EFFECT OF *AZOSPIRILLUM* INOCULATION ON THE GROWTH AND NITROGEN ASSIMILATION OF SORGHUM [*SORGHUM BICOLOR*) (C_4)] AND FINGER MILLET [*ELEUSINE CORACANA* (C_3)]

by

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A Thesis submitted to the Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, in partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

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CERTIFICATE

This is to certify that this thesis entitled **EFFECT OF *AZOSPIRILLUM* INOCULATION ON THE GROWTH AND NITROGEN ASSIMILATION OF SORGHUM [*SORGHUM BICOLOR* (C_4) AND FINGER MILLET [*ELEUSINE CORACANA* (C_3)]** submitted to the Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, for the award of the degree of Doctor of Philosophy in **BIOCHEMISTRY**, is a record of original research work done by **G.SUDHA** during the period of her study in the Department of Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree/Diploma/ Associateship/Fellowship or similar title to any candidate of any University.

17.7.95

Signature of the Guide

DECLARATION

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, under the supervision of Dr.S.Saroja,M.Sc.,M.Phil.,Ph.D (Madras), Reader in Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, and it has not been submitted for the award of any Degree/Diploma/Associateship/Fellowship etc., of any other University or Institute.

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ABBREVIATIONS USED

DT : Drought tolerant variety

DS : Drought susceptible variety

AZ204 : *Azospirillum lipoferum*

AZ208 : *Azospirillum halopraferens*

FT326 : *Azospirillum brasilense*

IAA-T : External application of IAA

T_1 : Control

T_2 : Inoculation with AZ204

T_3 : Inoculation with AZ208

T_4 : Inoculation with FT326

T_5 : IAA-T

DAS : Days after sowing

S_1 : 30 DAS

S_2 : 45 DAS

S_3 : 60 DAS

S_4 : 75 DAS

S_5 : 90 DAS

S_6 : 105 DAS

GS : Glutamine synthetase

GOGAT : Glutamate synthase

GDH : Glutamate dehydrogenase

AAT : Aspartate amino transferase

AS : Asparagine synthetase

XDH : Xanthine dehydrogenase

IAA : Indole acetic acid

T : Treatment

V : Variety

S : Stage

INTRODUCTION

INTRODUCTION

Recognition of the importance of nitrogen in increasing crop production has led, in the last few decades, to an increased demand for nitrogen fertilizers. It is the most expensive and major input in present day agricultural systems. In foreseeable future, demands for fertilizer nitrogen are likely to progressively increase to meet the food requirements of ever expanding global population. All over the world today, production of nitrogenous fertilizers is insufficient to compensate the nitrogen removed from the soil by cropping and through other means. Thus, without nitrogen fertilization it is impossible to feed the world's population.

The Green revolution in India succeeded in alleviating hunger and this success was through the use of fertilizers, particularly nitrogenous fertilizers which obligate a high monetary and more energy inputs through Haber - Bausch processes. Technologies that could be less dependent on fertilizer nitrogen have been explored and studied for quite sometime.As a consequence, the momentum or the thrust on Biological Nitrogen Fixation (BNF) kept spinning up the ladder of research. BNF contributes 69% of the global nitrogen fixation and non biological processes account for 31%. Improved use of BNF could be the only possible avenue to minimise the chemical fertilizer use and ecological hazards (Verma, 1993).

There has been a revival of interest in the study of nitrogen fixing micro organisms in agriculture due to the escalation in the price of chemical fertilizers in recent years. Biofertilizers (microbial inoculants) are the products containing living cells of different types of micro organisms which have an ability to mobilise nutritionally important elements from non-usable to usable form through biological process (Subbarow, 1993). Biofertilizers are environment friendly. They are low cost agricultural inputs playing a significant role in improving nutrient availability to the crop plants (Tilak and Singh, 1994).

Besides the traditional *Rhizobium* - legume symbiotic system having its own realm of applications, other biological nitrogen fixation systems have also gained a great amount of interest especially in crop fields, forests and other grass lands. The possibility of providing nitrogen for important food crops using the associative system and free - living system hold a great promise. This is based on the fact that rice has been grown for centuries, with dependable yields, without the application of nitrogenous fertilizers and with no reduction in soil fertility.

Certain nitrogen - fixing organisms live in association with various grasses and the association is termed "associative symbiosis". The term "Associative symbiosis" refers to a loose association of nitrogen fixers with plant parts of an angiosperm. No visible structures viz., nodules, pouches, coralloid outgrowths etc., are produced to protect

the microsymbiont from competition with other microorganisms. The loose symbiotic association in the rhizosphere of grasses and sedges is gaining importance because of the fact that the tropical grasses possess C_4 type of photosynthetic pathway and have an obvious advantage over other plants with C_3 type of photosynthesis for supporting nitrogen fixation by associative microsymbiont (Tilak and Negi, 1987).

Azospirillum a potent associative symbiont, described by Dobereiner and Day (1976) from the roots of *Digitaria decumbens*, attracted considerable attention as a possible source of biological fertilizer (Dart, 1986). *Azospirillum* species are free living nitrogen fixing bacteria (Dobereiner, 1989) widely distributed in the rhizosphere of several tropical grasses (Dell-Gallo and Fendrik,1994). They are found in association with roots of grasses (Sangawan and Kundu, 1992), enhance the growth of plants (Puente and Bashan, 1993; Zaddy *et al.*,1993) and participate in several transformations in the nitrogen cycle (Heulin *et al.*, 1989).

Azospirillum inoculation had increased the yields of forage grasses and cereals (Fages, 1994). The effect of *Azospirillum* inoculation on the total yield increase of field-grown plants generally ranged from 10-30% (Kapulnik *et al.*, 1987). The most marked effects of *Azospirillum* inoculation on plants are the various morphological changes in the root system. These changes were reported to be related to inoculum concentration (Bashan *et al.*, 1989a). Cereals like corn and wheat could harbour 10^4 to 10^7 cells per gram dry weight of roots (Dobereiner and Pedrosa, 1987).

The success of *Azospirillum* and plant interaction depends on the survival and persistence of these bacteria in the soil and effective colonization of the rhizosphere (Victoria and Lovell, 1993). Chemotaxis is one of the several mechanisms that may contribute to the survival, rhizosphere colonization, and initiation of mutualistic interactions by *Azospirillum* spp (Reinhold *et al.*, 1985). The utilization of several aromatic components as carbon and energy sources by *Azospirillum* spp was recently demonstrated by Chen *et al.* (1993). The associative symbiotic micro organisms have been found to contribute to the nitrogen nutrition of the soil, because of their ability to colonize the roots by utilizing the root exudates for growth and nitrogen fixation (Kipe-Nolt *et al.*, 1985).

Plant nutrient uptake can be stimulated by inoculation with *Azospirillum* (Bashan, 1990). Association of *Azospirillum* with plant roots might increase the permeability of host roots to nitrogen, phosphorus and potassium (Okon and Kapulnik, 1986), alter plant root growth and root bacterial enzyme activities (Kucey, 1988a).

Apart from the nitrogen fixing capacity, *Azospirillum* also produces a variety of plant growth regulating compounds (Fallik *et al.*, 1994). Indole acetic acid, gibberelins, kinetin and abscisic acid were a few such plant growth regulating compounds reported to be secreted by the diazotrophic bacteria in the culture medium (Arshad and Frankenberger,1992. Sarwar *et al.*, 1992). Plant hormone

enhanced the nitrogen fixation capability of *Azospirillum* (Christiansen - Weniger, 1988). Applications of external hormones either synthetic or purified from bacterial culture, to seedlings completely reproduced the effects of *Azospirillum* on root development and morphology (Harari *et al., 1988*. Zimmer and Bothe, 1988).

Sorghum and Finger millet are the important crops of semi arid tropics. Nitrogen supply to sorghum and finger millet is generally a limiting factor, particularly when they are grown on low fertile soils. The apparent potential for biological nitrogen fixation associated with cereals exceeds its present utilization, but knowledge in this field is not enough to exploit these associations fully. Exploitation of rhizospheric nitrogen fixation may prove beneficial for crop improvement. There is lack of information on the physiological and biochemical interactions between the host plant and the microsymbiont.

In *Rhizobium* the fixed nitrogen is reduced by matured bacteriod suspension by nitrogenase activity and excretes the product as NH^{+}_{4} . A similar process is known to occur in legume nodules with ammonia assimilation into amino acids and ureides (Pate, 1989). Although these studies have been carried out in detail in *Rhizobium* - legume symbiosis, no detailed study has been made on this aspect in *Azospirillum* particularly in association with C_3 and C_4 plants. *Azospirillum* is known to fix dinitrogen, but how that fixed nitrogen is being assimilated and transported to various plant

parts is not understood.

This investigation was therefore undertaken to study the process of nitrogen assimilation on application of three different *Azospirillum* strains to Sorghum(C_4) plants and Finger millet (C_3) and the mode of transport of the fixed nitrogen.

The objectives of the present study were:

1. To assess the production of IAA by different strains of *Azospirillum* viz., *A. lipoferum, A. brasilense and A. halopraeferens.*

2. To study the different nitrogen assimilatory enzymes viz., Glutamine synthetase (GS), Glutamate synthase (GOGAT), Glutamate dehydrogenase (GDH), Asparatate amino transferase (AAT), Asparagine synthetase (AS), Nitrate reductase, Nitrite reductase, Asparaginase and Glutaminase.

3. To assay the enzymes involved in ureide biogenesis viz., Xanthine dehydrogenase (XDH), Uricase, Allantoinase and Urease.

4. To identify and determine the nitrogen transporting compounds.

5. To assess the quantity of plant hormones produced by the plant.

6. To study the impact of external application of IAA.

7. To estimate the total protein, total phenol and orthodihydric phenol contents in the plant.

8. To study the macro elements nitrogen,phosphorus, and potassium.

9. To study the influence of *A. brasilense, A. lipoferum* and *A. halopraeferens* strains on the growth of sorghum (C_4) and finger millet (C_3) plants in terms of physiological changes (Biometric observations).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The review of literature pertaining to the present study entitled "Effect of *Azospirillum* inoculation on the growth and nitrogen assimilation of sorghum [*Sorghum bicolor*(C_4)] and finger millet [*Eleusine coracana* (C_3)] is discussed under the following headings.

1. The genus *Azospirillum*
2. Effect of *Azospirillum* inoculation on plants
3. Effect of inoculation on root development
4. Colonization of roots by *Azospirillum*
5. Mode of action of *Azospirillum* on plant growth

5.1 Nitrogen fixation

5.1.1 Enzymes involved in nitrogen metabolism

5.1.2 Nitrogen transporting compounds

5.2 Production of growth promoting substances

5.3 Improvement of root development and mineral uptake by *Azospirillum*

5.4 Production of phenolic compounds in response to plant microbial interaction

6. Specificity and variability in *Azospirillum*

1. THE GENUS *AZOSPIRILLUM*

The first species of *Azospirillum* was isolated by Beijerinck (1925) from N-poor sandy soil in the Netherlands and was originally named *Spirillum lipoferum*. This bacterium was later isolated from soil, (Schroder,1932) and as a phyllosphere bacterium of tropical plants (Becking, 1982).

Dobereiner and Day (1976) isolated the bacterium and were the first to report that it was widely distributed in the rhizosphere of several tropical grasses. Since then, *Azospirillum* has been isolated from the roots of numerous wild and cultivated grasses, cereals, legumes, and from tropical, subtropical and temperate soils worldwide (Ladha *et al* .,1987; Li and Casellano, 1987; Horemans *et al*.,1988; Sundaram *etal* ., 1988).

Tarrand *et al* . (1978) proposed *Azospirillum* as the genus and distinguished two species: *Azospirillum brasilense* and *A. lipoferum*, based on physiological and morphological differences between various strains and on DNA homology experiments (Falk *et al* ., 1986). Later, two additional *Azospirillum* species were described: *A.amazonense* (Magalhaes *et al* ., 1983; Falk *et al* ., 1985), isolated from many grasses in the Amazonian area of Brazil, and the salt tolerant species. *A.halopraeferans*, associated exclusively with roots of kallar grass (Reinhold *et al* ., 1987). However, most strains are referred to as either *A.brasilense* or *A.lipoferum* (Krieg and Dobereiner, 1986). Another species of *Azospirillum irakense* has been isolated from the roots and rhizosphere of rice in the region of Diwaniyah in Iraq (Khammas et al., 1989).

2. EFFECT OF *AZOSPIRILLUM* INOCULATION ON PLANTS

Inoculation of plants with *Azospirillum* can result in a significant change in various plant growth parameters. Most studies of the *Azospirillum* plant association have been conducted on cereals and grasses (Patriquin *et al* ., 1983)

and only a few other plant families have been investigated (Kolb and Martin, 1985; Saha *et al* ., 1985; Crossman and Hill, 1987; Bashan *et al*., 1989a). Following inoculation there is increase in total plant dry weight, amount of nitrogen in shoots and grains, total number of tillers, grain weight, greater plant height and leaf size. and higher germination rates (Bashan, 1986a; Mertens and Hess, 1984; Millet and Feldman, 1986; Pacovsky *et al* ., 1985; Warembourg *et al* ., 1987; Yahalom *et al* ., 1984).

The effect of *Azospirillum* inoculation on the total yield increase of field-grown plants generally ranged from 10-30% (Kapulnik *et al* ., 1987; Rao *et al*., 1983; Watanabe and Lin, 1984). Even moderate yield increases (up to 20%) attributed to inoculation with *Azospirillum* are considered commercially valuable to modern agriculture, if obtained consistently. Okon (1985) evaluated the worldwide success of *Azospirillum* inoculation and concluded that positive effects on yield were obtained in approximately 65% of all field experiments.

Two basic variables that contribute to the complexity of plant yield response to inoculation are the level of nitrogen fertilization and the plant cultivars, which often showed differential response to inoculation (Millet *et al* ., 1986). The highest yield increases were obtained when the levels of nitrogen fertilization were sub optimal for maximum yield (Kapulnik *et al* ., 1981a; Lau-wong, 1987; O' Hara *et al* ., 1987). Therefore, *Azospirillum* inoculation was considered a partial substitute for nitrogen fertilization.

3. EFFECT OF INOCULATION ON ROOT DEVELOPMENT.

The most marked effects of *Azospirillum* inoculation on plants are the various morphological changes in the root system. (Pacovsky 1990; Sarig *et al.*, 1992). These changes are directly related to inoculum concentrations: higher than optimal levels had inhibitory effects, while low bacterial doses had no effect and, on the *Azospirillum* strain used, each producing different amounts of IAA (Barbieri *et al.*, 1990). The optimal inoculum level for seeds or seedlings of many cereals and industrial crop plants was 10^5 - 10^6 cfu/ml (Bashan *et al.*, 1989b).

Effects of inoculation had been demonstrated on various root parameters, including increase in root length, particularly of the root elongation zone (Sarig *et al.*, 1988; Levanony and Bashan, 1989), increase in number and length of lateral roots, which increased the root volume (Venkateswarlu and Rao, 1983; Barbieri *et al.*, 1988), increases in root dry weight (Schank *et al.*,1981; Hadas and Okon,1987), increase in the number, density and early appearance of root hairs (Umali-Garcia *et al.*,1980; Martin and Glatzle,1982), increase in root surface area (Bashan, 1986a), enhanced cell division in the root meristem (Levanony and Bashan, 1989), changes in cell arrangements in the cortex and stimulation of root exudation (Lee and Gaskins,1982; Heulin *et al.*, 1987). These remarkable morphological changes in the root system led to the hypothesis (Okon, 1985) that improvement in plant growth and yield increase are due to a

general non specific improvement of the root system (Lal and Rao 1990; Panwar and Sirohi,1989; Panwar *et al.*, 1992).

4. COLONIZATION OF ROOTS BY *AZOSPIRILLUM*

Azospirillum can colonize roots externally and internally. In external colonization, the bacteria form mainly small aggregates, although many single cells may also be scattered on the root surface. These externally colonizing bacteria are embedded in the mucigel layer of the root surface (Umali-Garcia *et al.*,1981; Bashan *et al.*, 1987; Murty and Ladha,1987). In internal colonization, *Azospirillum* cells can colonize roots by penetrating into the root intercellular spaces (Patriquin and Dobereiner, 1978; Levanony *et al.*, 1989).

Scanning electron microscopic studies have shown that *Azospirillum* cells are connected to the root surface and to each other within the bacterial aggregate by a network of fibrillar material (Gafni *et al.*, 1986; Hadas and Okon, 1987).

The specific mechanism by which *Azospirillum* attaches itself to the roots remains unknown. Lectin binding has been suggested as a possible mechanism (Tabary *et al.*, 1984) and it was speculated that agglutinins might be located in the fibrillar material, helping cell anchorage (Bashan and Levanony, 1989).

5. MODE OF ACTION OF *AZOSPIRILLUM* ON PLANT GROWTH

The principal mechanism by which *Azospirillum* enhances plant growth is undetermined. However, several possible modes of action have been proposed, as discussed below:-

5.1. NITROGEN FIXATION

All wild type *Azospirillum* strains fix atmospheric nitrogen efficiently either as free living bacteria or in association with plants and participate in several transformations in the nitrogen cycle (Hurek *et al.*, 1988; Heulin *et al.*, 1989). Following inoculation, there is an increase in the total nitrogen of shoots and grains of inoculated plants (Wani *et al.*, 1985; Boddey *et al.*,1986; Baldani *et al.*,1987).Therefore, nitrogen fixation was naturally the first major mechanism of action suggested for the enhancement of plant growth by *Azospirillum*. Conclusive proof that plants derive some of their nitrogen from the atmosphere came from the use of isotopic 15N incorporation and 15N-dilution techniques (Boddey, 1987; Boddey and Dobereiner, 1988).

Evidence that nitrogen fixation contributes to the nitrogen balance of plants was based on the common observation of an increase in the nitrogenase activity within inoculated roots (Cohen *et al.*, 1980; Rao and Rao, 1983). This well-documented enzymatic activity was of sufficient magnitude to account for the increase in total nitrogen yield of inoculated plants if all the fixed nitrogen was incorporated into the plants (Mertens and Hess, 1984; Sarig *et al.*, 1984). Inoculation of wheat and maize has indicated that upto 18% of the plant nitrogen was derived from nitrogen fixation (Rennie *et al.*, 1983; Rennie and Thomas,1987). In addition,inoculated plants grew normally with only a partial amount of the nitrogen fertilizer usually required for such

growth (Nur *et al.*, 1980 ; Kapulnik *et al.*, 1981b). On the other hand, studies have shown low or even negligible nitrogenase activity in plants positively responding to inoculation (Lethbridge and Davidson, 1983; Kapulnik *et al.*, 1985a). Furthermore, of all the nitrogen fixed by the bacteria, less than 5% was incorporated into the host plants (Eskew *et al.*, 1981; Okon *et al.*, 1983). These amounts of fixed nitrogen are insufficient to explain total increases in nitrogen content of inoculated plants. Finally, high nitrogen fertilization levels, which inhibit nitrogen fixation, did not eliminate the plant response to inoculation (Avivi and Feldman, 1982; Mertens and Hess, 1984).

The ultimate control to distinguish the contribution via nitrogen fixation from other effects of bacterial inoculation is to use NIF^- mutants incapable of fixing nitrogen but otherwise isogenic with respect to parental strains. Inoculation of cereals with NIF^- mutants caused the same effects as the parental strains (O' Hara *et al.*, 1981; Barbieri *et al.*, 1986; Morgenstern and Okon 1987). Recently, tomato seedlings responded to inoculation with the site-directed NIF^- mutant of *A.brasilense cd* in a manner similar to their response to the wild type (Bashan *et al.*, 1989b). These observations indicate that the plant response was caused by factors other than nitrogen fixation. Nevertheless, the possibility remains that nitrogen fixation contributes to the plant small amounts of nitrogen, which may be important in critical stages of plant development, such as the reproductive and the tillering stages. Thus nitrogen

fixation was found to occur in many *Azospirillum* associations.

5.1.1.ENZYMES INVOLVED IN NITROGEN METABOLISM

Nitrogen metabolising enzymes have been studied in detail in the rhizobial cells under free living conditions as well as in the root nodules. But not much information is available in case of associative symbiotic micro organism *Azospirillum*.

Several workers have reviewed the ammonia assimilation in legume-Rhizobium symbiosis as well as in rhizobial cells in defined media (Brown and Dilworth, 1975; Scott *et al.*, 1976; Ludrig, 1978; Sen and Schulman, 1980).

The enzymes involved in nitrogen metabolism was reported in nodules and also in different parts of various legumes viz., pigeonpea (Amarjit and Singh, 1985), cowpea (Woo *et al.*, 1981), pea and soybean (Polayes and Schubert, 1984).

Luthra *et al.* (1983) studied the ontogenetic changes in the level of ureides and enzymes of their metabolism in various plant parts of pigeonpea and reported increase in the activity of glutamine synthetase in nodules and shoot.

Purushothaman *et al.* (1979) studied the path way of ammonia assimilation in C_3 and C_4 plants and reported that ammonia assimilation was chiefly mediated through coupled reaction of Glutamine synthetase and Glutamate synthase.

Ohyama and Kumazawa (1980) reported that ammonia

produced by nitrogen fixation in soybean nodule was assimilated by the GS/GOGAT system to glutamic acid and then assimilated to various amino acids in situ.

Lea *et al.* (1982) studied the role of ammonia assimilating enzymes during nitrogen fixation in legume root nodules and suggested that ammonia was initially assimilated into the amide position of glutamine by GS and then into the 2-amino position of glutamate by GOGAT.

Loginora *et al.* (1982) studied the enzymes of ammonia assimilation in bacteria with different pathways of C_1 metabolism. They observed that bacteria with serine cycle and ribulose diphosphate pathway assimilated ammonia by GS and GOGAT cycle while, reductive amination of 2-keto glutarate is the pathway of ammonium nitrogen assimilation in the bacteria hexulose phosphate cycle.

