacteria* enhance the nitrogen fixation of soybean when co-inoculated with *Bradyrhizobium japonicum*. Biol.Fertilizer.Soil.34,6:427-432.

Cooper, J.B. and Long S.R.(1988). Morphogenesic rescue of *Rhizobium meliloti* nodulation mutants by trans-zeatin secreation. Plant cell. 6:215-225.

Datta,C.and P.S.Basu 2000.Indole acetic acid productionby a *Rhizobium* species from root nodules of a leguminous shrub *cajanus cajan*. Microbiol.res.155,2:123-127.

Di-Bonito, R.,Porreca, G. and S. Mannelli 1990. Competition for nodulation of Rhizobium spp.inoculated on different cultivars of chickpea (*Cicer arietinum* L.) Proceeding of the International Conference on The Mechanisms between Soil Microorganisms in the Rhizosphere. 405-409.

Elsheikh, E.A.E. and Osman, A.G. 1995. *Rhizobium leguminosarum* inoculation decrease damage to *Vicia faba* caused by broad bean mottle, bromovirus and bean yellow mosaic potyvirus. World J. Microbiol Biotechnol. 11,2:181-183.

Gandhi, A.P.,M. M.Nenwant, and A.Nawab 1985. Some physiochemical characteristics of soybean. Food chemistry. 17: 71- 74.

Gupta,U.S. 1995. Production and Improvement of Crops for Drylands. Oxford IBH Publications Co. PVT. LTD. New Dehli, Bombay, Calcutta.

Harborne, J.B. 1997. Biochemical Plant Physiology. In: Plant Biochemistry.P.M.Dey and J.B.Harborne. Academic press London.
Hreman, J.C. 1992. How asoybean plant developes. Iuma State Univ. Coop. Exten. Special Report No. 53. 20p.

Hoeflich,G., and S.Rupple 1994. Growth stimulation of pea after inoculation with associative bacteria. Microbiol. RES. 149.1: 99-104.

Hsiao, T.C. 1973. Plant response to water stress. Annv. Rev. Plant Physiology. 24: 519-570.

Ishitani M., T.Nakamura, S.Y.Han and T. Takbae 1995. Expression of the betain aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid. Plant Mol Biol. 27: 307-315.

Itai, C. 1999. Role of Phytohormones in Plant Responses to Stress. In :Plant Responses to Environmental Stresses. H.R.Lerner (Eds). Marcel Dekker, Inc. USA.

Javanovic, L. and Quarrie. 1990. The distribution of ABA in maize plants in response to drought stress. British Society for plant growth regulation. 21: 322-323. .

Jensen, C.R., V.O.. Morgensen, H.H. Ponlsen, I.E. Henson, S. Aagot 1998. Soil water metric potential rather than water contentdetermines drought responses in field grown lupins (*Lupinus angustifolius* L.). Aust. J. Plant.Physiol. 25,2253-363.

Kirda, C., S.K. Danso, and F.Zapta 1989. Temporal water stress effects on nodulation, nitrogen accumulation and growth if soybean. Plant Soil. 120,1: 49-55.

Kulkarni, J.H.., Joshi and D.M. Bhatt, 1988. Effect of water stress on nodulation and nitrogen contents of groundnut (*Arachis hypogea*). Zbi. Mikrobiol. 143:299.

Landi,P.,S.Conti,F.Gherardi,M.sanguineti 1995.Genetic analysis of leaf ABA concentration and of agronomic traits in maize hybrid grown under different water regimes.MAYDICA.40,2:179-186.

Lavisolo,C., H.Wolfram, and A.Schuberi 2002,Whole Plant Hydraulic conductance and root-to-shoot flow of abscisic acid are independently affected by water stress in grapevines, Funet.Plant Biol,29,1349-1356.

Lermer, H.R., G.N.Amzallag, Y.Friedman, and P.Goloubinoff 1994. The response of plants to salinity from turgor adjustments to genome modification. Isr. J. Plant Sci 42: 285-300.

Leung,J.and J.Giraudat 1998. Abscisic acid signal transduction Annual Review of Plant Physiology and Molecular Biology 49, 199-222.

Lu, D.B.,R.G.Sears and G.M.Paulsen 1990. Increasing stress resistance by in-vitro selection for abscisic acid insensitivity in wheat. Crop. Sci. 29,4:939-943.

Masyhudi,M.E. and R.P. Patterson 1991. The effect of water stress on nitrogen absorption of soybean. Indonesian J. Crop Sci. 6, 2: 43- 63.

Morgan, P. 1990. Effect of abiotic stresses on plant hormone systems. In Alsher R.G. Cumming J. R.(Eds) Stress Responses in Plants : Adaptations and Acclimation Mechanism. New York, Wiely-Liss, pp 113-146.

Naqvi, S.M.S. 1994. Plant Hormones and Stress Phenomena. In: Hand Book of Plant Crop Stress. Passarak,M.(Eds). Pp 83-400. Mercel Dekker, In New York.

Nehru,S.D.and S.Rangaiah 1998.effect of *Rhizobium japonicum* on nodulation and yield of soybean .Crop.Improv.25,2:235-236

Noel T.C .,C.Sheng,C.K.Yast, R.P.Pharis and M.F.Hynes 1995.*Rhizobium leguminosarum* as a plant growth –promoting *rhizobacterium*; direct growth promotion of canola and lettuce .Can.J.Microbiol.42:279-283.