Magalhaes and Huber (1989) studied the ammonia assimilation in rice, maize and tomato plants and observed that GDH activity in roots of the three plants was higher. Glutamine synthetase activity in roots and shoots of rice was much higher than in tissues of tomato and maize.

The pattern of nitrate reduction and glutamine synthetase activity as a function of leaf development were studied in sorghum (Scott and Neyra, 1979). They found that leaf nitrate reductase activity increased during early leaf development and reached a maximum at full leaf expansion which was followed by a decline with leaf maturation. Glutamine synthetase activity increased during early leaf development

but then remained constant until senescence. Enzymes of nitrogen assimilation in maize roots were studied by Oaks *et al*. (1980). They reported that nitrate reductase, glutamate synthase and glutamine synthetase activities were found to be higher in the apical region than in the mature regions of the root, but glutamate dehydrogenase and asparagine synthetase were more in mature regions of the root than in the apical region.

Fallik *et al*. (1988) found that the specific activity of Glutamine synthetase was increased when *A.brasilense* was applied to maize plants and also reported that the specific activity of the enzyme was significantly higher between 2nd and 3rd week after sowing in inoculated roots as compared to uninoculated controls.

5.1.2.NITROGEN TRANSPORTING COMPOUNDS

A number of legumes especially tropical species produce allantoin and allantoic acid from fixed nitrogen in nodules and use these compounds for transport and storage in different plant species. Several reports have indicated the occurrence of ureides and ureide producing enzymes in various legumes such as Pigeonpea (Hegde, 1982), soybean (Christensen and Jochimsen, 1983; Singh *et al*., 1983), and Cowpea (Atkins *et al*., 1982).

According to New comb *et al*.(1985) these legumes could be divided into various groups based on nitrogenous compounds transported from the root nodules to the shoots and they suggested that pea and lupin be grouped as amide exporters and

soybean and cowpea as ureide exporters.

The ureide production in legumes is mainly correlated with nitrogenase activity, nitrogen fixation and other physiological activities. Several reports indicate the positive correlation between ureide synthesis and nitrogen fixation in legumes (Argiller *et al*,1983; Yoneyama *et al*., 1985; Singh,1986). Luthra *et al*. (1983), reported that the levels of allantoin and allantoic acid in shoots, roots, nodules and leaves of Pigeonpea increased progressively during the growth.

The major nitrogen compounds in xylem sap of most of tropical legumes were reported to be ureides (Pate *et al*., 1980). Formation of ureides and their presence in the cell sap as dominant nitrogen solutes were found to be closely related to the presence of effective nitrogen fixing nodules, (Matsumoto *et al*., 1977a;1977b), suggest that the level of allantoin and allantoic acid in xylem sap or in particular tissue might reflect current rate of nitrogen fixation and could be used as a convenient and simple assay for nitrogen fixation status of field grown plants.

Nitrogen accumulation in plants following inoculation of *Azospirillum* strains is not solely via nitrogen fixation but also due to an increase in nitrate assimilation. The parental NR strain aided nitrate reduction in the roots and thus decreased nitrate translocation to the leaves, while inoculation with NR mutant caused direct translocation and reduction of nitrate in the

plant foliage (Ferreira *et al.*,1987). Boddey and Dobereiner, 1988). They have also reported increased nitrogen accumulation in shoots due to nitrate reductase activity besides the unaffected nitrogen fixation ability.

5.2. PRODUCTION OF GROWTH PROMOTING SUBSTANCES

Many *Azospirillum* strains produce several plant hormones in liquid culture. The major hormone produced is indole-3-acetic acid (IAA) (Jain and Patriquin, 1985; Plazinski and Rolfe, 1985; Ruckdaschel *et al.*, 1988). Other hormones detected at much lower, but biologically significant levels were indole lactic acid (Tien *et al.*, 1979), indole- 3-butyric acid (IBA) (Fallik *et al.*, 1989), indole-3-ethanol, indole-3-methanol (Crozier *et al.*, 1988), as yet unidentified indole compounds (Hartmann *et al.*, 1983), several gibberellins (Bottini *et al.*, 1989), abscisic acid (ABA) (Kolb and Martin, 1985) and cytokinins (Horemans *et al.*, 1986).

Plant hormones were found to affect the Nitrogen fixation capability of *Azospirillum* (Christiansen-Weniger,1988). Application of external hormones, either synthetic or purified from bacterial culture, to seedlings completely reproduce the effects of *Azospirillum* on root development and morphology (Harari *et al.*, 1988; Kucey, 1988b). In particular, it caused changes in root length (Kolb and Martin, 1985; Morgenstern and Okon, 1987), produced more root hairs (Kapulnik *et al.*, 1985c) and branching of root hairs (Jain and Patriquin, 1984), produced more lateral roots (Barbieri *et al.*, 1986) and enhanced the rates of cell

division and differentiation in meristematic tissues (Fallik *et al.*, 1989). An *Azospirillum* strain and a mutant which overproduced IAA in culture strongly affected plant root morphology (Kolb and Martin, 1985), whereas mutants that failed to produce IAA in culture had no effect on root morphology (Barbieri *et al.*, 1986). Inoculation with *Azospirillum* improved the hormonal balance of a hormone-defective mutant of wheat (Inbal and Feldman, 1982). Higher amounts of IAA and IBA were identified in inoculated maize roots than in uninoculated plants (Fallik *et al.*, 1989).

Horemans and Vlassak (1985) demonstrated that *A.brasilense* could produce IAA in the absence of tryptophan when grown aerobically in presence of NH_4, while De Francesco *et al.* (1985) showed that the highest levels of auxin were produced in both Nitrogen fixing conditions and limiting ammonia with stationary cultures of *A.brasilense* strain Sp6. Increased diameters of maize stems and increased tillering and number of ears in wheat as a result of inoculation have also been implicated to be due to hormonal effects (Kapulnik *et al.*, 1982; Reynders and Vlassak, 1982; Zambre *et al.*, 1984).

Kolb and Martin (1985) have shown that spraying a solution of IAA on roots of wheat growing in root boxes resulted in a significant increase in root length, which mimicked *Azospirillum* inoculation. Fallik *et al.* (1989) showed that the roots of *Azospirillum* inoculated maize seedlings contained elevated levels of both bound and free IAA and IBA two weeks after sowing.

Tien *et al.* (1979) have reported the production of IAA, gibberellins and cytokinins by *A.brasilense*. They found that inoculation of pearlmillet with *Azospirillum* strains greatly increased proliferation of lateral root and root hairs. Horemans *et al.* (1986) found that IAA producing capacity of *A.brasilense* was more than that of *A.lipoferum* and no gibberellins were produced by both the species. Prabhu (1989) also reported the production of IAA by *A.lipoferum*. He found that inoculation of rice varities (IR 50,IR 20 and CO 40) with *Azospirillum* isolates enhanced the growth of rice seedlings due to the excretion of plant growth regulating substances by the *Azospirillum* isolates.

Sheela (1991) reported that *Azospirillum* inoculation improved plant growth and rice yield and also found that *Azospirillum* strains with higher IAA production promoted the root growth better which resulted in better establishment of seedlings and increased uptake of nutrients from the soil.

Zimmer and Bothe (1988) reported that the cultures of *A.brasilense* Sp7 and *A. lipoferum* Sp 59 excreted IAA in the logarithmic phase but no gibberellins and cytokinins were produced. Harari *et al.* (1988) studied the involvement of IAA in the interaction between *A.brasilense* and *Panicum miliaceum* roots.

5.3. IMPROVEMENT OF ROOT DEVELOPMENT AND MINERAL UPTAKE BY *AZOSPIRILLUM*.

In addition to increasing (Kapulnik *et al.*, 1985b) many root parameters, plant inoculation with *Azospirillum* affected many foliage parameters. These changes were directly attributed to positive bacterial effects on mineral uptake by the plant. Enhancement in uptake of NO^{+}_{3}, NH^{+}_{4}, PO^{-}_{4}, K^{+}, Rb^{+} and Fe^{++} by *Azospirillum* (Barton *et al.*, 1986; Jain and Patriquin, 1984. Kapulnik *et al.*, 1985c; Sarig *et al.*, 1988 was proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves, which could have then been transferred to the panicles and spikes and finally resulted in a higher yield.

Inoculation of maize with *A. brasilense* strain or Sp7 increased uptake of nitrate, potassium, and phosphate into excised root segments by 30 to 50% over the controls (Okon,1982; Okon and Kapulnik, 1986). Mechanisms other than nitrogen fixation have been advanced to explain inoculation responses to *Azospirillum*, such as enhanced uptake of nitrate, phosphate and potassium by roots of maize, wheat and sorghum (Lin *et al., 1983;* Kapulnik *et al.*, 1985 a) which varied with the strains, Sp 245 increased and strain Sp7 decreased phosphate and potassium uptake (Jain and Patriquin,1984). Murty and Ladha (1988) have also reported yield increases in rice inoculated with *A.lipoferum* strain 34-H associated with an increased root surface area and increased rates of ammonium and phosphate uptake. This enhanced nutritional status, which probably resulted from improved root surface area and activity, often resulted in yield increases which, in

wheat were likely to stem from the increased number of fertile-tillers per unit area in inoculated plants (Kapulnik *et al.*, 1983; Millet *et al.*, 1985), have increased contents of nitrogen and phosphorus in the leaves of sorghum following inoculation with *A. brasilense*, which corresponded to the increases in biomass observed.

It was further suggested that *Azospirillum* inoculation may promote availability of ions in the soil by helping the plant scavenge limiting nutrients (Lin *et al.*, 1983). This might explain accumulation of nitrogen compounds in the plant without any apparent nitrogen fixation. The plant might take up nitrogen more efficiently from the limited supply in the soil, resulting in a lower requirement of nitrogen fertilization to attain a certain yield. Supporting evidence for increased mineral uptake by inoculated roots was provided by enhancement in proton efflux activity of wheat roots inoculated with *Azospirillum* (Bashan *et al.*, 1989b; Bashan, 1990). Proton efflux activity was found to be directly related to the balance of ions in plant roots. Some *A. brasilense* strains failed to improve uptake of several ions but nevertheless improved plant growth (Bashan *et al.*, 1990). Therefore, the study of the individual strains on various plant species becomes important.

5.4. PRODUCTION OF PHENOLIC COMPOUNDS IN RESPONSE TO PLANT MICROBIAL INTERACTION

Phenolic compounds have gained considerable interest in the control of insect pests and diseases of crop plants (Panda,1979). When phenolics occur in fairly large

concentrations, because of their direct toxicity, insect pests are warded off (Todd *et al.*, 1971).

Mote *et al.*(1985) observed that in *Azospirillum* applied sorghum crop, the incidence of shoot fly was strikingly very low and also reported that the increased plant vigour due to *Azospirillum* inoculation might be responsible for the low incidence of shoot fly. Uritani (1976) illustrated that post infectionally synthesized phenolic compound played an important role in disease resistance.

Harborne (1980) reported that although several enzymes involved in phenolic biosynthesis in plants, phenylalanine ammonia lyase was found to be the most important enzyme. Khurana and Verma (1983) observed that the phenol content was negatively correlated to sorghum shootfly susceptibility. Mohan *et al.* (1987) observed that the use of *Azospirillum* biofertilizer for sorghum, by the seed as well as soil inoculation increased the total phenolic content in young sorghum plants offering resistance against major pests. Mohan *et al.*(1988) reported that *Azospirillum* inoculation activates the enzyme phenyl alanine ammonia lyase implicated in the biosynthesis of phenolics resulting in increased phenolics in plant and that would be the reason for resistance towards shoot fly incidence.

Mathar and Vidhyasekaran (1978) studied the physiology of resistance to rust in sunflower. They reported that leaves of resistant variety contained more O.D.phenol and

total phenolic content than the susceptible variety.

6.SPECIFICITY AND VARIABILITY IN AZOSPIRILLUM

One of the most important factors concerning the *Azospirillum* association is that of plant-strain specificity. Specific differences between responses of C_3 and C_4 plants was suggested. *A. lipoferum* was the predominant species colonizing C_4 plants and *A. brasilense* was the predominant species associated with C_3 plants in tropical zones (Doberiner and De polli, 1980; Baldani *et al.*, 1986). Similar host plant preference was also found in temperate zones (Haahtela *et al.*, 1981; Lamm and Neyra, 1981). When the bacterial species was inoculated on to the respective plant species, success was more frequent when the proper plant-bacterial species combination was used (Baldani *et al.*, 1987; Pereira *et al.*,1988). Specificity can occur at the plant cultivar level. only a few of the many tested plant cultivars responded to inoculation with a given strain of *Azospirillum* (Wani *et al.*, 1985; Millet *et al.*,1986).

The requirement of" homologous" strain (isolated from surface sterilized roots of the same crop) for successful inoculation has been well established, with non homologous strains proving to be much less effective (Dobereiner and Baldani,1981; Patriquin *et al.*, 1983). Further evidence of the specificity of *Azospirillum* strains were cited by Baldani *et al.* (1986) in relation to chemotaxis to various organic acids and to root tip exudates in relation to attachment to roots.

Response to inoculation appeared to depend on both the bacterial and the host genomes at the cultivar and subspecies levels as well as on a multitude of other factors.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

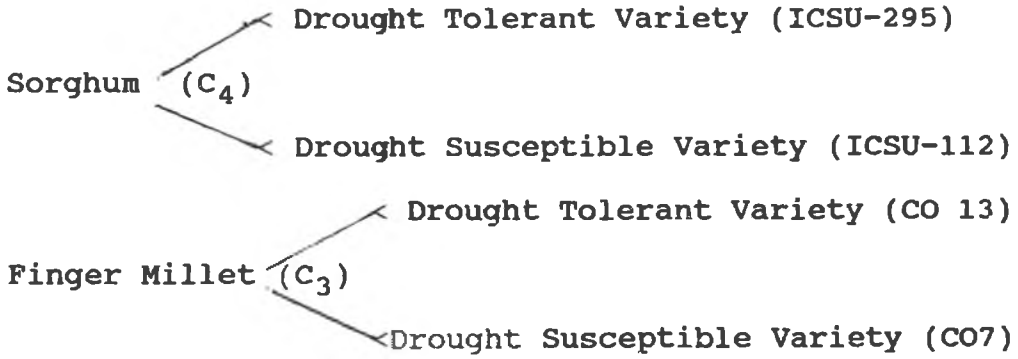
This study was carried out to assess the effect of *Azospirillum* inoculation on the growth and nitrogen assimilation of Sorghum [*Sorghum bicolor*(C_4)] and Finger millet [*Eleusine coracana* (C_3)]. The experimental details of the present study are as follows.

A.LOCATION

Pot culture experiments were carried out at the university campus.

B. SELECTION OF THE PLANTS

C_4 and C_3 plants with two different varieties representative of each were chosen for the study.



C. SEED COLLECTION

The seeds of sorghum were collected from ICRISAT, Hyderabad and the seeds of finger millet were obtained from millet breeding station, Tamil Nadu Agricultural University, Coimbatore. The seeds were surface sterilized using 0.1 Per cent mercuric chloride and then rinsed several times with sterile distilled water. The seeds were then treated with selected biofertilizer strains.

D.CULTURES

Azospirillum cultures [*A. lipoferum* (AZ 204), *A. halopraeferens* (AZ 208) and *A. brasilense* (FT326)] used in the present study were obtained from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. *Azospirillum* cultures were subcultured on Dobereiner's Nitrogen free malate medium.

Multiplication of Azospirillum.

The medium (Okon *et al.*, 1977) of the following composition was used to multiply *Azospirillum* culture:

Nitrogen free malate medium

Malic acid	5.0 g
Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	0.1 g
Calcium chloride	0.2 g
Sodium molybdate	0.002 g
Manganese sulphate	0.001 g
Iron EDTA (1.64%)	4.0 ml
Bromothymol blue (0.5 percent W/V in ethanol)	3.0 ml
Potassium hydroxide	4.9 ml
Trace element solution (composition given below)	2.0 ml
Vitamin solution (composition given below)	1.0 ml
Distilled water	1000 ml
pH	6.8

Composition of Trace element solution

Magnesium sulphate	235 mg

Sodium molybdate	200 mg
Boric acid	280 mg
Copper sulphate	8 mg
Zinc sulphate	24 mg
Distilled water	200 ml
Composition of vitamin solution	
Biotin	10 mg
Pyridoxine	20 mg
Distilled water	100 ml

E. BIOFERTILIZER TREATMENTS

The seeds of the selected plants were treated with different *Azospirillum* strains, as detailed below:

T1- Uninoculated control.

T2- Inoculation with *Azospirillum lipoferum* (AZ204)

T3- Inoculation with *Azospirillum halopraeferens* (AZ208)

T4- Inoculation wtih *Azospirillum brasilense* (FT326)

T5- IAA - T (IAA was applied at a concentration of 10^{-8}M to the plant on the 8th,16th and 24th day of growth).

F.INOCULATION PREPARATION

Azospirillum cell suspension were mixed with finely seived,autoclaved,sterilised peat, adjusted to pH 6.8 with calcium carbonate at a final concentration of about 10^9 colony forming units (cfu) per gram of peat. The moisture content of the mixture was 40% and the inoculant was stored at room temperature in sterile polythene bags. At the time of use,the peat contained 10^6 cfu per gram of peat.

G.SELECTION OF SOIL FOR POT CULTURE EXPERIMENTS

Red soil and sand were mixed in the ratio of 3:1 and the mixture was taken in pots.This mixture is advantageous to root proliferation by preventing clumping of red soil.

H. EXPERIMENTAL DESIGN

The experimental design was a Factorialy Completely Randomized Design with 10 replications at each elevation.

I. BIOMETRIC OBSERVATIONS

Plants were carefully uprooted from the pots at 15 days interval till the harvesting period (105 days), starting from the 30th day. They were washed and then observed for the physiological parameters such as shoot length, root length and plant fresh weight.

(i) Shoot and root length

The shoot and root lengths were measured separately and expressed in cm.

(ii) Plant fresh weight

The plant samples were weighed and expressed in gram.

J. BIOCHEMICAL ANALYSIS

Plant samples were uprooted on 30,45,60,75,90 and 105th day after sowing (DAS). They were analysed for the following biochemical parameters in leaf, shoot and root:-

Glutamine synthetase, Glutamate synthase, Glutamate dehydrogenase, Aspartate amino transferase, Asparagine

synthetase, Nitrate reductase, Nitrite reductase,Asparaginase, Glutaminase, Urease,Xanthine dehydrogenase, Allantoinase, Uricase, ureides, IAA, Gibberellins,total proteins, phenols and orthodihydric phenols. Nitrogen, phosphorus and potassium levels were estimated in the whole plants after drying.

(i) Estimation of Glutamine synthetase (GS)

Glutamine synthetase activity was assayed by the method of Shapiro and Stadtman(1970). The details of the procedure are given in Appendix I.

(ii) Glutamate synthase(GOGAT)

Glutamate synthase activity was assayed by the method of Tempest *et al.*,(1970). The details of the method are given in Appendix II.

(iii)Glutamate dehydrogenase (GDH)

Glutamate dehydrogenase activity was assayed by the method of Doherty (1970). The procedure of the method is presented in Appendix III.

(iv)Aspartate amino transferase (AAT)

Aspartate amino transferase activity was assayed by the method of Bergmeyer and Bernt (1974) as explained in Appendix IV.

(v) Asparagine synthetase (AS)

Asparagine synthetase activity was determined by the method of Ravel (1970). Appendix V gives the details of the method.

(vi)Nitrate reductase

Nitrate reductase activity was assayed by the method of Hageman and Reed (1980). which is described in

Appendix VI.

(vii) Nitrite reductase

Nitrite reductase activity was determined by the method of Vega *et al.*,(1980) which is detailed in Appendix VII.

(viii) Asparginase

Asparaginase activity was determined by Nesslerisation method (Farnden and Robertson, 1980) as given in Appendix VIII.

(ix) Glutaminase

Glutaminase activity was determined by Nesslerisation method (Farnden and Robertson, 1980) as explained in Appendix IX.

(x) Urease

Urease activity was determined by the method of (Sumner, 1955) as shown in Appendix X.

(xi) Xanthine dehydrogenase (XDH)

Xanthine dehydrogenase activity was assayed by the method of Boland (1981). The details of the method are given in Apppendix XI.

(xii) Allantoinase

Allantoinase acitivity was assayed by the method of Vogels and van der Drift (1970) as given in Appendix XII.

(xiii) Uricase

Uricase activity was assayed by the method of Muller and Moller (1969). This is described in Appendix XIII.

(xiv) Ureides

Ureides were determined by the method of Young and Conway (1942). The details of the method are

given in Appendix XIV.

(xv) Indole acetic acid (IAA)

Indole acetic acid was determined by the method of Gorden and Paleg (1957). The details of the method are given in Appendix XV.

(xvi) Gibberellins

Gibberellins was determined by the method of Graham and Henderson (1961) as given in Appendix XVI.

(xvii) Total Proteins

The total Protein content was determined by Bradford's method (1976), which is described in Appendix XVII.

(xviii) Total phenol content

The total Phenol content of the plant is reported to render the plant resistance to certain insects. In the present study the total Phenol and OD Phenol contents were analysed in order to elucidate whether the *Azospirillum* application influenced the plant's defence against insects. Phenols were determined by Folin-Ciocalteu method (Bray and Thorpe,1954). The details of the procedure are given in Appendix XVIII.

(xix) Ortho dihydric phenols

OD phenols was determined by Arnow's method (Mahadevan, (1966), as given in Appendix XIX.

Nutrient Analysis

The saplings were dried in an oven at 80 °C for 24 hours. The dried samples were powdered with a mortar and pestle. The powdered samples were used for the estimation of nitrogen,phosphorus and potassium.

(xx) Total nitrogen

Total nitrogen content of the plant was estimated by Microkjeldahl method (Humphries, 1956). The details of the procedure are given in Appendix XX.

(xxi) Phosphorus

Phosphorus content was estimated by the method of Jackson (1973), the details of which are given in Appendix XXI.

(xxii) Potassium

Potassium content of the plant sample was estimated by the method of Jackson (1973). The details of the procedure are given in Appendix XXII.

(xxiii)IAA from culture filtrate

Indole acetic acid was determined by the method of Gorden and Paleg (1957). The detailed procedure is given in Appendix XXIII.

K.STATISTICAL ANALYSIS

The data are subjected to statistical scrutinity by following standard statistical procedures as given by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The present investigation was undertaken to study the process of nitrogen assimilation and the mode of transport of fixed nitrogen in Sorghum (C_4) and Finger millet (C_3) plants. The results of series of experiments conducted are presented in this chapter.

4.1.INDOLE ACETIC ACID PRODUCTION BY *AZOSPIRILLUM* STRAINS

Indole acetic acid production by the *Azospirillum* strains was determined and the results are presented in Table I.

TABLE - I
IAA PRODUCTION BY *AZOSPIRILLUM* STRAINS

STRAINS	IAA PRODUCTION µg/ml
AZ204	41.2
AZ208	38.6
FT326	61.3

The strains used in the present study included *A.lipoferum* (AZ204), *A.halopraeferens* (AZ208) and *A.brasilense* (FT326). The strain FT326 recorded the highest IAA production in seven days. Strains were different in their ability to produce IAA.

4.2.GLUTAMINE SYNTHETASE

Tables II and III and Figures-1 and 2 present the activities of glutamine synthetase (GS) in leaf, shoot and root of the selected varieties of sorghum and finger millet as a result of inoculation with different strains of *Azospirillum* and external application of IAA.

Sorghum

Maximum activity of GS in leaf was found in FT326 inoculated plants. In shoot and root, AZ204 treated plants showed the maximum activity. All the treatments showed a significant increase in the activity of GS when compared to the control. Among the various stages, activity was found to be more at 45 DAS in all parts of the plant. There was significant interaction between treatments and varieties, stages and varieties as well as treatments and stages. In leaf, shoot and root drought tolerant variety (DT) registered higher activity than the drought susceptible variety (DS). In DT, activity of GS exhibited a biphasic behaviour with peaks at days 45 and 90 in leaves and days 45 and 75 in shoots and roots. In DS also GS activity exhibited a biphasic behaviour with peaks at days 45 and 90 in all parts of the plants. Maximum activity was observed in shoots, followed by roots and leaves.

Finger millet

All treatments influenced the leaf GS activity significantly with FT326 inoculation being superior followed

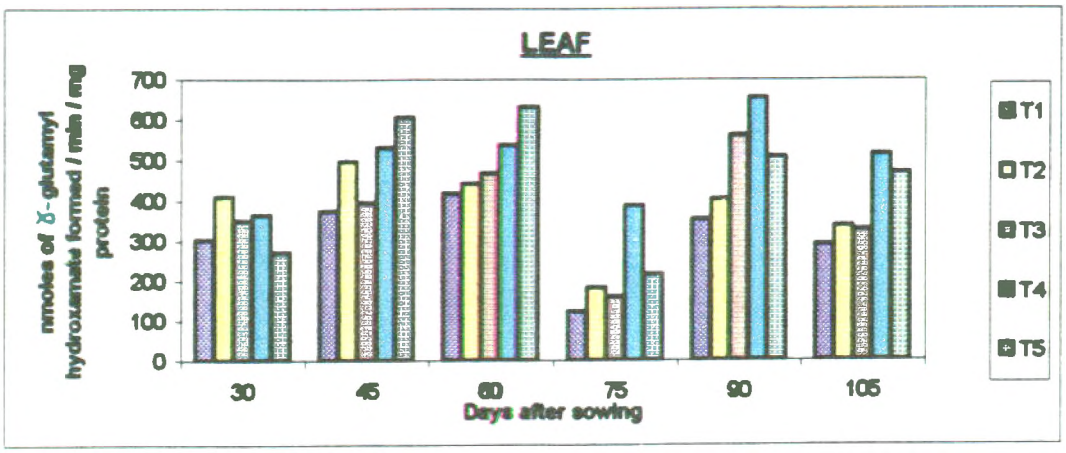
TABLE-II

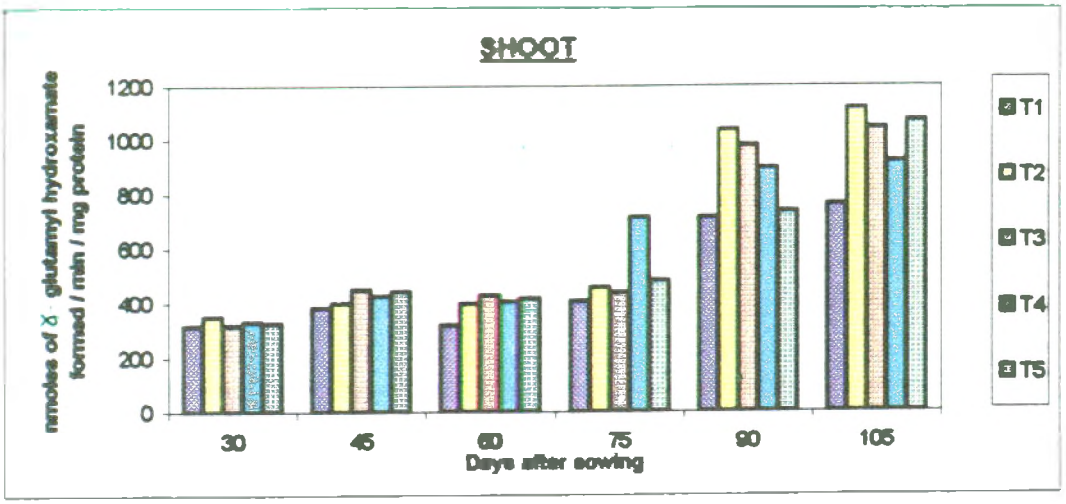
GLUTAMINE SYNTHETASE ACTIVITY (n moles of γ-glutamyl hydroxamate formed/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	989.68	1187.41	942.50	780.70	856.53	524.19	880.17	1124.21	1243.19	675.99	1102.42	1002.34	972.57	1020.12	949.13	1011.31	606.64	1404.84	759.91	869.06	933.48
	DS	1094.99	1251.68	854.99	448.13	633.19	362.81	774.30	860.82	1001.23	1673.86	455.81	649.27	280.73	820.29	898.13	1263.49	1099.30	671.85	885.20	305.98	853.99
	MEAN	1042.34	1219.55	898.75	614.42	744.86	443.50	827.23	992.52	1122.21	1174.93	779.12	825.81	626.65	920.20	923.63	1137.40	852.97	1038.35	822.56	587.52	893.74
AZ204 (T2)	DT	1243.83	1339.00	1054.72	1079.37	1179.84	588.22	1079.75	2044.18	2185.99	1097.89	2086.69	1315.45	1111.40	1640.27	1163.42	2306.84	782.89	2550.71	804.81	943.15	1425.30
	DS	1289.23	1426.75	909.77	648.13	863.52	440.61	929.67	1021.80	1605.96	1985.90	675.21	838.96	685.83	1135.63	1193.27	1624.49	1498.75	758.09	951.17	742.11	1127.98
	MEAN	1266.28	1382.88	982.25	863.75	1021.68	511.41	1004.71	1532.99	1895.97	1541.90	1381.00	1077.21	898.62	1387.95	1178.35	1965.67	1140.82	1654.40	877.99	842.63	1276.64
AZ208 (T3)	DT	801.95	1361.27	1014.19	965.41	994.18	704.88	973.65	1241.61	1383.03	727.58	2169.25	1097.64	1070.05	1281.53	1452.36	1581.68	814.09	1846.08	993.16	1270.96	1326.39
	DS	1088.53	1293.11	918.28	585.13	682.32	482.54	841.65	978.96	1241.96	1833.97	603.40	675.25	577.89	985.24	1123.44	1876.55	1562.71	882.69	912.59	569.33	1154.55
	MEAN	945.24	1327.19	966.24	775.27	838.25	593.71	907.65	1110.29	1312.50	1280.78	1386.33	886.45	823.97	1133.38	1287.90	1729.12	1188.40	1364.39	952.88	920.15	1240.47
IFT 326 (T4)	DT	1288.41	1320.10	1155.98	871.85	1084.41	636.52	1059.55	1647.69	2113.33	903.84	1892.55	956.18	777.96	1381.92	1061.88	1568.49	841.60	2422.67	1082.51	1183.15	1360.05
	DS	1204.92	1604.92	919.67	579.64	901.96	654.24	977.56	992.33	1565.25	1705.28	491.61	984.54	713.17	1075.36	971.00	1889.97	1492.74	909.46	1074.78	354.53	1115.41
	MEAN	1246.67	1462.51	1037.83	725.75	993.18	645.38	1018.55	1320.01	1839.29	1304.56	1192.08	970.36	745.57	1228.64	1016.44	1729.23	1167.17	1666.07	1078.65	768.84	1237.73
IAA/T (T5)	DT	1195.00	1284.57	851.65	704.88	754.61	597.86	898.10	1149.45	1466.19	750.42	1615.51	923.34	803.42	1118.06	999.98	1084.07	693.18	1515.36	913.37	1008.79	1035.79
	DS	1112.16	1294.10	1060.26	649.89	713.07	586.52	902.67	974.96	1014.85	2184.47	664.45	961.59	449.20	1041.59	911.04	1755.38	1675.22	701.68	1042.43	450.16	1089.32
	MEAN	1153.58	1289.34	955.96	677.39	733.84	592.19	900.38	1062.21	1240.52	1467.45	1139.98	942.47	626.31	1079.82	955.51	1419.73	1184.20	1108.52	977.90	729.48	1062.56
	MEAN	1130.82	1336.29	968.20	731.31	866.36	557.24	931.70	1203.60	1482.10	1353.92	1175.70	940.46	744.22	1150.00	1072.37	1596.23	1106.71	1366.34	941.99	769.72	1142.23
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.03	0.02	0.04	0.05	0.05	0.08	0.11	0.04	0.03	0.05	0.06	0.07	0.10	0.15
	CD(5%)	0.07	0.05	0.08	0.10	0.11	0.18	0.25	0.06	0.04	0.07	0.09	0.10	0.16	0.22	0.09	0.05	0.09	0.12	0.13	0.21	0.30

Figure - 1

GS ACTIVITY IN DTV OF FINGER MILLET





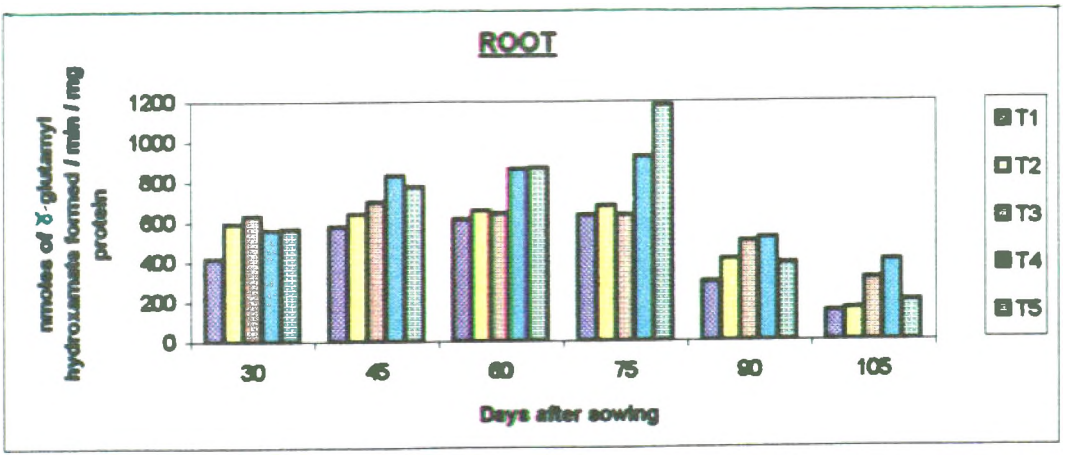


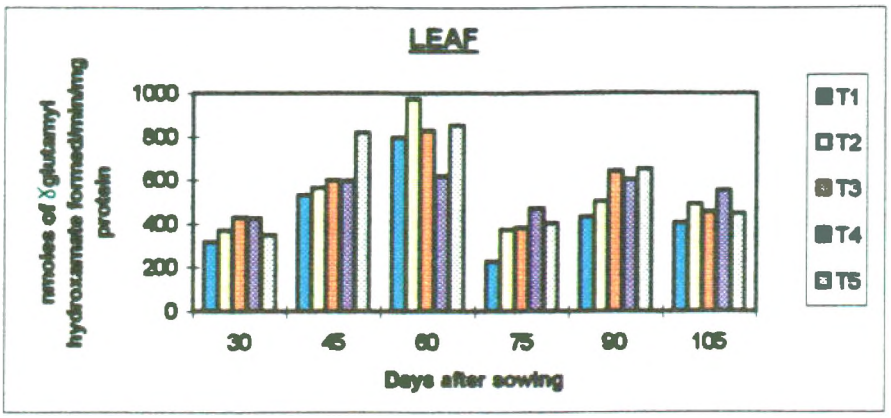
TABLE – III

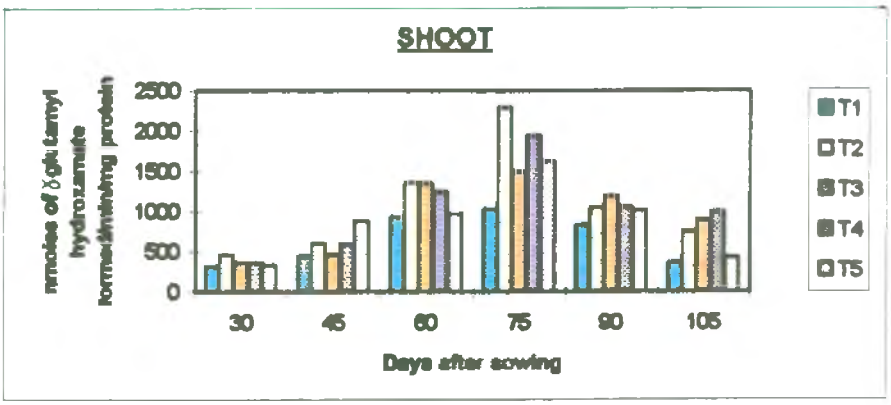
GLUTAMINE SYNTHETASE ACTIVITY (n moles of γ-glutamyl hydroxamate formed/min/mg prot.) IN DIFFERENT PARTS OF DROUGHT TOLERANT(DT) AND DROUGHT SUSCEPTIBLE(DS) VARIETIES OF FINGER MILLET

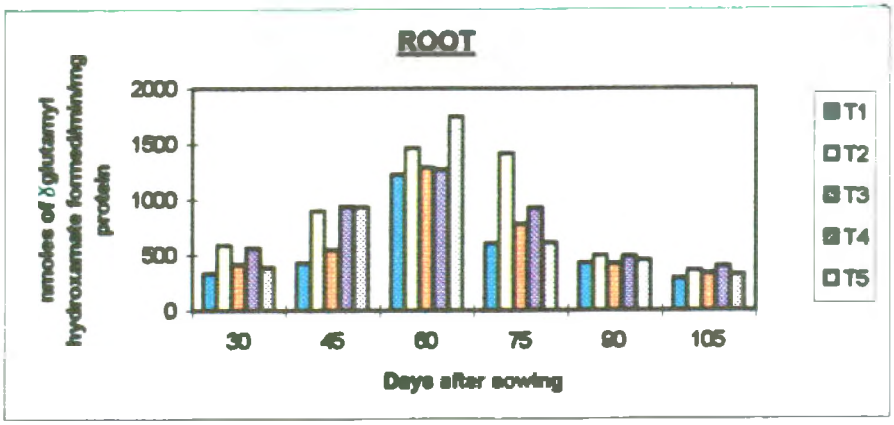
	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	1303.12	374.74	418.10	122.12	353.18	290.23	1310.25	1317.00	383.36	318.05	409.02	713.22	761.14	483.63	1415.67	580.14	614.30	632.75	298.60	150.06	1448.59
(T1)	DS	1316.15	532.80	794.85	227.07	430.55	404.43	1450.98	1312.05	447.49	939.06	1031.04	830.91	365.35	654.32	1333.46	428.55	1226.02	604.15	430.59	289.03	1551.97
	MEAN	1309.64	453.77	606.48	174.60	391.87	347.33	1380.61	1314.53	415.43	628.56	720.03	772.07	563.25	568.97	1374.57	504.35	920.16	618.45	364.60	219.55	1500.28
AZ204	DT	1409.54	496.93	441.22	182.14	403.20	336.80	1378.31	1350.47	401.50	398.91	458.26	1037.92	1114.85	626.99	1591.15	639.36	656.56	678.21	410.35	161.13	1522.79
(T2)	DS	1370.50	565.05	973.24	371.32	508.97	490.54	1545.60	1456.88	608.06	1364.48	2293.04	1050.65	758.68	1087.63	1588.15	895.80	1465.68	1417.29	490.72	356.78	1869.07
	MEAN	1390.02	530.99	707.23	276.73	453.09	413.67	1461.95	1403.68	504.78	881.70	1375.65	1044.29	933.77	857.31	1589.65	767.58	1061.12	1047.75	450.54	258.96	1695.93
AZ208	DT	1350.50	394.72	467.56	159.74	561.07	327.88	1376.91	1318.03	451.25	429.68	439.50	979.47	1043.85	610.30	1629.82	702.98	644.23	634.89	501.66	315.33	1571.49
(T3)	DS	1428.36	600.36	827.87	381.18	641.94	455.65	1555.89	1362.77	464.47	1356.67	1496.04	1191.53	893.79	960.88	1419.19	542.96	1288.08	780.42	420.36	302.08	1630.51
	MEAN	1389.43	497.54	647.72	270.46	601.51	391.77	1466.40	1340.40	457.86	893.18	967.77	1085.50	968.82	785.59	1524.51	622.97	966.16	707.66	461.01	323.71	1601.00
FT 326	DT	1364.31	529.92	537.55	387.25	655.58	514.81	1498.24	1330.33	426.70	405.64	713.47	896.13	919.67	615.32	1559.93	829.83	864.44	924.06	518.53	402.99	1683.30
(T4)	DS	1495.61	597.34	619.11	471.58	602.19	553.53	1556.56	1361.26	595.54	1247.72	1945.87	1058.68	1002.72	1035.30	1560.48	935.83	1275.42	927.10	485.23	401.24	1764.22
	MEAN	1429.96	563.63	578.33	429.42	628.89	534.17	1527.40	1345.80	511.12	826.68	1329.67	977.41	961.20	825.31	1560.21	882.83	1069.93	925.58	501.88	402.12	1723.76
IAA/T	DT	1270.74	606.46	631.92	215.59	507.43	469.13	1450.21	1325.46	443.25	416.97	483.55	735.38	1071.49	579.35	1567.50	777.48	870.55	1179.11	389.91	200.76	1664.22
(T5)	DS	1348.57	819.76	853.40	401.23	649.62	447.96	1586.76	1327.03	891.12	977.30	1631.01	1015.52	426.94	878.15	1387.77	932.94	1744.96	608.91	449.39	325.21	1741.53
	MEAN	1309.65	713.11	742.66	308.41	578.53	458.55	1518.48	1326.25	667.19	697.14	1057.28	875.45	749.22	728.75	1477.64	855.21	1307.76	894.01	419.65	262.99	1702.87
	MEAN	1365.74	551.81	656.48	291.92	530.77	429.10	1470.97	1346.13	511.27	785.45	1090.08	950.94	835.25	753.19	1505.31	726.59	1065.02	838.69	439.53	293.46	1644.77
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.05	0.07	0.11	0.03	0.02	0.03	0.04	0.04	0.07	0.09	0.04	0.02	0.04	0.05	0.06	0.09	0.13
	CD(5%)	0.06	0.04	0.07	0.09	0.09	0.15	0.21	0.05	0.03	0.06	0.08	0.08	0.13	0.19	0.08	0.05	0.08	0.11	0.12	0.18	0.26

Figure - 2

GS ACTIVITY IN DSV OF FINGER MILLET







by IAA -T, AZ208, AZ204 inoculation and then control. Almost the same trend was observed in root. Application of AZ204 exhibited the maximum effect in shoot. Significant difference in GS activity was recorded over the various time points with a peak activity after 60 DAS. Significant interaction was recorded between all the combinations studied. DS expressed greater activity of GS than DT. In DT, activity of GS in leaf and root increased with plant growth, attaining a peak at 90 days and declined at 105, but in shoot there was decline at 60 DAS. In DS, activity declined at 75 and 105 DAS in leaf, but in shoot and root decline in activity was seen during maturity.

Our results are in agreement with the report of Luthra *et al.*(1983) who observed increased GS activity in pigeon pea shoots attaining a peak at 75 days and declining thereafter till maturity. However, in leaves and roots the enzyme exhibited a biphasic behaviour at 60 and 105 DAS in leaves and 75 and 105 DAS in roots.

Amarjit and Singh (1985) reported maximum GS activity in nodules of pigeon pea at 15th day. Thereafter it declined and remained almost constant till 45th day and again increased.

GS is the major enzyme responsible for the primary assimilation of ammonia. Greater activity in shoot could be of significance for reassimilation of fixed nitrogen, which might then be transported to the root.

GS activity increased during early development but then remained at a constant level throughout the time of study. This maintenance of GS activity with increase in age would enable the leaf to assimilate ammonia arising from sources other than nitrate assimilation.