Okon, Y., J. Kigel and Sarig, S. (1996). Field inoculation of common bean and chickpea with *Azospirillum brasilense* strain. Symbiosis. 21,1:41-48.

Pena-Cabriales, J.J. and J.Z. Castellanos 1993. Effect of water stress on nitrogen fixation and grain yield of *Phaseolus vulgaris* L. Plant and Soil. 152, 1:151- 15.

Peuke, A.D.,C. Schraml and W.Hartung 2002. Identification of drought- sensitive beech ecotypes by physiological parameters. New-Phytol. 154, 2: 373-387.

Pustovojtova,T.N., G.V.Ermin,E.G. Rassvetaeve, N.E.Zhdanova and V.N.Zolkevich 1996. Drought resistance, recovery capacity and phytohormone content in polyploid plum leaves. Russian J.Plant Physiology. 43,2:232-235.

Pustovojtova, T.N., N.E.Zhdanova, V.N. Zolkevich 2000. The importance of phytohormone in Adaptation of plants to drought. Proceedings of 3rd Conference, Immunoassay of plant physiology plant growing and Biotechnolog. 200-224.

Quarrie, S.A. 1984. Abscisic acid and drought resistance in crop plants. British plant growth regulator group. 7,1: 550-565.

Rahman, S., M.S. Shaheen, M. Rahman and T.A. Malik 2000. Evaluation of excised leaf water loss and relative water contents, as screening techniques for breeding drought resistant wheat. Pak. J. boil. Sci., 3(4) :663- 665.

Salama, A.M. and T.R.,Sinclair, (1994). Soybean nitrogen fixation and growth as affected by drought stress and potassium fertilization. Journal of Plant Nutrition. 17, 7:1193-1203.

Salim, M.H. , G.W. Todd and A.M. Schlehvber 1965. Root development of Wheat, Oat and Barley under conditions of soil moisture stress. Agron. J., 57: 603- 607.

Salisbury, F.B. and Ross 1992. Hormones and Growth Regulators: auxins and G.A. In: Plant Physiology. (Eds).F.B. Salisbury and C.W. Ross. 372- 407.

Salisbury F.B. 1994. The Role of Plant Hormones in Plant Environment Interaction. Marred Dekker Avenue New York. USA.

Shabaeu V.P., V.Y.Somlin and V.A.Mudrik 1995. CO_2 exchange in soybean plant and symbiotic nitrogen fixation upon joint inoculation with nodule bacteria and either rhizosphere pseudomonas or endomycorrhizal fungi. Izvestiia Akademii Nauk.Sssr.Seriia. Biologi. Cheskaia. 6,4:693-701.

Sharma, H.R. and S. Suneja (1994). Effect of dual inoculation of *Rhizobium* and *Azotobacter* in chickpea. Environ. Ecol. 12,4: 865-868.

Tardieu, F.,W.J. Davies 1993. Integration of hydraulic and chemical signaling in control of stomatal conductance and water status of droughted plants. Cell and environmental 16,341-349.

Taiz, L. and E. Zeiger 1998. Plant Physiology. Sinauer Associates, Inc. Publishers. Sunderlands, Massachuestts.

Thomas, H. 1991. Accumulation and consumption of solutes in swards of *Lolium perenne* during drought and after rewatering. NEW PHYTOL. 118, 1: 35- 48.

Towenley-Smith, T.F. and E.A. Hurd 1979. Testing and Selecting for Resistance in Wheat. In: Stress Physiology in Crop Plants. H. Mussell and R.C. Staples, (Eds). A Wiley-International Publication. Jhon Wiley Sons, New York, Chichester, Toranto.

Vevkateswark, B. and A.V.Rao 1987. Quantitative effects of field water deficiets on $N_2(CH_2H_2)$ fixation in selected legume grown in Indian deserts. Biol. Fertil. Soil. 5:18.
Aspirall, D.and I. Hussain 1970. The influence of flowering by water stress . Aust. J. Biol. Sci. 23: 925-936.

Victorova, L.V.and N.N.Maksutova 2002. Effect of absisic acid on protein synthesis in wheat caryopsis under drought. The Proceeding of 3rd Conference. Immunoassay of plant growth regulators in Plant Physiology, Plant growing and Biotechnology. Ufa. 22 p.
Wang-Peiwu, A.Isoda, and Wei-Guozhi 1995. Growth and adaptation of soybean cultivars under water stress conditions. Japanese Journal of Crop Science. 6,4: 777-783.

Yanni,Y.G.,A.A.Hussan, M.M.El-Beheiry and M.E.Hassan 1991. Relation between soybean nodulation by Bradyrhizobium japonicum and intensity of infestation with the spider mite under different sowing dates and increasing amount of nitrogen fertilizer. World –J. Microbiol. Biotechnol. 7,1: 37-42.

Zahran,H.H. 1999. *Rhizobium* legume symbiosis and Nitrogen fixation under serve conditions and in an arid climate .Microbiol .Mol.Biol.REV.63,4:968-989.

Zou, O.I., G.Y.Sun and T.A.O.Wang 1994. Water relations and osmotic adjustments in soybean under drought stress. Soybean Science. 13,4:312-320.