4.3.GLUTAMATE SYNTHASE

Tables IV and V and Figures-3 and 4 depict the activities of glutamate synthase (GOGAT) in different parts of drought susceptible and resistant varieties of sorghum and finger millet upon inoculation of different strains of *Azospirillum* and external application of IAA.

Sorghum

FT326 inoculated plants showed significantly higher GOGAT activity. Significant increase in the activity was seen in all the treatments when compared to the control. Among the various stages,activity was found to be more at 90 DAS in all the three parts of the plant. In both the varieties, GOGAT activity showed a maximum increase as a result of FT326 treatment and was higher in the DS than in DT.

Significant interaction was observed between treatments and varieties, stages and varieties and varieties and stages. The specific activity of the enzyme showed alternate decrease and increase at various time points of the study in leaf and shoot, while in root, an initial decrease and then a continuous increase followed by a final dip was observed. Maximal GOGAT activity was observed in roots followed by shoot and then leaf.

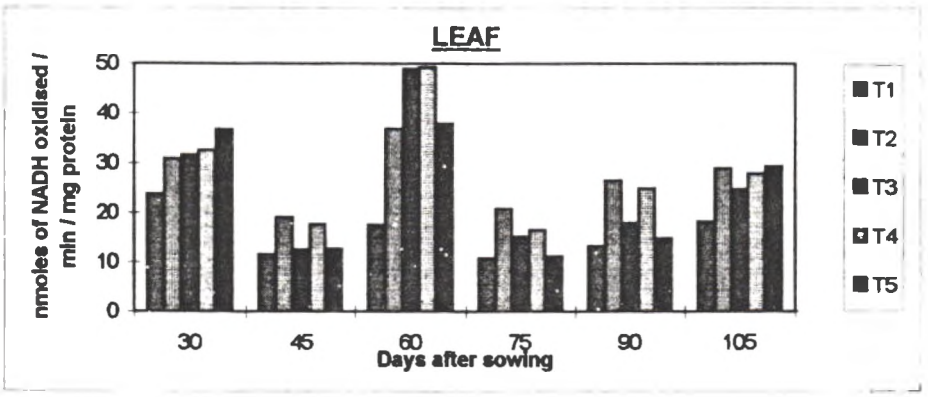
TABLE-IV

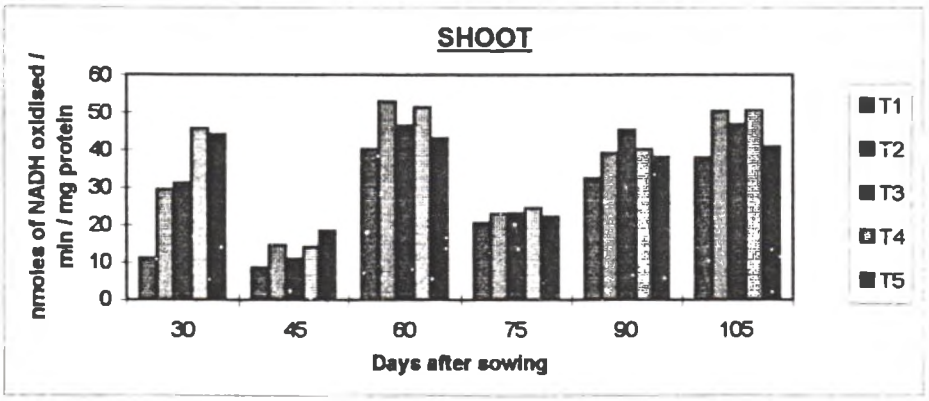
GLUTAMATE SYNTHASE ACTIVITY (n moles of NADH oxidised/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

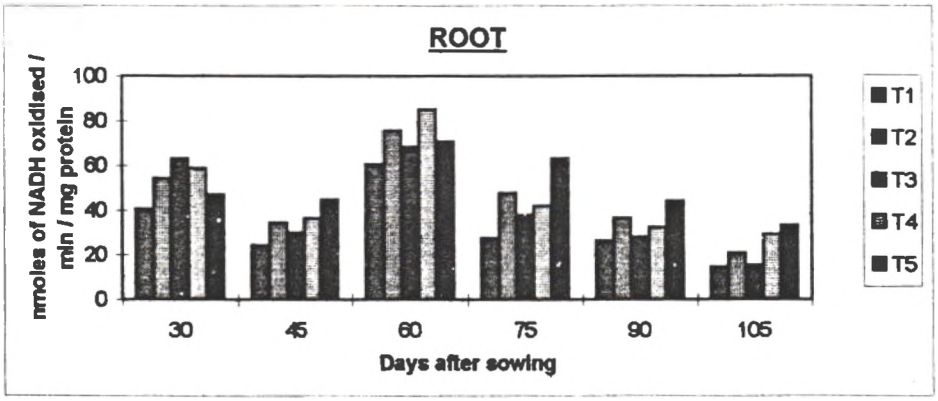
	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	140.56	12.55	16.98	8.08	10.83	11.21	116.70	131.64	9.49	16.75	12.32	18.48	38.84	121.25	138.43	15.50	24.98	33.34	42.82	39.69	32.46
(T1)	DS	117.91	12.61	24.58	33.71	68.09	63.67	136.76	135.58	15.98	57.58	74.77	86.80	71.63	157.06	154.86	39.13	57.57	85.93	104.38	80.05	70.32
	MEAN	129.24	12.58	20.78	20.90	39.46	37.44	126.73	133.61	12.74	37.17	43.55	52.64	55.24	139.16	146.65	27.32	41.28	59.64	73.60	59.87	51.39
AZ204	DT	155.36	18.31	21.85	11.82	15.04	18.92	123.55	164.70	22.56	33.82	19.79	22.72	52.00	135.93	152.73	21.01	31.05	58.47	65.97	43.20	45.41
(T2)	DS	123.63	16.91	34.39	41.47	76.42	91.53	147.39	161.39	24.27	65.43	93.14	123.44	88.02	175.95	193.33	51.33	73.61	108.67	132.64	96.77	92.73
	MEAN	139.50	17.61	28.12	26.65	45.73	55.23	135.47	163.05	23.42	49.63	56.47	73.08	70.01	155.94	173.03	36.17	52.33	83.57	99.31	69.99	69.07
AZ208	DT	159.40	14.55	29.63	14.33	16.57	17.35	125.31	156.46	13.78	22.22	17.54	19.40	61.98	131.90	160.25	15.56	31.84	37.24	53.99	38.70	39.60
(T3)	DS	139.20	18.65	41.78	78.18	94.60	79.13	158.59	140.09	27.52	74.62	113.89	120.78	78.10	175.83	168.59	76.88	82.54	199.99	113.65	87.59	104.87
	MEAN	149.30	16.60	35.71	46.26	55.59	48.24	141.95	148.28	20.65	48.42	65.72	70.09	70.04	153.86	164.42	46.22	57.19	118.61	83.82	63.15	72.23
FT 326	DT	158.61	16.95	47.45	13.58	23.88	26.94	131.23	166.65	20.65	61.83	13.14	39.52	40.19	140.33	162.21	19.25	41.39	46.37	58.30	51.81	46.55
(T4)	DS	144.18	24.50	78.17	86.46	103.04	71.37	167.95	166.55	32.97	70.52	118.15	142.38	98.10	188.11	197.55	70.26	83.97	126.37	141.33	104.05	103.92
	MEAN	151.40	20.73	62.81	50.02	63.46	49.16	149.59	166.60	26.81	66.18	65.65	90.95	69.15	164.22	179.88	44.76	62.68	86.37	99.82	77.93	75.24
IAA/T	DT	164.14	13.98	31.29	13.63	14.94	17.90	125.98	137.44	17.36	39.45	27.53	47.82	56.24	137.64	158.07	22.49	43.89	40.52	44.56	40.01	41.59
(T5)	DS	149.47	17.78	53.64	64.01	84.79	74.05	157.29	158.59	28.63	81.20	88.07	103.78	90.54	175.14	178.31	72.64	81.41	105.33	129.47	97.82	94.16
	MEAN	156.81	15.88	42.47	38.82	49.87	45.98	141.63	148.02	23.00	60.33	57.80	75.80	73.39	156.39	168.19	47.57	62.65	72.93	87.02	68.92	67.88
	MEAN	145.25	16.68	37.98	36.53	50.82	47.21	139.08	151.91	21.32	52.34	57.83	72.51	67.56	153.91	166.43	40.41	55.23	84.22	88.71	67.97	67.16
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.04	0.06	0.09	0.03	0.02	0.04	0.05	0.05	0.08	0.11	0.03	0.02	0.03	0.04	0.04	0.07	0.10
	CD(5%)	0.05	0.03	0.06	0.07	0.08	0.12	0.17	0.06	0.04	0.07	0.09	0.10	0.16	0.22	0.06	0.04	0.06	0.08	0.09	0.14	0.19

Figure - 3

GOGAT ACTIVITY IN DTV OF FINGER MILLET







TABLE– V

GLUTAMATE SYNTHASE ACTIVITY (n moles of NADH oxidised/min/mg prot.) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF *FINGER MILLET*

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	123.71	11.59	17.47	10.75	13.27	18.24	115.84	111.28	8.62	40.11	20.59	32.42	37.94	125.16	140.43	24.45	60.60	27.70	26.38	14.62	132.36
	DS	128.89	18.85	32.26	42.28	25.33	30.23	129.64	133.09	7.71	13.35	51.01	23.59	40.35	128.18	143.36	34.07	40.79	63.82	50.20	72.86	150.85
	MEAN	126.30	15.22	24.87	26.52	19.30	24.24	122.74	122.19	8.17	26.73	35.80	28.01	39.15	126.67	141.90	29.26	50.70	45.76	38.29	43.74	141.61
AZ204 (T2)	DT	130.94	19.03	36.84	20.83	26.54	28.93	127.19	129.43	14.57	52.97	23.00	39.18	50.29	134.91	154.43	34.50	75.51	47.59	36.43	21.03	144.92
	DS	132.73	21.97	41.14	60.75	38.48	40.13	142.53	151.50	13.61	35.56	75.36	30.39	65.82	148.71	153.90	38.75	62.15	89.20	65.95	86.45	166.07
	MEAN	141.84	20.50	38.99	40.79	32.51	34.53	134.86	140.47	14.09	44.27	49.18	44.79	58.06	141.81	154.17	36.63	68.83	68.40	51.19	53.74	155.49
AZ208 (T3)	DT	131.71	12.35	48.95	15.17	17.90	24.84	125.19	131.26	10.95	46.60	23.08	45.28	46.90	134.01	163.34	30.11	68.35	37.98	28.10	15.47	140.56
	DS	131.41	17.35	33.19	56.17	20.74	35.38	132.37	137.18	12.48	22.48	63.00	30.72	52.03	136.32	150.84	34.29	70.07	79.69	69.05	91.03	165.83
	MEAN	131.56	14.95	41.07	35.67	19.32	30.11	128.78	134.22	11.72	34.54	43.04	38.00	49.47	135.16	157.09	32.20	69.21	58.84	48.58	53.25	153.19
FT 326 (T4)	DT	132.55	17.70	49.22	16.54	24.98	27.96	128.16	145.67	14.07	51.42	24.54	40.22	50.51	137.74	159.01	36.55	84.94	41.96	32.38	29.23	147.35
	DS	146.18	18.93	39.36	68.44	49.16	51.50	148.93	143.38	37.66	42.52	79.24	46.33	60.96	151.68	162.10	50.05	81.13	95.14	64.76	97.75	175.16
	MEAN	139.37	18.32	54.29	42.49	37.07	39.73	138.54	144.53	25.87	46.97	51.89	43.28	55.74	144.71	160.56	43.30	83.04	68.55	48.57	63.49	161.25
IAA/T (T5)	DT	136.74	12.74	37.89	11.22	14.82	29.29	123.78	144.10	18.37	43.15	22.30	38.04	40.97	134.49	147.03	44.79	70.68	68.27	44.31	33.36	150.57
	DS	146.75	19.32	33.38	55.99	30.32	39.13	137.48	146.06	38.23	45.23	63.30	51.41	55.93	150.03	154.09	44.26	68.94	83.68	61.79	85.65	166.40
	MEAN	141.75	16.03	35.64	33.61	22.57	34.21	130.63	145.08	28.30	44.19	42.80	44.73	48.45	142.26	150.56	44.53	69.81	73.48	53.05	59.51	158.49
	MEAN	136.16	17.00	38.97	35.81	26.15	32.56	131.11	137.30	17.63	39.34	44.54	39.76	50.17	138.12	152.85	37.18	68.32	63.00	47.94	54.75	154.01
		T	V	B	TV	VB	TB	TVB	T	V	B	TV	VB	TB	TVB	T	V	B	TV	VB	TB	TVB
	SED	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.03	0.02	0.04	0.05	0.05	0.08	0.11	0.03	0.02	0.04	0.05	0.05	0.08	0.11
	CD(5%)	0.07	0.05	0.08	0.10	0.11	0.18	0.25	0.06	0.04	0.07	0.09	0.10	0.16	0.22	0.07	0.04	0.07	0.09	0.10	0.16	0.23

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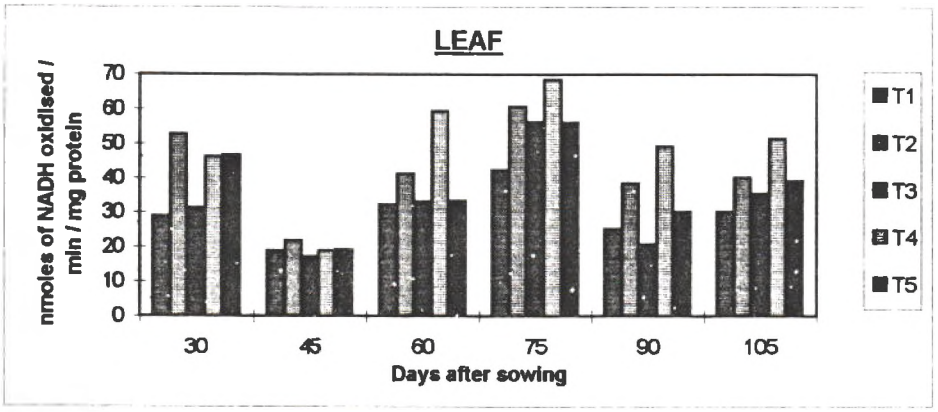
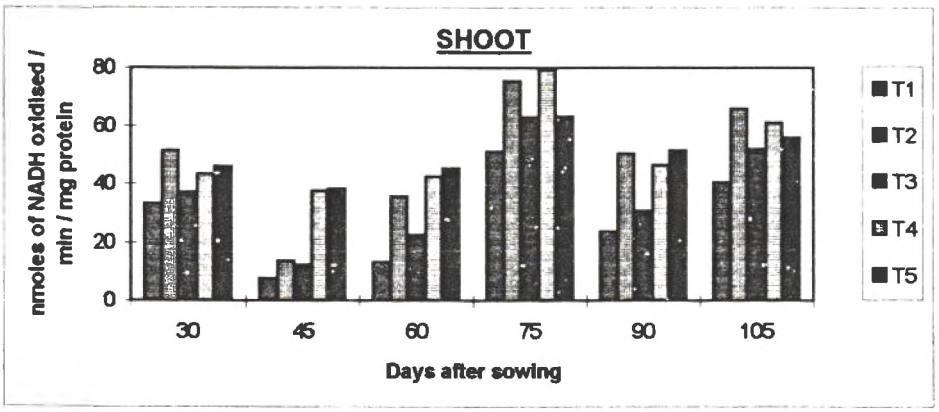
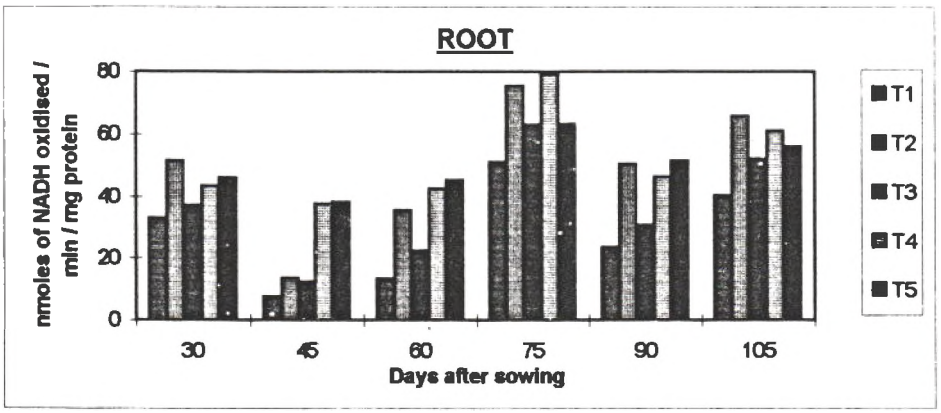
Figure - 4

GOGAT ACTIVITY IN DSV OF FINGER MILLET







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Finger millet

A similar trend was observed as in sorghum except in leaves where AZ204 caused the maximal increase. Significant increase in activity was seen in all the treatments when compared with the control. Activity peaked at 60 DAS in leaf and root and at 105 DAS in shoot. Significant interaction was seen between treatments and varieties, stages and varieties and treatments and stages. DS registered higher activity than DT in all the parts of the plant. Similar to sorghum, maximum activity was recorded in root, followed by shoot and leaf.

Tobin *et al.* (1985) reported that the GOGAT activity increased considerably during leaf development in the presence of light. Amarjit and Singh (1985) reported that the activity in pigeon pea nodules fluctuated through various stages of growth.

4.4.GLUTAMATE DEHYDROGENASE

The activities of GDH in different parts of drought susceptible and tolerant varieties of sorghum and finger millet on inoculation of different strains of *Azospirillum* and also on external application of IAA are given in Tables VI and VII.

Sorghum

The different treatments of the plant evinced statistically significant difference in the GDH activity of all the tissues as compared to control. FT326 application

TABLE – VI

GLUTAMTE DEHYDROGENASE ACTIVITY (n moles of NADH oxidised/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT(DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	129.36	13.26	47.31	36.99	50.29	32.35	134.93	122.21	13.04	35.26	19.83	43.19	30.01	127.26	141.01	33.58	46.03	66.19	75.04	53.20	152.51
	DS	126.36	35.76	64.60	13.33	26.36	12.93	129.89	115.14	23.99	51.93	44.47	60.03	14.39	134.99	121.31	48.58	57.85	46.70	65.81	52.12	148.73
	MEAN	127.86	24.51	55.96	25.16	38.33	22.64	132.41	118.68	18.52	43.60	32.15	51.61	22.20	131.12	131.16	41.08	51.94	56.45	70.43	52.66	150.62
AZ204 (T2)	DT	149.76	17.98	59.56	40.51	75.76	53.07	149.44	138.36	25.42	49.58	30.05	68.03	49.76	143.53	152.11	43.51	61.00	84.05	107.18	77.54	170.90
	DS	136.81	41.48	84.79	22.74	45.21	33.75	144.13	131.85	57.73	73.14	70.87	83.16	19.67	156.07	136.14	53.58	72.13	61.24	74.35	60.46	159.65
	MEAN	143.29	29.73	72.18	31.63	60.49	43.41	146.79	135.11	41.58	61.36	50.46	75.60	34.72	149.80	144.13	48.55	66.57	72.65	90.77	69.00	165.27
AZ208 (T3)	DT	137.28	23.64	64.03	30.27	67.50	52.84	145.93	140.68	19.11	27.64	21.64	57.85	40.85	134.63	160.56	36.50	56.33	75.89	90.90	62.70	163.81
	DS	153.56	60.44	81.93	37.71	59.26	17.10	151.67	124.65	27.86	67.98	67.32	98.27	13.26	148.89	147.33	61.67	73.79	54.87	68.17	53.48	159.80
	MEAN	145.42	42.04	72.98	33.99	63.38	34.97	148.80	132.67	23.49	47.81	44.48	75.06	27.05	141.76	153.95	49.09	65.06	65.13	79.54	58.09	161.81
IFT 326 (T4)	DT	159.66	24.24	84.16	44.65	73.92	66.40	158.87	138.18	28.35	50.48	29.80	65.75	54.10	144.44	165.54	45.84	60.79	82.61	97.61	80.27	172.11
	DS	151.65	65.48	94.63	25.31	37.96	45.39	153.40	145.84	52.86	80.01	57.23	74.81	29.48	156.71	148.42	64.76	80.22	71.66	80.45	65.91	168.57
	MEAN	155.66	44.86	89.40	34.98	55.94	56.00	156.14	142.01	40.61	65.25	43.52	70.88	41.79	150.57	156.98	55.30	70.51	77.14	89.03	73.09	170.34
IAA/T (T5)	DT	130.03	15.00	71.95	53.80	64.80	55.36	151.82	130.33	12.85	55.51	22.19	55.12	43.88	136.55	150.89	35.88	50.21	78.74	88.52	73.54	162.03
	DS	151.15	66.39	90.20	13.01	57.08	31.04	151.48	130.89	34.71	70.32	46.17	72.35	26.67	146.85	141.34	35.17	69.74	57.33	71.70	66.79	160.35
	MEAN	150.59	40.70	81.08	33.41	60.94	43.20	151.65	130.61	23.78	62.92	34.18	63.74	34.97	141.70	146.12	45.23	59.98	65.54	80.11	70.17	161.19
	MEAN	144.56	36.37	74.32	31.83	55.81	40.04	147.16	131.81	29.59	56.19	40.96	67.26	32.15	142.99	146.47	47.85	62.81	67.38	81.97	64.60	161.85
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.04	0.03	0.04	0.06	0.06	0.10	0.14	0.03	0.02	0.03	0.04	0.04	0.07	0.10	0.04	0.03	0.04	0.06	0.06	0.10	0.14
	CD(5%)	0.08	0.05	0.09	0.12	0.13	0.20	0.28	0.06	0.04	0.06	0.08	0.09	0.14	0.20	0.08	0.05	0.09	0.11	0.12	0.19	0.27

TABLE–VII

GLUTAMATE DEHYDROGENASE ACTIVITY (n moles of NADH oxidised/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT(DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

		LEAF							SHOOT							ROOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	17.27	41.26	15.47	28.14	17.16	38.84	26.36	27.10	38.83	27.26	11.53	21.49	46.49	28.78	25.72	49.53	33.91	15.25	35.62	26.08	31.02
	DS	7.54	16.21	31.01	48.52	13.54	35.25	25.35	23.12	15.94	40.84	67.94	26.57	47.13	36.92	39.69	45.50	54.27	72.36	33.95	63.27	51.51
	MEAN	12.41	28.74	23.24	38.33	15.35	37.05	25.85	25.11	27.39	34.05	39.74	24.03	46.81	32.85	32.71	47.52	44.09	43.81	34.79	44.68	41.26
AZ204 (T2)	DT	21.30	45.62	27.34	37.30	23.66	59.84	35.84	45.20	60.71	50.86	17.48	42.58	64.52	46.89	51.82	64.06	52.54	22.14	51.30	41.17	47.17
	DS	15.42	30.95	39.02	58.80	22.19	56.71	37.18	40.75	20.61	64.63	80.73	42.35	69.16	53.04	54.50	65.11	80.70	91.72	60.26	79.71	72.00
	MEAN	18.36	38.29	33.18	48.05	22.92	58.28	36.51	42.98	40.66	57.75	49.11	42.47	66.84	49.97	53.16	64.59	66.62	56.93	55.78	60.44	59.59
AZ208 (T3)	DT	38.81	54.02	22.21	26.32	20.37	62.32	37.34	30.13	48.64	31.73	18.39	24.26	55.12	34.71	45.36	52.95	46.33	22.11	40.47	31.98	39.87
	DS	14.71	26.81	42.41	60.36	14.07	60.07	36.41	42.12	18.63	74.29	81.55	44.37	58.20	53.19	46.43	54.10	64.73	83.21	50.38	74.77	62.27
	MEAN	26.76	40.42	32.31	43.34	17.22	61.20	36.87	36.12	33.64	53.01	49.97	34.32	56.66	43.95	45.90	53.53	55.53	52.66	45.43	53.38	51.07
IFT 326 (T4)	DT	21.67	49.84	20.17	29.04	18.41	70.89	35.00	30.77	43.00	31.26	22.03	30.90	65.30	37.18	54.39	69.58	60.07	21.62	50.73	41.26	49.61
	DS	17.26	27.28	35.72	67.25	16.81	67.79	38.68	49.90	30.78	65.55	88.08	41.64	61.00	56.16	59.70	64.47	75.68	88.43	58.75	81.54	71.43
	MEAN	19.47	38.56	27.95	48.15	17.61	69.34	36.84	40.34	36.89	48.41	55.06	36.17	63.15	46.67	57.05	67.02	67.88	55.03	54.74	61.40	60.52
IAA/T (T5)	DT	37.86	58.02	36.04	41.38	15.06	66.85	42.54	36.23	53.68	29.47	12.85	41.69	58.07	38.67	48.54	57.28	41.85	32.73	47.83	38.43	44.44
	DS	9.24	32.73	48.89	58.85	27.18	53.96	38.48	48.45	15.06	50.37	71.80	54.23	58.47	49.73	50.67	53.46	70.24	81.18	47.71	68.72	62.00
	MEAN	23.55	45.38	42.47	50.12	21.12	60.41	40.51	42.34	34.37	39.92	42.33	47.96	58.27	44.20	49.61	55.37	56.05	56.96	47.77	53.58	53.22
	MEAN	20.11	38.27	31.83	45.60	18.84	57.25	35.32	37.38	34.59	46.63	47.24	36.97	58.35	43.52	47.68	57.60	58.03	53.08	47.70	54.69	53.13
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.03	0.03	0.05	0.06	0.03	0.02	0.03	0.04	0.04	0.07	0.10	0.02	0.01	0.02	0.03	0.03	0.05	0.07
	CD(5%)	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.06	0.03	0.06	0.08	0.09	0.13	0.19	0.04	0.02	0.04	0.05	0.06	0.09	0.13

resulted in significantly higher activity of GDH in all the selected tissues of the plant than by other treatments. Maximal activity of the enzyme was noticed in leaf after 60 DAS and after 90 DAS in shoot and root. GDH activity was greater in leaf and root of DT while a reverse effect was observed in DS.

The interaction between treatments and varieties was found to be superior in leaf and shoot of the plants applied with FT326. Significantly higher activity of GDH was recorded after 90 days when the stage and variety interaction was considered. The overall picture revealed maximum GDH activity in root followed by leaf and then shoot.

In both the varieties of sorghum the specific activity of GDH increased continually and steadily from vegative stage till pod-setting stage and then decreased in root. But in leaves and shoot it declined during the flowering stage and increased in the pod-setting stage with a final drop at maturation.

Finger millet

GDH activity was found to be greater in leaf of plants inoculated with IAA, while shoot and root of FT326 treated plants exhibited maximal activity. All the treatments were more effective in elevating the GDH activity than the control. With regard to the stages, GDH activity was maximum in leaf and shoot at 105 DAS, whereas,it peaked at 60 DAS in the root. Between the DT and DS no significant difference in the activity existed in leaves while it was

found to be significantly different in root and shoot.

The interaction between stages and varieties and treatments and stages was found to be significant in all the tissues. However, no significant difference was recorded between treatments and varieties in leaves. GDH activity was maximum in root, similar to sorghum. Undulations in the activity was found during the various time points with a drop during pod-setting and a subsequent increase.

The above results are in agreement with the reports of various workers (Groat and Vance, 1982; Loyola- Vargas and Jimenez, 1984; Smirnoff and Stewart, 1987). Roots have been demonstrated to be the major site of ammonia assimilation and GDH levels are higher in this tissue and in senescing organs (Westby *et al.*,1987).

In the present study, GS activity was more than GDH and GOGAT. The same was reported in various legume nodules by Boland *et al.* (1978). Higher activity of GOGAT was observed than the GDH activity in both the varieties. Higher activity of GS and GOGAT than GDH suggested that ammonia assimilation was carried out in the plant cytoplasm by the coupled enzyme system of the two enzymes GS-GOGAT. Sivakumar (1993) also reported that there was increased activity of GOGAT and GS when compared to GDH in rice roots.

Purushothaman *et al.*(1979) reported that ammonia assimilation was chiefly mediated through coupled reaction of GS and GOGAT. Yuan et al. (1990) reported that GS-GOGAT cycle

appeared to be the only pathway for ammonia assimilation in the developing rice grain. Ohyama and Kumazawa (1980) also reported that ammonia was assimilated by GS-GOGAT system. Kaush *et al.* (1984) found that assimilation of ammonia formed in the process of nitrogen fixation occurs primarily in the reaction catalysed by glutamine synthetase.

For efficient functioning of GDH, relatively high concentration of ammonia is reported to be present. Such levels of ammonia exceeded the assimilation capacity (Boland et al., 1980). In the present study, increased activity of GDH on 30 DAS may be due to the presence of high levels of ammonia produced by *Azospirillum*. The GDH system might have been consequently turned on actively in the initial stages of nitrogen fixation.

However, due to the transport of ammonia to other parts of plants and due to utilisation, ammonia concentration might have decreased at later stages and hence GS-GOGAT system probably started functioning at low concentration of ammonia effectively. This might probably be the reason for the low activity of GDH. This is in agreement with the findings of Magalhaes and Huber (1989) who noticed high GDH activity in roots of rice, maize and tomato plants in presence of ammonia.

4.5.ASPARTATE AMINO TRANSFERASE

Tables VIII and IX and Figures-5 and 6 present the activities of Aspartate amino transferase (AAT) in leaf,shoot and root of drought tolerant and susceptible varieties of sorghum and finger millet on inoculation of the different

strains of *Azospirillum* and also that due to external application of IAA.

Sorghum

In leaf the activity of AAT was found to be significantly higher in FT326 (T4) treated plants followed by plants treated with AZ208(T3), AZ204(T2) and IAA-T(T5). In shoot and root, AZ204 application induced maximal activity and the external application of IAA caused minimal though statistically significant increase over control. AZ208 application induced AAT to a higher extent than FT326 in shoots but this effect was reversed in the roots.

All the treated plants showed a significant increase in the activity of AAT in both varieties of sorghum when compared to the control at all the time intervals tested. Among the various stages, activity was found to be more at 90 days in leaf followed by 45, 30, 60, 105 and 75 days respectively. In shoot maximum activity was recorded at 45 DAS followed by 90, 105, 75, 30 and 60 DAS. Increased activity was observed at 75 DAS in root followed by 60, 75, 95, 30 and 105 DAS.

There was significant interaction between treatments and varieties, stages and varieties and treatments and stages. In DT, inoculation of AZ204 and in DS, inoculation of FT326 increased the activity of AAT. In general, AAT activity was significantly higher in DS than in DT of sorghum. AAT activity increased with stages of growth and there was a decline during the harvesting period. FCRD analysis of AAT

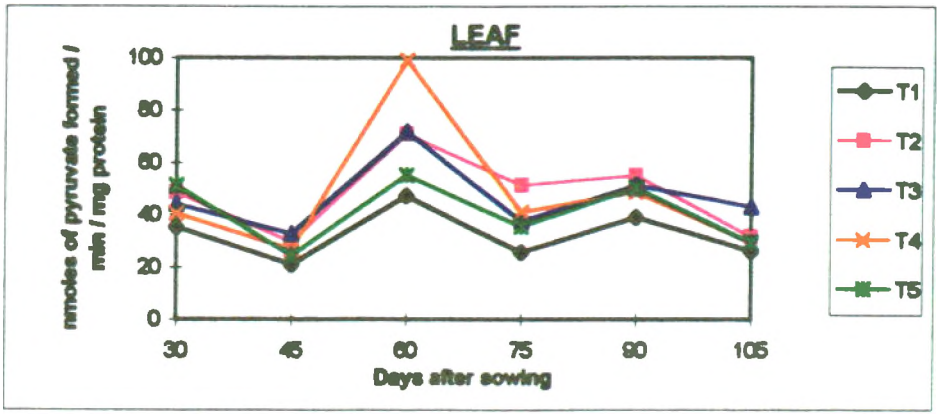
TABLE-VIII

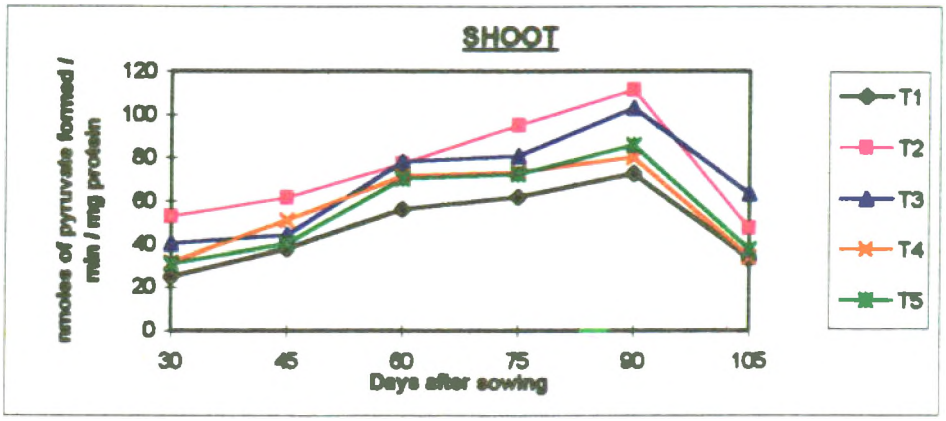
ASPARTATE AMINO TRANSFERASE ACTIVITY (n moles of pyruvate formed/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE(DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	35.49	21.12	47.23	25.71	39.02	26.17	32.46	25.05	37.94	56.08	61.72	72.74	33.19	47.79	13.55	40.13	80.12	108.10	29.80	16.46	48.03
(T1)	DS	40.21	77.44	18.80	23.28	54.24	41.08	42.51	60.44	103.31	31.41	32.00	43.15	70.46	56.80	55.69	64.29	18.18	51.11	56.14	47.62	48.84
	MEAN	37.85	49.28	33.02	24.50	46.63	33.63	37.48	42.75	70.63	43.75	46.86	57.95	51.83	52.29	34.62	52.21	49.15	79.61	42.97	32.04	48.43
AZ204	DT	48.40	29.67	70.72	51.15	54.91	31.75	47.77	52.85	61.42	77.04	94.87	111.28	47.33	74.13	23.08	46.70	90.99	161.31	69.09	29.00	70.03
(T2)	DS	47.89	97.79	23.81	28.74	66.23	51.51	52.66	102.42	157.61	34.17	46.35	72.77	81.59	82.49	73.08	89.91	24.18	64.66	69.35	56.14	62.89
	MEAN	48.15	63.73	47.27	39.95	60.57	41.63	50.21	77.64	109.52	55.61	70.61	92.03	64.46	78.31	48.08	68.31	57.59	112.99	69.22	42.57	66.46
AZ208	DT	44.03	32.97	71.74	37.65	51.15	43.02	46.76	40.18	44.22	78.01	80.81	102.81	63.18	68.20	29.90	58.07	106.98	113.31	49.13	52.75	68.36
(T3)	DS	46.30	84.71	13.82	34.99	78.75	68.08	54.44	97.40	122.67	32.61	57.20	49.93	107.81	77.94	58.68	78.43	25.63	78.89	68.95	52.38	60.49
	MEAN	45.17	58.84	42.78	36.32	64.95	55.55	50.60	68.79	83.45	55.31	69.01	76.37	85.49	73.07	44.29	68.25	66.31	96.10	59.04	52.57	64.43
IFT 326	DT	40.63	26.74	98.96	41.13	48.65	29.48	47.60	31.37	51.14	71.42	73.06	80.06	33.78	56.81	17.04	45.35	92.50	115.96	60.19	21.75	58.80
(T4)	DS	60.32	85.92	34.99	36.98	95.40	75.40	64.84	101.76	170.25	42.82	54.34	72.67	85.94	87.96	72.93	102.93	28.48	66.95	83.33	79.57	72.37
	MEAN	50.48	56.33	66.98	39.06	72.03	52.44	56.22	66.57	110.70	57.12	63.70	76.37	59.86	72.38	44.99	74.14	60.49	91.46	71.76	50.66	65.58
IAA/T	DT	51.15	24.42	55.08	35.61	50.20	29.34	40.97	30.87	40.35	70.10	72.34	85.78	37.42	56.14	19.13	42.30	92.97	110.39	38.27	25.79	54.81
(T5)	DS	64.12	88.41	33.33	37.69	60.36	50.58	55.75	64.75	115.80	36.98	38.46	51.73	92.12	66.64	82.96	90.54	36.39	54.86	71.68	58.57	65.83
	MEAN	57.64	56.42	44.21	36.65	55.28	39.96	48.36	47.81	78.08	53.54	55.40	68.76	64.77	61.39	51.05	66.42	64.68	82.63	54.98	42.18	60.32
	MEAN	47.85	56.92	46.85	35.29	59.89	44.64	48.57	60.71	90.47	53.06	61.12	74.29	65.28	67.49	44.60	65.87	59.64	92.53	59.59	44.00	61.04
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.11	0.07	0.12	0.15	0.17	0.27	0.38	0.02	0.01	0.02	0.03	0.04	0.06	0.08	1.05	0.67	1.15	1.49	1.63	2.58	3.65
	CD(5%)	0.22	0.14	0.24	0.31	0.34	0.53	0.75	0.05	0.03	0.05	0.06	0.07	0.11	0.16	2.11	1.33	2.31	2.98	3.26	5.16	7.29

Figure 5

AAT ACTIVITY IN DTV OF SORGHUM





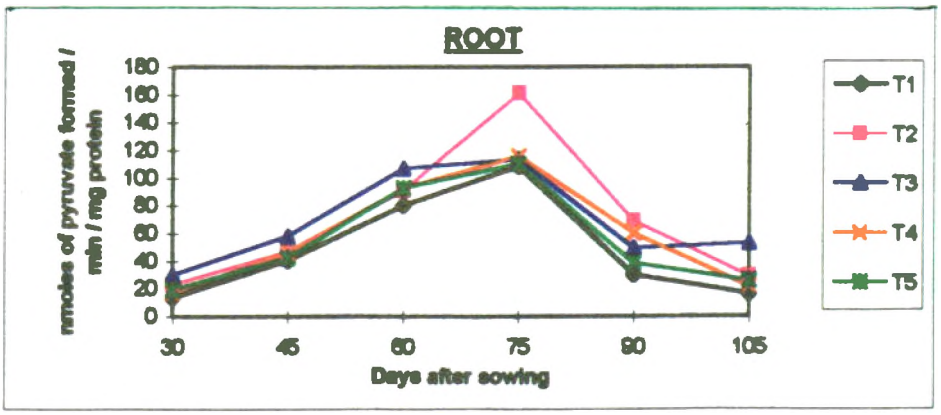


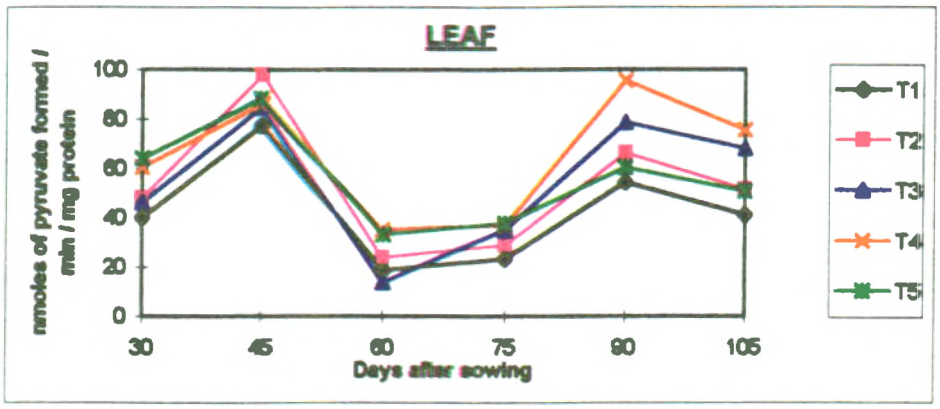
TABLE - IX

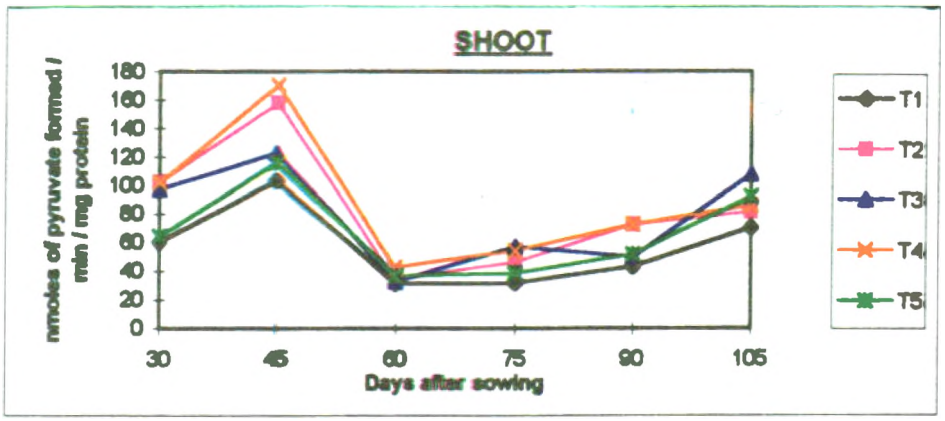
ASPARTATE AMINO TRANSFERASE ACTIVITY (n moles of pyruvate formed /min/mg prot) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

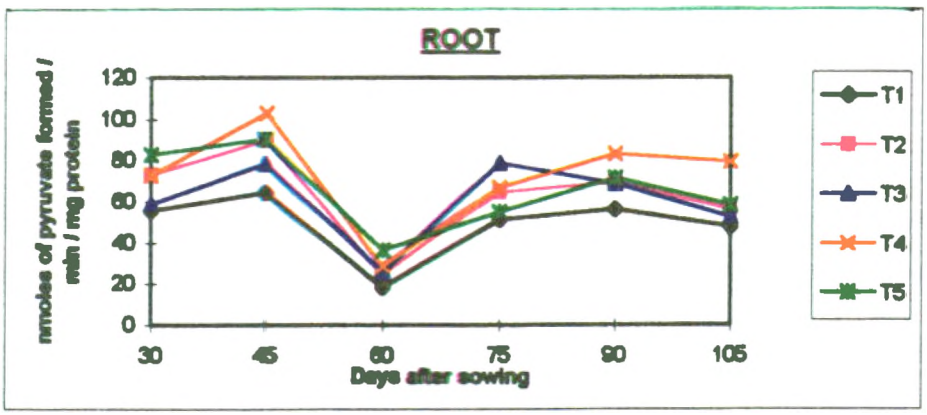
	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	48.24	38.80	63.01	19.69	41.56	29.92	40.20	87.12	42.16	71.22	29.35	46.74	82.29	59.81	71.23	52.18	73.69	29.46	23.09	71.95	153.60
(T1)	DS	36.88	69.23	51.40	55.95	29.88	50.22	48.93	46.58	96.56	44.29	149.57	87.11	72.49	82.77	28.21	98.23	49.71	23.41	52.38	47.96	149.98
	MEAN	42.56	54.02	57.21	37.82	35.72	40.07	44.57	66.85	69.36	57.76	89.46	66.93	77.39	71.29	49.72	75.21	61.70	26.44	37.74	59.96	151.79
AZ204	DT	51.28	43.06	91.77	32.33	38.40	37.93	49.13	92.47	56.87	133.68	48.85	100.05	126.98	93.15	87.04	62.20	81.55	56.50	24.61	87.07	166.49
(T2)	DS	98.09	86.90	64.61	107.70	60.74	78.87	74.65	68.06	114.30	94.27	178.57	98.84	94.85	107.20	51.23	142.86	56.22	51.13	98.84	53.57	175.64
	MEAN	53.19	64.98	78.19	70.02	49.57	55.40	61.89	77.27	85.69	114.03	113.71	99.46	110.92	100.18	69.14	102.53	68.89	53.82	61.72	70.32	171.07
AZ208	DT	59.82	37.88	79.02	22.08	43.13	36.51	46.41	91.46	67.27	123.86	66.73	77.69	127.39	92.40	102.83	62.87	109.03	48.67	35.71	71.15	171.71
(T3)	DS	40.33	75.31	57.44	79.30	31.89	37.17	53.57	57.41	104.44	45.79	114.29	106.80	91.52	86.71	32.69	102.91	67.16	56.55	61.69	50.65	161.94
	MEAN	50.08	56.60	68.23	50.69	37.51	36.84	49.99	74.44	85.86	84.83	90.51	92.25	109.46	89.55	67.76	82.89	88.10	52.61	48.70	60.90	166.83
IFT 326	DT	59.31	35.35	86.81	57.56	68.10	52.55	59.98	92.55	63.49	116.53	57.29	61.10	89.08	80.01	85.47	79.27	150.88	56.12	54.48	149.35	195.93
(T4)	DS	42.30	77.21	64.06	119.05	43.19	96.06	73.65	52.24	109.72	75.68	276.40	121.21	115.15	125.07	39.68	158.47	125.19	30.78	127.48	107.43	198.17
	MEAN	50.81	56.38	75.44	88.31	55.65	74.31	66.81	72.40	86.61	96.11	166.85	91.15	102.12	102.54	62.58	118.87	138.04	43.45	90.98	128.39	197.05
IAA/T	DT	56.46	44.03	88.84	41.09	54.89	48.13	55.57	105.12	67.93	95.76	63.59	68.57	103.52	84.08	74.23	86.67	110.99	42.28	29.47	96.68	173.39
(T5)	DS	42.35	87.70	60.25	65.31	52.60	53.08	60.22	43.38	118.41	70.29	179.42	143.48	123.92	113.15	53.49	123.13	71.94	22.73	92.81	86.58	175.11
	MEAN	49.41	65.87	74.55	53.20	53.74	50.61	57.89	74.25	93.17	83.03	121.51	106.03	113.72	98.62	63.86	104.90	91.47	32.51	61.14	91.63	174.25
	MEAN	49.21	59.57	70.72	60.01	46.44	51.44	56.23	73.04	84.14	87.15	116.41	91.16	102.72	92.43	62.61	96.88	89.64	41.76	60.06	82.24	172.20
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.03	0.03	0.04	0.06
	CD(5%)	0.04	0.03	0.04	0.06	0.06	0.10	0.14	0.04	0.03	0.05	0.06	0.06	0.10	0.14	0.04	0.02	0.04	0.05	0.06	0.09	0.13

Figure 6

AAT ACTIVITY IN DSV OF SORGHUM







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activity showed that maximum activity was associated with shoot followed by root and leaf.

Finger millet

The activity of AAT was found to be significantly higher in leaves on application of FT326 followed by AZ204, IAA-T, AZ208 and control. Same trend was observed in shoot and root. Significant increase was induced by all the treatments when compared to control. Among the various stages, activity was found to be more at 60 DAS in leaf followed by 75, 45, 105, 90 and 30 days. In shoot also maximum activity was observed at 75 DAS followed by 105, 90, 60, 45 and 30 days. But in root 45 days recorded maximal activity followed by 60, 105, 30, 90 and 75 DAS.

Significant interaction was recorded between all the combinations. Similar to the sorghum plants in finger millet also, DS was found to express higher AAT activity than DT and the FCRD analysis showed that maximal activity was expressed in shoots followed by roots and leaves.

Our results are in agreement with the report of Christensen and Jochimsen (1983) who reported an increased AAT activity in stems of soybean and pea plants followed by roots and leaves.

Our results showed that the AAT expressed a fluctuating activity at various stages of growth. The trend of increase or decrease in the activity was however, more or less similar within a given variety, irrespective of the treatment in all

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the parts. Similar fluctuation in AAT activity has been reported by Amarjit and Singh (1985) in pigeon pea nodules and by Reynolds *et al*. (1982) in developing soybean nodules.

4.6.ASPARAGINE SYNTHETASE

Tables X and XI depict the activities of AS in different parts of drought tolerant and susceptible varieties of sorghum and finger millet on inoculation of different strains of *Azospirillum* and external application of IAA.

Sorghum

In shoot AZ204 treated plants recorded a higher value of AS activity followed by FT326, IAA-T, AZ208 treated plants and control. In leaf and root also AZ204 inoculation showed maximum activity and the effect of the rest of the treatments followed almost the same pattern as observed in shoot. When all the treatments were compared with the control, significant increase in activity was observed. AS activity steadily increased up to 60 DAS and then declined in both leaf and shoot while in root it started declining after 75 DAS. The difference in the activity between DT and DS was significant in leaf and shoot with DS having higher activity. However, DT showed higher activity in root. Among the different parts of the plant, root showed maximum activity followed by shoot and leaf.

Finger millet

Maximum AS activity was noticed in all the parts of AZ204 treated plant. Most of the treatments were on par with each other in leaf and shoot. In leaf and shoot, increased

TABLE – X

ASPARAGINE SYNTHETASE ACTIVITY (n moles of β – aspartyl hydroxamate formed/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	0.07	0.12	0.13	0.09	0.06	0.01	0.08	0.06	0.11	0.20	0.15	0.10	0.08	0.12	0.13	0.16	0.19	0.25	0.37	0.10	0.20
(T1)	DS	0.05	0.07	0.13	0.19	0.07	0.05	0.09	0.07	0.13	0.19	0.25	0.13	0.09	0.14	0.12	0.20	0.28	0.33	0.21	0.10	0.21
	MEAN	0.06	0.09	0.13	0.14	0.07	0.03	0.09	0.07	0.12	0.20	0.20	0.12	0.09	0.13	0.13	0.18	0.24	0.29	0.29	0.10	0.20
AZ204	DT	0.12	0.16	0.19	0.15	0.11	0.03	0.13	0.08	0.16	0.31	0.24	0.19	0.17	0.19	0.18	0.25	0.33	0.41	0.49	0.16	0.30
(T2)	DS	0.07	0.10	0.16	0.24	0.14	0.09	0.13	0.13	0.18	0.25	0.30	0.20	0.16	0.20	0.19	0.32	0.41	0.49	0.26	0.15	0.30
	MEAN	0.10	0.13	0.18	0.20	0.13	0.06	0.13	0.11	0.17	0.28	0.27	0.20	0.17	0.20	0.18	0.29	0.37	0.45	0.38	0.16	0.30
AZ208	DT	0.12	0.12	0.17	0.11	0.07	0.02	0.10	0.07	0.15	0.22	0.19	0.13	0.10	0.14	0.13	0.17	0.29	0.37	0.44	0.22	0.27
(T3)	DS	0.07	0.12	0.16	0.20	0.15	0.06	0.13	0.08	0.14	0.24	0.28	0.23	0.12	0.18	0.15	0.28	0.35	0.42	0.34	0.12	0.28
	MEAN	0.09	0.12	0.17	0.16	0.11	0.04	0.11	0.08	0.15	0.23	0.24	0.18	0.11	0.16	0.14	0.23	0.32	0.40	0.39	0.17	0.27
IFT 326	DT	0.09	0.11	0.16	0.11	0.08	0.03	0.10	0.07	0.12	0.35	0.25	0.16	0.13	0.18	0.16	0.17	0.43	0.47	0.41	0.24	0.31
(T4)	DS	0.08	0.10	0.19	0.23	0.18	0.08	0.14	0.09	0.17	0.23	0.26	0.25	0.09	0.18	0.17	0.25	0.37	0.39	0.25	0.11	0.26
	MEAN	0.09	0.11	0.18	0.17	0.13	0.06	0.12	0.08	0.15	0.29	0.26	0.21	0.11	0.18	0.17	0.21	0.40	0.43	0.33	0.18	0.28
IAA/T	DT	0.09	0.17	0.18	0.09	0.07	0.01	0.10	0.08	0.13	0.23	0.20	0.18	0.17	0.17	0.17	0.20	0.24	0.30	0.38	0.14	0.24
(T5)	DS	0.06	0.09	0.15	0.20	0.16	0.07	0.12	0.09	0.14	0.31	0.32	0.14	0.10	0.18	0.13	0.31	0.35	0.38	0.23	0.16	0.26
	MEAN	0.08	0.13	0.17	0.15	0.12	0.04	0.11	0.09	0.14	0.27	0.26	0.16	0.14	0.17	0.15	0.26	0.30	0.34	0.31	0.15	0.25
	MEAN	0.08	0.12	0.16	0.16	0.11	0.05	0.11	0.08	0.14	0.25	0.24	0.17	0.12	0.17	0.15	0.23	0.32	0.38	0.34	0.15	0.26
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SEd	0.01	0.00	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.01	0.01	0.01	0.02	0.02	0.03	0.04
	CD(5%)	0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.02	0.01	0.02	0.03	0.03	0.04	0.06	0.02	0.01	0.02	0.03	0.03	0.05	0.08

TABLE – XI

ASPARAGINE SYNTHETASE ACTIVITY (n mole of β-aspartyl hydroxamate formed/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	0.08	0.07	0.10	0.12	0.06	0.04	0.07	0.04	0.10	0.13	0.16	0.10	0.07	0.10	0.35	0.42	0.47	0.29	0.11	0.07	0.29
	DS	0.03	0.06	0.07	0.09	0.11	0.06	0.07	0.06	0.12	0.17	0.21	0.17	0.09	0.14	0.11	0.21	0.27	0.31	0.38	0.14	0.24
	MEAN	0.03	0.07	0.09	0.11	0.09	0.05	0.07	0.05	0.11	0.15	0.19	0.14	0.08	0.12	0.23	0.32	0.37	0.30	0.25	0.11	0.26
AZ204	DT	0.04	0.08	0.13	0.15	0.09	0.06	0.09	0.07	0.16	0.19	0.22	0.16	0.12	0.15	0.54	0.61	0.69	0.45	0.16	0.12	0.43
(T2)	DS	0.06	0.08	0.10	0.11	0.13	0.09	0.10	0.09	0.22	0.28	0.35	0.25	0.13	0.22	0.23	0.28	0.40	0.45	0.53	0.29	0.36
	MEAN	0.05	0.08	0.12	0.13	0.11	0.08	0.09	0.08	0.19	0.24	0.29	0.21	0.13	0.19	0.39	0.45	0.55	0.45	0.35	0.21	0.40
AZ208	DT	0.05	0.07	0.11	0.12	0.06	0.04	0.08	0.04	0.13	0.16	0.17	0.14	0.10	0.12	0.46	0.50	0.57	0.35	0.12	0.09	0.35
(T3)	DS	0.04	0.06	0.09	0.10	0.12	0.07	0.08	0.07	0.17	0.21	0.24	0.21	0.11	0.17	0.19	0.27	0.32	0.37	0.45	0.19	0.30
	MEAN	0.05	0.07	0.10	0.11	0.09	0.06	0.08	0.06	0.15	0.19	0.21	0.18	0.11	0.15	0.32	0.39	0.45	0.36	0.29	0.14	0.32
FT 326	DT	0.04	0.11	0.13	0.14	0.08	0.04	0.09	0.05	0.14	0.17	0.19	0.16	0.09	0.13	0.50	0.57	0.63	0.35	0.17	0.12	0.39
(T4)	DS	0.05	0.08	0.11	0.12	0.14	0.08	0.10	0.09	0.14	0.27	0.32	0.25	0.19	0.21	0.15	0.26	0.34	0.49	0.51	0.28	0.34
	MEAN	0.05	0.10	0.12	0.13	0.11	0.06	0.09	0.07	0.14	0.22	0.26	0.21	0.14	0.17	0.33	0.42	0.49	0.42	0.34	0.20	0.36
AA/T	DT	0.07	0.09	0.13	0.14	0.07	0.05	0.09	0.05	0.11	0.16	0.19	0.15	0.09	0.13	0.41	0.47	0.50	0.38	0.15	0.08	0.33
(T5)	DS	0.04	0.07	0.10	0.12	0.14	0.07	0.09	0.10	0.18	0.21	0.27	0.23	0.17	0.19	0.12	0.25	0.30	0.40	0.50	0.25	0.30
	MEAN	0.06	0.08	0.12	0.13	0.11	0.06	0.09	0.08	0.15	0.19	0.23	0.19	0.13	0.16	0.27	0.36	0.40	0.39	0.33	0.17	0.32
	MEAN	0.05	0.08	0.11	0.12	0.10	0.06	0.09	0.07	0.15	0.20	0.23	0.18	0.12	0.16	0.31	0.38	0.45	0.38	0.31	0.16	0.33
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.01	0.00	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.01	0.02	0.02	0.02	0.04	0.05
	CD(5%)	0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.02	0.01	0.02	0.02	0.03	0.04	0.06	0.03	0.02	0.03	0.04	0.05	0.07	0.10

activity was recorded at 75 DAS and in root it was at 60 DAS. Interaction between treatment and variety and treatment and stages was not found to be significant. As in sorghum, root showed maximum activity followed by shoot and leaf. Drought tolerance or susceptibility had no influence on AS activity in the leaf. In shoot DS expressed higher activity than DT and in root it was vice versa. The activity showed a gradual increase over time and reached maximum level at 75 DAS in DT and 90 DAS in DS and declined thereafter. In the present study AS showed an increase in specific activity, although the extent of increase lagged behind that of other ammonia assimilatory enzymes.

Reynolds *et al.* (1982) reported that after inoculation with *Rhizobium*, enzyme activity in soybean nodules increased from 11 to 17 days and then declined at 19 days, after that slight increase was seen till 23rd day. The activity was less when compared to the activities of other ammonia assimilating enzymes. Oaks *et al.* (1980) reported that the AS activity was relatively low in the tips and increased as the root tissue matures. Oaks and Ross (1984) also found that mature root had more activity than the other parts, the pattern of alteration in AS activity over the selected time points is almost similar to that reported by Amarjit and Singh (1985) in pigeon pea nodules.

4.7.ASPARAGINASE

The activities of asparaginase in leaf, shoot and root of drought tolerant and susceptible varities of sorghum and finger millet upon inoculation of different *Azospirillum*

TABLE – XII

ASPARAGINASE ACTIVITY (n moles of ammonia liberated/min/mg prot.) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	75.87	65.69	78.70	99.31	111.31	64.57	82.58	73.88	61.86	67.07	83.59	67.13	45.81	66.56	111.27	82.58	120.62	180.06	99.85	90.11	114.08
	DS	59.72	45.74	55.74	97.25	66.78	33.87	59.85	27.52	24.61	50.41	101.83	73.24	41.22	53.14	61.63	44.26	75.37	114.49	44.04	37.35	62.86
	MEAN	67.80	55.72	67.22	98.28	89.05	49.22	71.21	50.70	43.24	58.74	92.71	70.19	43.52	59.85	86.45	63.42	98.00	147.28	71.95	63.73	88.47
AZ204 (T2)	DT	88.51	81.48	93.58	107.69	125.69	87.22	97.36	109.13	68.55	79.60	107.61	75.40	67.22	84.59	130.90	94.56	177.93	261.36	187.97	151.29	167.34
	DS	74.51	56.15	76.15	131.44	72.49	35.59	74.39	54.90	29.74	97.05	106.18	93.78	70.41	75.34	73.98	73.66	107.16	206.87	51.41	48.18	93.54
	MEAN	81.51	68.82	84.87	119.57	99.09	61.41	85.88	82.02	49.15	88.33	106.90	84.59	68.82	79.96	102.44	84.11	142.55	234.12	119.69	99.74	130.44
AZ208 (T3)	DT	92.06	77.05	90.75	109.83	131.18	92.94	98.97	115.88	73.94	78.90	121.10	83.88	69.35	90.51	185.51	111.25	130.81	217.18	152.53	112.42	151.62
	DS	72.40	46.28	64.45	100.70	75.32	48.10	67.88	42.64	35.81	62.47	124.16	87.49	45.37	66.32	74.60	59.52	115.29	167.92	62.29	55.28	89.15
	MEAN	82.23	61.67	77.60	105.27	103.25	70.52	83.42	79.26	54.88	70.69	122.63	85.69	57.36	78.42	130.06	85.39	123.05	192.55	107.41	83.85	120.38
IFT 326 (T4)	DT	91.33	79.08	99.26	121.52	168.54	85.99	107.62	104.68	73.89	88.79	99.56	86.63	75.91	88.24	145.52	122.87	211.24	252.90	189.35	147.37	178.21
	DS	81.38	56.71	81.71	99.06	67.94	47.05	72.31	46.53	37.57	59.09	123.89	78.14	65.90	68.52	88.40	59.36	82.56	162.31	48.20	41.15	80.33
	MEAN	86.36	67.90	90.49	110.29	118.24	66.52	89.96	75.61	55.73	73.94	111.73	82.39	70.91	78.38	116.96	91.12	146.90	207.61	118.78	94.26	129.27
IAA/T (T5)	DT	80.09	76.64	83.28	104.65	143.90	72.73	93.55	98.13	81.86	97.05	105.26	76.98	56.73	86.00	131.49	116.84	188.32	192.34	176.01	135.41	156.74
	DS	80.46	48.52	64.49	101.30	77.48	50.37	70.44	50.24	47.94	51.82	135.28	80.69	52.35	69.72	80.89	51.74	84.72	152.98	50.63	140.56	93.59
	MEAN	80.28	62.58	73.89	102.98	110.69	61.55	81.99	74.19	64.90	74.44	120.27	78.84	54.54	77.86	106.19	84.29	136.52	172.66	113.32	137.99	125.16
	MEAN	79.63	63.33	78.81	107.27	104.06	61.84	82.49	72.35	53.58	73.23	110.85	80.34	59.03	74.89	108.42	81.66	129.40	190.84	106.23	95.91	118.74
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.06	0.04	0.06	0.08	0.09	0.14	0.20	0.03	0.02	0.03	0.04	0.05	0.07	0.10	0.03	0.02	0.03	0.04	0.04	0.07	0.10
	CD(5%)	0.12	0.07	0.13	0.16	0.18	0.28	0.40	0.06	0.04	0.07	0.09	0.09	0.15	0.21	0.06	0.04	0.06	0.08	0.09	0.14	0.19

TABLE – XIII

ASPARAGINASE ACTIVITY (n moles of ammonia liberated /min/mg prot.) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE VARIETIES (DS) OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (DT1)	DT	117.36	20.43	11.30	22.00	18.10	15.08	117.38	131.03	25.45	28.99	51.29	58.66	33.04	138.08	114.03	20.40	17.42	35.09	30.33	13.69	121.83
	DS	9.08	12.23	17.38	43.13	33.39	29.40	124.10	117.05	38.50	23.67	63.03	74.35	33.13	141.62	120.18	43.78	22.85	36.35	40.09	37.49	133.46
	MEAN	113.22	16.33	14.34	32.57	25.75	22.24	120.74	124.04	31.98	26.33	57.16	66.51	33.09	139.85	117.11	32.09	20.14	35.72	35.21	25.59	127.64
AZ204 (T2)	DT	135.36	41.27	17.69	35.29	29.73	26.16	130.92	135.19	28.27	43.26	65.09	74.25	40.14	147.70	116.08	30.33	36.39	56.91	38.62	21.52	133.31
	DS	119.51	20.29	25.45	71.65	60.27	41.82	139.83	121.48	49.07	34.85	72.33	83.57	35.94	149.54	125.49	50.61	39.09	53.54	55.29	46.35	145.06
	MEAN	127.44	30.78	21.57	53.47	45.00	33.99	135.37	128.34	38.67	39.06	68.71	78.91	38.04	148.62	120.79	40.47	37.74	55.23	46.96	33.94	139.19
AZ208 (T3)	DT	127.16	33.48	12.48	28.53	25.71	21.39	124.79	141.75	32.35	34.04	57.75	69.96	38.13	145.66	121.86	24.87	28.76	49.04	37.64	16.93	129.85
	DS	115.70	28.90	15.82	64.96	37.99	31.62	132.50	125.83	52.79	39.48	85.97	89.99	31.46	154.25	124.87	61.80	30.92	56.83	60.58	51.76	147.79
	MEAN	121.43	31.19	14.15	46.75	31.85	26.51	128.65	133.79	42.57	36.76	71.86	79.98	34.80	149.96	123.37	43.34	29.84	52.94	49.11	34.35	138.82
FT 326 (T4)	DT	125.92	28.92	21.23	33.86	31.75	27.30	128.16	138.38	34.69	43.07	62.34	80.03	43.51	150.34	124.47	37.99	41.99	55.18	45.66	23.48	138.13
	DS	114.70	33.45	35.51	59.07	54.17	40.19	139.52	121.00	56.89	35.51	86.35	91.15	25.32	152.70	135.52	63.63	31.91	43.88	58.85	51.35	147.52
	MEAN	120.31	31.19	28.37	46.47	42.96	33.75	133.84	129.69	45.79	39.29	74.35	85.59	34.42	151.52	130.00	50.81	36.95	49.53	52.26	37.41	142.83
AA/T (T5)	DT	138.44	44.87	21.09	41.38	21.57	19.91	131.21	143.03	36.91	38.09	57.09	63.38	43.05	146.93	115.66	21.69	26.61	44.45	27.87	19.79	126.01
	DS	127.91	31.15	34.26	45.88	40.44	33.28	135.49	125.50	53.76	24.26	78.24	81.41	37.77	150.16	133.07	66.85	28.58	41.17	51.30	45.28	144.38
	MEAN	133.18	38.01	27.68	43.63	31.01	26.60	133.35	134.27	45.34	31.18	67.67	72.40	40.41	148.54	124.37	44.27	27.60	42.81	39.59	32.54	135.19
	MEAN	123.11	29.50	21.22	44.58	35.31	28.62	130.39	130.02	40.87	34.52	67.95	76.68	36.15	147.70	123.12	42.20	30.45	47.24	44.62	32.76	136.73
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.79	0.50	0.87	1.12	1.23	1.94	2.74	0.02	0.02	0.03	0.03	0.04	0.06	0.08	0.03	0.02	0.03	0.04	0.04	0.06	0.09
	CD(5%)	1.58	1.00	1.74	2.24	2.45	3.88	5.49	0.05	0.03	0.05	0.07	0.07	0.12	0.17	0.05	0.03	0.06	0.07	0.08	0.12	0.18

strains and also that due to external application of IAA is given in Tables XII and XIII.

Sorghum

Inoculation of FT326 induced asparaginase activity to a maximal extent in leaf. In shoot and root AZ204 application exhibited higher activity. AZ208 and FT326 treatment were on par with each other in shoot. Statistically significant increase over control was seen in all the treatments. 75 DAS was found to be the time point of highest asparaginase activity in all the organs. The activity was greater in DT than in DS.

Significant interaction was seen between treatments and varieties, stages and varieties, treatments and stages. In both the varieties, the activity decreased during the vegetative stage and then peaked at flowering and thereafter declined during maturity.

Finger Millet

AZ204 inoculation recorded significantly higher activity in leaf whereas it was FT326 inoculation which induced maximum activity in shoot and leaf. All the treatments showed significantly increased activity over the control. Enzyme activity peaked at 75 DAS in leaf and root and at 90 DAS in shoot. DS expressed more activity than DT in all parts of the plants. Significant interaction was seen between treatments and varieties, stages and varieties, and stages and treatments. The activity of asparaginase was more in shoot

followed by root and leaf. The activity increased as the growth progresssed and decreased during maturation with a slight decline at 60 days in shoot and root.

Tonin and Sodek (1990) reported that the asparaginase activity increased in older cotyledons, when soybean cotyledons were grown *invitro* with asparagine. Gomes and Sodek (1984) reported that the activity of the enzyme increased in legume cotyledons during development.

4.8.GLUTAMINASE

The impact of inoculation of different strains of *Azospirillum* and external application of IAA on leaf, shoot and root of sorghum and finger millet is presented in Tables XIV and XV.

Sorghum

All the treatments resulted in significantly higher glutaminase activity in all parts of the plants than in controls. Between treatments also significant difference was observed with T2 (AZ204) having maximum activity in leaf and T4 (FT326) in shoot and root.The pattern of glutaminase activity in leaf and shoot was similar with an initial increase, a decline at S2 (45 DAS) followed by a steep increase upto 75 DAS and then a decline. While the final value dropped even below the initial activity in leaf, it continued to be high in shoot. Glutaminase activity steadily increased in root and was about 3 times the initial value at 75 DAS and later decreased slightly .

TABLE – XIV

GLUTAMINASE ACTIVITY (n moles ammonia liberated /min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	136.94	45.11	27.59	42.35	50.00	28.17	138.36	148.29	64.00	50.51	68.05	86.77	61.47	163.18	122.29	63.29	79.69	109.88	80.42	59.15	169.18
	DS	115.97	19.08	17.84	24.83	31.84	23.77	122.22	131.32	38.79	29.08	46.85	53.59	28.64	138.05	131.12	31.27	25.46	65.07	67.45	48.82	144.87
	MEAN	126.46	32.10	22.72	33.59	40.92	25.97	130.29	139.81	51.40	39.80	57.45	70.18	45.06	150.61	126.71	47.28	52.58	87.48	73.94	53.99	156.99
AZ204 (T2)	DT	137.19	39.39	34.29	54.75	62.15	40.10	147.16	160.84	69.75	57.05	91.40	114.31	75.57	178.15	146.52	78.39	85.49	125.05	92.64	78.09	184.36
	DS	135.62	44.88	32.86	46.08	59.39	34.31	142.12	143.19	54.27	34.30	51.62	67.79	45.20	149.40	135.43	40.87	36.37	76.35	97.05	73.59	159.94
	MEAN	136.41	49.34	33.63	50.42	60.77	37.30	144.64	152.02	62.01	45.68	71.51	91.05	60.39	163.77	140.98	59.63	60.93	100.70	94.85	75.84	172.15
AZ208 (T3)	DT	142.03	55.37	25.84	48.96	54.39	35.89	143.75	153.61	71.91	61.80	75.46	97.48	69.56	171.64	130.47	87.82	91.42	118.46	108.64	64.18	183.50
	DS	119.88	22.22	20.52	35.53	53.32	28.99	130.08	133.61	47.34	37.06	65.97	57.46	31.43	145.48	133.28	36.23	25.78	69.47	85.58	59.17	151.59
	MEAN	130.96	38.80	23.18	42.25	53.86	32.44	136.91	143.61	59.63	49.43	70.72	77.47	50.50	158.56	131.88	62.03	58.60	93.97	97.11	61.68	167.54
FT 326 (T4)	DT	148.80	61.09	43.33	52.69	62.49	41.19	151.60	165.75	82.19	71.35	81.69	134.49	83.31	186.46	125.69	94.46	116.67	123.73	101.29	71.46	188.88
	DS	123.15	32.22	26.25	36.88	51.32	26.49	132.72	142.96	58.49	37.17	69.88	76.04	58.82	157.23	141.00	55.46	38.78	77.88	94.06	70.89	163.01
	MEAN	135.98	46.66	34.79	44.79	56.91	33.84	142.16	154.35	70.34	54.26	75.79	105.27	71.07	171.84	133.35	74.96	77.73	100.81	97.68	71.18	175.95
IAA/T (T5)	DT	152.72	69.18	42.89	59.88	65.97	33.20	153.97	158.37	80.37	76.16	84.88	111.17	70.00	180.16	128.12	87.35	108.24	110.57	99.56	66.26	183.35
	DS	123.82	34.58	27.59	35.57	42.79	35.48	133.31	137.39	50.58	46.46	58.63	69.07	38.18	150.05	140.00	54.37	41.13	66.63	77.43	66.87	157.74
	MEAN	138.27	51.88	35.24	47.73	54.38	34.34	143.64	147.88	65.48	61.31	71.76	90.12	54.09	165.11	134.06	70.86	74.69	88.60	88.50	66.57	170.54
	MEAN	133.61	43.75	29.91	43.75	53.37	32.78	139.53	147.53	61.77	50.09	69.44	86.82	56.22	161.98	133.39	62.95	64.90	94.31	90.41	65.85	168.64
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.22	0.14	0.24	0.31	0.34	0.53	0.75	0.03	0.02	0.03	0.04	0.04	0.07	0.10	0.03	0.02	0.03	0.04	0.05	0.07	0.10
	CD(5%)	0.43	0.27	0.47	0.61	0.67	1.06	1.50	0.06	0.03	0.06	0.08	0.09	0.13	0.19	0.06	0.04	0.07	0.09	0.09	0.15	0.21

TABLE - XV

GLUTAMINASE ACTIVITY (n moles ammonia liberated /min/mg prot) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

		LEAF							SHOOT							ROOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	121.08	29.30	19.50	27.46	41.82	38.90	129.71	164.72	71.44	58.59	68.06	88.39	62.58	68.96	61.09	77.36	88.62	124.67	135.95	92.19	96.65
	DS	119.00	41.12	23.74	56.74	63.51	23.72	137.97	160.00	65.84	58.74	124.88	139.70	105.79	92.49	87.65	103.74	44.83	67.05	127.73	105.79	89.47
	MEAN	120.04	35.31	21.62	42.10	52.67	31.31	133.84	162.36	68.64	58.67	96.47	114.05	84.19	80.73	74.37	90.55	66.73	95.86	131.84	98.99	93.06
AZ204 (T2)	DT	141.29	43.08	33.87	39.44	61.31	50.89	144.98	189.11	107.41	88.59	91.71	107.65	72.41	92.81	71.26	87.36	117.90	159.53	185.05	129.87	125.16
	DS	128.09	50.87	47.44	68.95	72.73	35.97	150.68	178.75	85.06	79.66	152.94	168.53	113.23	113.03	103.13	118.86	72.44	84.06	136.47	113.23	104.70
	MEAN	134.69	46.98	40.66	54.20	67.02	43.43	147.83	183.93	96.24	84.13	122.33	138.09	92.82	102.92	87.20	103.11	95.17	121.80	160.76	121.55	114.93
AZ208 (T3)	DT	138.63	44.76	22.65	40.47	63.20	49.09	143.13	169.36	89.35	64.01	71.63	145.21	69.27	84.81	64.98	92.69	106.64	135.19	147.93	129.40	112.81
	DS	123.69	52.99	35.42	60.61	72.04	29.22	145.65	178.45	112.27	61.90	135.87	151.21	116.47	109.36	98.45	117.49	57.23	72.79	145.43	106.47	99.64
	MEAN	131.16	48.83	29.04	50.54	67.62	39.16	144.39	173.91	100.81	62.96	103.75	148.21	92.87	97.08	81.72	105.09	81.94	103.99	146.68	117.94	106.22
FT 326 (T4)	DT	127.20	38.18	29.32	54.82	68.27	61.02	146.47	175.91	93.18	82.21	115.25	136.36	75.77	96.45	74.84	80.48	97.05	168.50	183.06	126.36	121.72
	DS	127.20	66.21	34.01	64.69	82.15	27.71	150.33	176.05	112.93	91.27	147.39	174.68	125.45	121.30	111.21	129.33	51.66	75.99	172.30	123.45	110.99
	MEAN	127.20	52.20	31.67	59.76	75.21	44.37	148.40	175.98	103.07	86.74	131.32	155.52	100.61	108.87	93.03	104.91	74.36	122.25	177.68	125.91	116.35
AA/T (T5)	DT	122.97	42.71	38.50	56.01	75.03	65.52	150.12	163.03	94.24	76.04	91.34	103.11	84.32	85.35	67.01	82.30	116.76	147.14	166.93	108.98	114.86
	DS	133.68	59.46	27.65	61.00	87.27	23.47	148.75	180.77	109.28	86.34	162.90	157.34	119.15	119.30	118.88	122.17	63.67	73.22	170.96	119.15	111.34
	MEAN	128.33	51.09	33.08	58.51	81.15	44.49	149.44	171.90	101.76	81.19	127.12	130.23	101.74	102.32	92.95	102.24	90.82	110.18	168.96	114.07	113.10
	MEAN	128.28	46.88	31.21	53.02	68.73	40.55	144.78	173.62	94.10	74.74	116.20	137.82	94.44	98.39	85.85	101.18	81.68	110.81	157.18	115.69	108.73
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.04	0.06	0.09	0.11	0.07	0.12	0.16	0.17	0.27	0.39	0.03	0.02	0.03	0.04	0.04	0.06	0.09
	CD(5%)	0.05	0.03	0.06	0.07	0.08	0.13	0.18	0.22	0.14	0.24	0.31	0.34	0.54	0.77	0.05	0.03	0.06	0.07	0.08	0.13	0.18

DT recorded significantly higher glutaminase activity than DS. When the interaction between treatments and varieties, stages and varieties and treatments and stages was considered (taking one combination at a time), a significant difference in glutaminase activity was observed.

Finger Millet

All the treatments caused significantly higher glutaminase activity in all the parts of the plants than in the controls. FT326 treated plants exhibited maximum activity in shoot and root followed by AZ204, IAA-T and AZ208 treated plants. In leaf, IAA treated plants had the maximum activity followed by FT326, AZ204 and AZ208 treated plants.The pattern of glutaminase activity was found to be similar to that in sorghum.Contrary to sorghum,DS recorded a higher activity than DT in leaf and shoot.

4.9.NITRATE REDUCTASE

Nitrate reductase activities in different parts of drought tolerant and susceptible varieties of sorghum and finger millet due to the inoculation of different strains of *Azospirillum* and external application of the hormone is given in Tables XVI and XVII.

Sorghum

All the treatments influenced leaf, shoot and root nitrate reductase activity significantly with AZ204 treated plants being superior. Individually in both DT and DS , AZ204 treated plants was found to be the most efficient inducer of nitrate reductase. Among the different periods of study higher

TABLE – XVI

NITRATE REDUCTASE ACTIVITY (n moles of nitrite produced/min/mg prot,) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	3.39	2.78	3.21	2.40	3.15	3.96	3.15	0.88	1.08	1.57	0.86	1.80	1.14	1.22	1.75	2.90	3.08	1.42	1.82	1.16	2.02
(T1)	DS	1.93	2.20	3.06	2.94	2.50	3.08	2.62	1.27	2.00	0.94	1.33	1.02	1.16	1.29	0.97	1.11	1.52	1.19	0.86	1.07	1.12
	MEAN	2.66	2.49	3.14	2.67	2.83	3.52	2.88	1.08	1.54	1.26	1.10	1.41	1.15	1.25	1.36	2.01	2.30	1.31	1.34	1.12	1.57
AZ204	DT	3.96	3.51	4.77	4.38	4.42	4.91	4.33	0.94	1.81	2.33	1.41	2.69	1.77	1.83	2.22	3.52	3.53	1.84	3.50	1.41	2.67
(T2)	DS	3.52	3.03	4.53	3.46	3.01	4.13	3.61	1.66	2.84	1.77	1.89	1.19	1.91	1.88	1.21	1.97	2.68	1.77	1.39	1.54	1.76
	MEAN	3.74	3.27	4.65	3.92	3.72	4.52	3.97	1.30	2.33	2.05	1.65	1.94	1.84	1.85	1.72	2.75	3.11	1.81	2.45	1.48	2.22
AZ208	DT	3.80	3.12	4.08	2.51	3.53	4.08	3.52	1.00	1.42	1.58	1.16	2.26	1.59	1.50	1.93	2.95	3.51	1.60	3.23	1.67	2.48
(T3)	DS	2.45	2.26	3.19	3.03	2.84	3.22	2.83	1.45	2.25	1.39	1.43	1.35	1.53	1.57	1.14	1.24	1.66	1.59	1.29	1.46	1.40
	MEAN	3.13	2.69	3.64	2.77	3.19	3.65	3.18	1.23	1.84	1.49	1.30	1.81	1.56	1.53	1.54	2.10	2.59	1.60	2.26	1.57	1.94
FT 326	DT	4.45	4.12	5.95	2.89	3.92	4.52	4.31	0.90	1.91	2.05	1.52	1.96	1.41	1.63	2.43	3.07	4.20	1.48	2.46	1.42	2.51
(T4)	DS	2.63	2.77	3.30	3.18	2.97	4.57	3.24	1.82	2.16	1.51	1.98	1.04	1.40	1.65	1.41	1.81	2.38	1.36	0.96	1.49	1.57
	MEAN	3.54	3.44	4.63	3.04	3.45	4.55	3.77	1.36	2.04	1.78	1.75	1.50	1.41	1.64	1.92	2.44	3.29	1.42	1.71	1.46	2.04
IAA/T	DT	3.98	3.47	3.97	3.20	3.54	4.74	3.82	0.93	1.84	1.97	1.11	2.45	1.33	1.61	2.03	3.27	3.74	1.51	1.90	1.32	2.30
(T5)	DS	2.85	2.56	3.31	3.09	2.81	3.72	3.06	1.93	2.20	0.95	1.58	1.12	1.21	1.50	1.28	1.59	1.74	1.25	1.01	1.20	1.35
	MEAN	3.42	3.02	3.64	3.15	3.17	4.23	3.44	1.43	2.02	1.46	1.35	1.79	1.27	1.55	1.66	2.43	2.74	1.38	1.46	1.26	1.82
	MEAN	3.30	2.98	3.94	3.11	3.27	4.09	3.45	1.28	1.95	1.61	1.43	1.69	1.45	1.57	1.64	2.34	2.80	1.50	1.84	1.37	1.92
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.04	0.07	0.10	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.11	0.07	0.12	0.15	0.16	0.26	0.36
	CD(5%)	0.06	0.04	0.06	0.08	0.09	0.14	0.20	0.04	0.02	0.04	0.05	0.06	0.10	0.13	0.21	0.13	0.23	0.30	0.33	0.52	0.73

TABLE - XVII

NITRATE REDUCTASE ACTIVITY (n moles of nitrite produced/min/mg/prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	2.81	2.00	2.19	1.11	2.45	1.91	2.08	1.31	1.08	1.58	1.75	1.01	0.81	1.26	2.18	0.95	1.70	2.40	2.61	2.11	1.99
	DS	2.84	2.17	3.13	3.45	2.15	1.98	2.62	2.84	1.11	1.75	2.81	1.60	0.99	1.85	1.90	1.25	1.34	2.24	3.19	2.00	1.99
	MEAN	2.83	2.09	2.66	2.28	2.30	1.95	2.35	2.08	1.10	1.67	2.28	1.31	0.90	1.55	2.04	1.10	1.52	2.32	2.90	2.06	1.99
A2204 (T2)	DT	3.46	3.06	3.37	2.31	3.07	3.00	3.08	2.93	1.46	1.88	1.76	1.61	1.25	1.82	2.74	1.44	1.99	2.72	2.89	2.70	2.41
	DS	3.73	2.45	3.99	4.18	3.95	2.27	3.43	2.70	1.99	2.09	3.28	2.95	1.98	2.68	1.97	1.57	2.57	2.87	4.13	3.43	2.76
	MEAN	3.70	2.76	3.68	3.25	3.51	2.64	3.25	3.32	1.73	1.99	2.57	2.28	1.62	2.25	2.36	1.51	2.28	2.80	3.51	3.07	2.59
A2208 (T3)	DT	3.21	2.61	3.50	1.49	2.43	2.27	2.59	1.41	1.05	2.07	2.11	1.16	0.97	1.46	2.87	1.96	2.08	2.34	3.08	2.74	2.51
	DS	3.33	2.69	3.73	4.09	2.67	2.09	3.10	3.10	1.53	1.81	3.19	1.77	1.25	2.11	2.41	1.28	1.44	2.80	3.22	2.22	2.23
	MEAN	3.27	2.65	3.62	2.79	2.55	2.18	2.84	2.26	1.29	1.94	2.65	1.47	1.11	1.78	2.64	1.62	1.76	2.57	3.15	2.48	2.37
FT 326 (T4)	DT	3.42	2.56	3.41	2.72	3.05	2.19	2.89	1.81	1.54	2.08	2.28	2.17	1.13	1.84	2.32	1.71	2.06	2.74	2.97	2.47	2.38
	DS	4.10	2.83	3.35	4.00	3.63	2.73	3.44	3.50	1.59	1.90	3.67	3.22	1.79	2.61	2.15	1.74	1.94	3.14	4.61	3.80	2.90
	MEAN	3.76	2.70	3.38	3.36	3.34	2.46	3.17	2.66	1.57	1.99	2.98	2.70	1.46	2.22	2.24	1.73	2.00	2.94	3.79	3.14	2.64
AA/T (T5)	DT	3.95	2.43	2.73	2.19	3.81	2.26	2.90	2.26	1.61	2.41	2.58	1.05	0.88	1.80	2.74	1.11	1.71	2.48	2.70	2.35	2.18
	DS	3.39	2.61	3.41	3.67	2.34	2.12	2.92	3.08	1.19	1.86	3.71	2.10	1.45	2.23	2.78	1.39	1.57	2.96	3.44	2.75	2.48
	MEAN	3.67	2.52	3.07	2.93	3.08	2.19	2.91	2.67	1.40	2.14	3.15	1.57	1.17	2.02	2.76	1.25	1.64	2.72	3.07	2.55	2.33
	MEAN	3.44	2.54	3.28	2.92	2.96	2.28	2.90	2.59	1.42	1.94	2.72	1.86	1.25	1.96	2.41	1.44	1.84	2.67	3.28	2.66	2.38
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.03	0.03	0.05	0.06	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.03	0.02	0.04	0.05	0.05	0.08	0.12
	CD(5%)	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.04	0.03	0.04	0.06	0.06	0.10	0.14	0.07	0.04	0.07	0.09	0.10	0.16	0.23

activity was observed in leaf at 105 DAS, shoot at 45 DAS and root at 60 DAS. There was a significant difference between DT and DS in leaf and root nitrate reductase activity, but no difference was seen in shoot. Leaf showed maximum activity followed by root and shoot.In DT, the activity in leaf dropped at 45 and 75 DAS and then increased during later stages. But in shoot and root the activity increased till 60 DAS, followed by a decrease at 75 DAS and again an increase at 90 DAS and then decreased at 105 DAS. In DS the activity in leaf peaked at 60 and 105 DAS. In shoot the activity decreased at 60 and 90 DAS and in root it decreased at 75 and 90 DAS.

Finger millet

AZ204 treated plants registered significantly increased activity in leaf and shoot. In leaf AZ204 and FT326 treated plants were on par. In root, FT326 and AZ204 inoculated plants, AZ208 treated and IAA-T plants were on par with each other in influencing the enzyme activity. At different stages of growth leaf at 30 DAS, shoot at 75 DAS and root at 90 DAS showed increased activity. DS recorded higher activity than DT in all parts of the plant. Similar to sorghum leaf exhibited maximum activity followed by root and shoot. In DT, enzyme activity increased as the days increased but declined at 45 and 75 DAS in leaf, 45 and 90 DAS in shoot and 45 DAS in root. In DS, the activity dropped at 45 DAS, increased until 75 DAS and then decreased till maturity.

Moorthy (1986) reported that the nitrate reductase activity in roots increased on application of Azospirillum to sorghum plants when compared to control from 15 to 45 DAS.

Scott and Neyra (1979) reported that the activity of the enzyme in sorghum leaf increased with leaf development, reached maximum at full leaf expansion and then declined as the leaf became older. The studies by several workers (Oaks *et al.*, 1980; Gaspariko *et al.*, 1978) reveal that the activity of the enzyme was higher in root tip region than in the mature root. Panwar (1992) reported that nitrate reductase activity was low in wheat under water stress conditions and its effect was mitigated by inoculation with *Azospirillum*.

4.10.NITRITE REDUCTASE

Tables XVIII and XIX illustrate the activities of nitrite reductase in different parts of tolerant and susceptible varieties of sorghum and finger millet on application of *Azospirillum* strains and external application of IAA.

Sorghum

In leaf nitrite reductase activity was highest in AZ208 treated plants and lowest in IAA-T plants. In shoot and root activity was highest in FT326 and AZ204 inoculated plants and lowest in AZ208 treated plants respectively. All the treatments showed significant increase in nitrite reductase activity when compared to control. Maximum enzyme activity was seen at 60 DAS in leaf, shoot and root. DT expressed higher activity than the susceptible variety in all the plant organs studied. Root showed maximum enzyme activity followed by shoot and leaf. In DT the enzyme showed a gradual increase over time in all the parts until 60 DAS and then decreased at 75 and 90 DAS and followed by an increase again at 105 DAS. In DS enzyme

TABLE – XVIII

NITRITE REDUCTASE ACTIVITY (n moles of nitrite reduced /min/mg prot.) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	53.54	141.38	179.81	89.12	52.57	87.61	100.67	93.01	150.12	194.65	138.77	74.02	123.00	128.93	80.97	105.12	196.63	124.02	91.91	112.88	118.59
	DS	58.54	70.58	104.89	145.72	83.98	34.90	83.10	68.09	118.39	171.28	89.30	66.52	50.28	93.98	75.29	97.51	174.24	128.17	109.49	115.18	116.65
	MEAN	56.04	105.98	142.35	117.42	68.28	61.26	91.89	80.55	134.26	182.97	114.04	70.27	86.64	111.45	78.13	101.32	185.44	126.10	100.70	114.03	117.62
AZ204 (T2)	DT	71.44	170.69	220.49	128.52	79.38	126.16	132.78	117.85	175.35	234.05	161.34	80.98	150.02	153.27	109.77	132.86	269.77	173.30	137.98	145.12	161.47
	DS	82.97	95.14	139.89	193.24	117.09	43.80	112.02	89.88	167.79	204.23	117.82	78.79	70.40	121.49	98.55	122.56	204.61	171.29	159.79	168.33	154.19
	MEAN	77.21	132.92	180.19	160.88	98.24	84.98	122.40	103.87	171.57	219.14	139.58	79.89	110.21	137.38	104.16	127.71	237.19	172.30	148.89	156.73	157.83
AZ208 (T3)	DT	71.45	173.10	196.89	94.78	64.87	107.00	118.02	116.28	167.50	210.58	185.13	84.29	143.00	151.13	87.63	124.02	251.47	140.37	112.51	125.38	140.23
	DS	70.32	88.74	150.00	180.11	89.21	48.20	104.43	74.99	176.71	233.75	96.56	73.50	57.75	118.88	83.94	103.26	197.54	155.28	140.68	151.76	138.74
	MEAN	70.89	130.92	173.45	137.45	77.04	77.60	111.22	95.64	172.11	222.17	140.85	78.90	100.38	135.00	85.79	113.64	224.51	147.83	126.60	138.57	139.49
IFT 326 (T4)	DT	63.22	168.29	219.86	172.56	70.25	100.89	132.51	136.86	180.07	271.61	174.93	74.93	132.10	161.75	102.42	150.05	226.53	155.96	107.94	135.17	146.35
	DS	79.88	98.74	168.03	179.02	103.02	39.42	111.35	75.98	173.34	237.26	128.17	96.17	76.19	131.19	87.58	112.47	218.00	170.38	137.48	186.53	152.07
	MEAN	71.55	133.52	193.95	175.79	86.64	70.16	121.93	106.42	176.71	254.44	151.55	85.55	104.15	146.47	95.00	131.26	222.27	163.17	122.71	160.85	149.21
IAA/T (T5)	DT	62.94	155.90	206.59	104.00	63.28	114.40	117.85	122.86	183.83	265.80	176.65	86.73	160.96	166.14	91.09	120.66	229.31	163.00	115.82	147.54	144.57
	DS	80.82	108.05	124.14	146.28	93.32	46.94	99.93	88.60	142.14	215.80	122.88	86.29	69.92	120.94	90.75	123.54	221.77	162.50	125.35	133.91	142.97
	MEAN	71.88	131.98	165.37	125.14	78.30	80.67	108.89	105.73	162.99	240.80	149.77	86.51	115.44	143.54	90.92	122.10	225.54	162.75	120.59	140.73	143.77
	MEAN	69.51	127.06	171.06	143.34	81.70	74.93	111.27	98.44	163.52	223.90	139.16	80.22	103.36	134.77	90.80	119.21	218.99	154.43	123.90	142.18	141.58
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.04	0.03	0.05	0.06	0.07	0.11	0.15	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.04	0.03	0.05	0.06	0.07	0.10	0.15
	CD(5%)	0.09	0.05	0.10	0.12	0.13	0.21	0.30	0.07	0.05	0.08	0.10	0.11	0.18	0.25	0.08	0.05	0.09	0.12	0.13	0.21	0.29

TABLE – XIX

NITRITE REDUCTASE ACTIVITY (n moles of nitrite reduced /min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	148.28	51.51	69.30	54.76	24.02	35.43	147.22	157.09	83.81	149.55	91.51	55.51	21.22	76.45	172.21	101.36	131.74	161.74	61.08	46.98	95.85
(T1)	DS	121.82	31.24	56.93	20.87	13.35	19.58	127.30	171.16	90.24	94.19	126.19	51.22	29.53	77.09	165.32	101.98	160.77	202.85	149.71	57.69	123.05
	MEAN	135.05	41.38	63.12	37.82	18.69	27.51	137.26	164.13	87.03	121.87	108.85	53.37	25.38	76.77	168.77	101.67	146.26	182.30	105.40	52.34	109.45
AZ204	DT	155.60	70.42	103.00	55.16	29.98	46.88	160.17	187.03	98.92	202.82	111.10	88.57	41.81	105.04	191.81	144.40	171.66	171.91	64.75	53.75	116.38
(T2)	DS	123.89	42.07	65.18	24.57	15.96	26.93	133.10	174.29	98.65	113.45	199.63	86.71	47.67	103.40	187.00	189.54	184.58	238.30	161.60	73.83	145.64
	MEAN	139.75	56.25	84.09	39.87	22.97	36.91	146.64	180.66	98.79	158.14	155.37	87.64	44.74	104.22	189.41	136.47	178.12	205.11	113.18	63.79	131.01
AZ208	DT	151.68	54.95	75.94	67.52	27.85	43.16	153.52	164.06	103.06	164.94	98.76	72.54	32.04	89.23	178.95	113.94	147.67	175.35	70.41	53.14	106.58
(T3)	DS	124.11	37.79	71.92	29.72	14.63	28.06	134.37	185.74	101.92	120.82	175.29	64.94	37.87	97.76	176.61	105.18	178.78	243.64	170.48	60.95	139.27
	MEAN	137.90	46.37	73.93	48.62	21.24	35.61	143.94	174.90	102.49	142.88	137.03	68.74	34.96	93.50	177.78	109.56	163.23	209.50	120.45	57.05	122.93
IFT 326	DT	164.44	70.89	83.06	62.79	28.58	58.91	161.45	170.82	115.30	177.20	116.89	91.94	40.81	102.16	190.87	116.32	142.39	187.89	83.06	67.64	114.70
(T4)	DS	128.39	36.54	62.65	38.54	14.43	29.29	134.97	189.26	101.09	142.94	165.56	72.18	38.52	101.59	179.24	113.95	185.90	226.68	158.42	62.41	137.77
	MEAN	146.42	53.72	72.86	50.67	21.51	44.10	148.21	180.04	108.20	160.07	141.23	82.06	39.67	101.88	185.06	115.14	164.15	207.29	120.74	65.03	126.23
AA/T	DT	157.72	61.96	92.72	80.95	30.99	40.29	160.77	162.69	84.12	190.24	104.24	80.29	28.22	91.63	183.46	124.30	159.97	170.24	69.77	48.08	109.30
(T5)	DS	122.53	49.22	70.39	25.32	20.04	31.04	136.46	182.53	97.20	100.53	160.75	58.29	41.77	90.18	183.16	126.57	171.96	214.64	162.73	61.09	136.69
	MEAN	140.13	55.59	81.66	53.14	25.52	35.67	148.61	172.61	90.66	145.40	132.50	69.29	35.00	90.91	183.31	125.44	165.97	192.44	116.25	54.59	123.00
	MEAN	139.85	50.66	75.13	46.02	21.98	35.96	144.93	174.47	97.43	145.67	134.99	72.22	35.95	93.45	180.86	117.65	163.54	199.32	115.20	58.56	122.52
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.02	0.03	0.03	0.04	0.06	0.08	0.03	0.02	0.04	0.05	0.05	0.08	0.12	0.03	0.02	0.03	0.04	0.04	0.06	0.09
	CD(5%)	0.05	0.03	0.05	0.07	0.08	0.12	0.17	0.07	0.04	0.07	0.10	0.10	0.16	0.23	0.05	0.03	0.06	0.07	0.08	0.13	0.18

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activity peaked at 75 DAS in leaf and at 60 DAS in shoot and root and thereafter decreased.

Finger millet

Leaf nitrite reductase activity ranked highest in IAA-T plants and lowest in AZ208 treated plants among all the treatment groups. Shoot and root enzyme activity was highest in AZ204 treated plants and lowest in IAA-T and AZ208 treated plants respectively. Comparison of all the treatments with control showed significant increase in all the plant parts studied. Enzyme activity reached maximum at 60 DAS in leaf and shoot and at 75 DAS in root. There was a significant interaction between treatments and varieties, stages and varieties and treatments and stages. Similar to sorghum, finger millet root showed greater activity followed by shoot and leaf. Leaf nitrite reductase activity was more in DT than in DS. The trend of the enzyme activity in DT increased until 60 DAS and declined at 75 and 90 DAS in both leaf and shoot.While the activity increased again at 105 DAS in leaf, such an increase was not observed in shoot. In root, the activity peaked at 75 DAS and declined thereafter. Similar trend was seen in DS also.

Moorthy (1986) reported that the nitrite reductase activity in roots increased on application of *Azospirillum* to sorghum plants when compared to control from 15 to 45 DAS.

4.11.XANTHINE DEHYDROGENASE

Tables XX and XXI give the activities of xanthine

TABLE –XX

XANTHINE DEHYDROGENASE ACTIVITY (n moles of NAD reduced/min/mg prot.) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	112.33	18.24	26.01	36.32	35.64	41.75	131.72	122.31	39.38	69.96	10.72	42.60	38.44	137.24	130.75	44.26	19.38	26.56	52.60	60.23	138.96
	DS	124.73	26.80	40.29	22.60	40.17	31.08	130.95	9.24	15.56	44.49	25.08	60.80	55.78	135.16	114.99	25.52	31.69	30.05	21.07	35.82	126.52
	MEAN	118.53	22.52	33.15	29.46	47.91	36.42	131.33	115.78	27.47	57.23	17.90	51.70	47.11	136.20	122.87	34.89	25.54	28.31	36.84	48.03	132.74
AZ2)A (T2)	DT	118.61	35.80	36.64	46.32	62.70	55.93	142.67	152.94	65.20	97.39	17.31	86.04	59.64	163.09	154.42	68.39	29.52	34.92	76.04	81.25	157.42
	DS	126.97	30.43	68.61	54.42	80.16	73.60	155.70	115.35	19.34	63.35	53.02	70.81	59.97	146.97	129.78	33.27	58.09	48.57	43.58	52.17	144.24
	MEAN	122.79	33.12	52.63	50.37	71.43	64.77	149.18	134.15	42.27	80.37	35.17	78.43	59.81	155.03	142.10	50.83	43.81	41.75	59.81	66.71	150.83
AZ208 (T3)	DT	112.78	28.21	34.59	39.74	70.63	48.62	139.10	143.28	53.40	81.67	26.03	85.08	61.24	158.45	143.36	58.82	18.39	30.42	59.20	68.95	146.52
	DS	115.88	20.09	32.15	30.71	71.43	61.66	138.65	115.97	21.22	61.80	32.94	72.35	43.12	141.23	124.16	33.22	48.23	34.07	27.30	37.17	134.03
	MEAN	114.33	24.15	33.37	35.23	71.03	55.14	138.87	129.63	37.31	71.73	29.49	78.72	52.18	149.84	133.76	46.02	33.31	32.25	43.25	53.06	140.27
FT 326 (T4)	DT	117.18	33.53	42.87	48.58	75.98	52.89	145.07	144.62	55.21	70.89	20.87	82.14	68.53	157.04	159.84	66.08	21.73	38.24	65.75	74.61	154.39
	DS	125.16	27.72	54.55	34.91	87.33	63.52	148.87	119.45	21.26	50.63	27.02	85.63	53.52	142.92	129.04	45.19	61.11	36.93	33.19	43.85	141.55
	MEAN	121.17	30.63	48.71	41.75	81.66	57.91	146.97	132.04	38.24	60.76	23.95	83.89	61.03	149.98	144.44	55.64	41.42	37.64	49.47	59.23	147.77
AA/T (T5)	DT	113.17	22.22	29.39	45.18	66.84	44.07	136.81	141.22	44.68	72.77	26.60	110.84	73.87	161.66	145.78	53.60	27.18	29.22	50.21	61.14	144.52
	DS	130.40	43.92	52.45	39.46	74.41	62.73	150.56	119.90	20.36	58.79	29.15	69.81	62.73	143.46	131.86	40.09	57.40	32.93	28.62	39.36	138.38
	MEAN	121.79	33.07	40.92	42.32	70.63	53.40	143.69	130.56	32.52	65.78	27.88	90.33	68.30	152.56	138.82	46.85	42.29	31.08	39.42	50.25	141.45
	MEAN	119.72	28.70	41.76	39.82	68.53	53.53	142.01	128.43	35.56	67.17	26.87	76.61	57.68	148.72	136.40	46.84	37.27	34.20	45.76	55.46	142.65
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.04	0.05	0.05	0.08	0.12	0.03	0.02	0.03	0.04	0.04	0.06	0.09	0.02	0.02	0.03	0.03	0.04	0.06	0.09
	CD(5%)	0.07	0.04	0.07	0.10	0.10	0.17	0.23	0.05	0.03	0.05	0.07	0.08	0.12	0.17	0.05	0.03	0.05	0.07	0.08	0.12	0.17

TABLE – XXI

XANTHINE DEHYDROGENASE ACTIVITY (n moles of NAD . reduced /min/mg prot.) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	5.69	8.62	11.37	17.38	16.44	13.89	112.23	110.03	14.77	23.43	26.13	14.86	22.40	118.60	116.86	25.16	37.70	43.05	25.34	21.55	128.28
	DS	9.59	15.54	21.67	23.20	20.99	24.64	119.27	110.98	20.14	22.20	27.28	20.46	26.46	121.25	118.81	20.95	25.44	27.28	12.64	25.37	120.45
	MEAN	7.64	12.08	16.52	20.29	18.72	19.27	115.75	110.51	17.46	22.82	26.71	17.66	24.43	119.93	113.94	23.06	31.57	35.17	18.99	23.46	124.36
AZ204 (T2)	DT	9.39	11.80	14.02	37.36	30.36	25.35	121.38	115.31	34.21	42.49	44.07	27.89	30.59	132.43	134.16	38.44	40.14	58.07	47.69	42.56	143.51
	DS	13.15	25.30	35.49	43.46	27.04	34.91	129.89	118.90	41.06	48.23	52.18	36.08	45.53	140.33	119.46	41.80	57.24	38.78	19.29	35.99	135.43
	MEAN	11.27	18.55	24.76	40.41	28.70	30.13	125.64	117.11	37.64	45.36	48.13	31.99	38.06	136.38	126.81	40.12	48.69	48.43	33.49	39.28	139.47
AZ208 (T3)	DT	6.28	9.60	25.85	30.17	22.60	20.67	119.20	110.21	28.83	40.15	52.44	23.92	35.84	131.90	125.09	31.68	47.24	50.14	36.05	33.16	137.23
	DS	11.00	18.99	24.84	36.56	23.05	28.08	123.75	113.44	30.31	44.94	48.08	29.35	35.33	133.58	111.40	30.38	47.33	35.03	27.79	29.55	130.25
	MEAN	8.64	14.30	25.35	33.37	22.83	24.38	121.47	111.83	29.57	42.55	50.26	26.64	35.59	132.74	118.25	31.03	47.29	42.59	31.92	31.36	133.74
IFT 326 (T4)	DT	11.40	15.74	21.18	27.70	21.44	19.74	119.53	117.54	26.56	37.66	47.39	18.81	34.80	133.79	137.91	44.71	49.95	47.66	41.56	29.23	141.84
	DS	12.03	24.30	33.15	38.52	27.48	37.60	128.85	116.43	32.38	49.38	54.52	29.74	66.58	141.51	117.15	39.60	54.77	32.79	15.41	35.98	132.62
	MEAN	11.72	20.02	27.17	33.11	24.46	28.67	124.19	116.99	29.47	43.52	50.96	24.27	60.69	137.65	127.53	42.16	52.36	40.23	28.49	32.61	137.23
IAA/T (T5)	DT	8.44	10.37	13.46	30.67	27.25	17.86	118.01	114.82	17.90	31.46	35.18	17.68	26.13	123.66	118.83	38.96	57.83	64.68	27.06	21.72	138.18
	DS	11.53	21.56	24.03	36.52	26.67	32.36	125.45	112.98	25.55	28.54	46.61	28.46	54.23	132.73	113.21	31.55	62.94	35.59	17.75	28.07	131.52
	MEAN	10.00	15.97	18.75	33.60	26.96	25.11	121.73	113.90	21.73	30.00	40.90	23.07	40.18	128.30	116.02	35.26	60.39	50.14	22.41	24.90	134.85
	MEAN	9.85	16.18	22.51	32.15	24.33	25.51	121.76	114.06	27.17	36.85	43.39	24.72	39.79	131.00	120.51	34.32	48.06	43.31	27.06	30.32	133.93
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.02	0.03	0.03	0.04	0.06	0.08	0.02	0.02	0.03	0.03	0.04	0.06	0.08	0.24	0.15	0.26	0.33	0.37	0.58	0.82
	CD(5%)	0.05	0.03	0.05	0.07	0.07	0.12	0.17	0.05	0.03	0.05	0.07	0.07	0.12	0.16	0.47	0.30	0.52	0.67	0.73	1.16	1.64

dehydrogenase (XDH) in leaf, shoot and root of selected varieties of sorghum and finger millet as a result of inoculation with different strains of *Azospirillum* and external application of IAA.

Sorghum

AZ204 treatment caused maximal increase of XDH activity while AZ208 induced the activity to a minimum extent in all parts of the plant. In leaf and shoot 90 DAS and in root 105 DAS were found to be the time points showing the highest XDH activity. DS registered increased activity in leaf, while in shoot and root DT recorded higher activity.

When treatments and varieties were considered inoculation with AZ204 exhibited maximal activity. There was a significant interaction between stages and varieties, and treatments and stages. Statistical analysis revealed maximum activity in shoot and more or less similar activity in both leaf and root. In DT the activity peaked at 90 DAS in all parts of the plant. The activity was minimum at 75 DAS and 60 DAS in shoot and root respectively. In DS the activity showed a biphasic behaviour with peaks at 60 and 90 DAS and a low activity at 75 and 105 DAS.

Finger millet

The activity of XDH was found to be significantly higher in leaf and root on application of AZ204 whereas FT326 induced maximum effect in shoot. All the treatments resulted in significant increase in the activity of XDH over control. Among the various stages, activity was found to be more at 75 DAS in leaf and shoot and at 60 DAS in root. XDH activity was

greater in leaf and shoot of DS while a reverse effect was observed in DT.

The interaction between treatments and varieties was found to be superior in leaf and root of the plants applied with AZ204. Significantly higher XDH activity was registered after 75 DAS when stages and varieties interaction was considered. The overall picture reveals that the activity was maximum in root followed by shoot and leaf. In both the varieties the enzyme activity showed an increasing trend until 75 DAS, dropped at 90 DAS and then increased at maturity.

An increase was observed in the specific activities of XDH in the plant cytosol fraction during soybean nodule development between 11 and 17 days which decreased at 19th day and remained constant till 23rd day (Reynolds *et al.*, 1982). Christensen and Jochimsen(1983),also found that the activity of XDH was more in root than in other tissues of pea and soybean plants .

Amarjit and Singh (1985) reported that XDH activity in pigeon pea exhibited two peaks at day 75 and other at day 105 with a decline at 45th and 90th day.

4.12.URICASE

The activities of uricase in leaf, shoot and root of drought tolerant and susceptible varieties of sorghum and finger millet upon inoculation of different *Azospirillum* strains and that due to external application of IAA is given

in Tables XXII and XXIII, and Figures - 7 and 8.

Sorghum

In both leaf and shoot the activity of uricase was found to be significantly higher in AZ204(T2) inoculated plants followed by plants inoculated with FT326(T4), IAA-T(T5) and AZ208(T3). In root FT326 application induced maximal activity. AZ208 and external IAA application were on par with each other although they showed a significant increase over the control.

All the treatments resulted in a significant increase in the activity of uricase in both varieties of sorghum when compared to the control at all the time intervals tested. Maximal activity of the enzyme was noticed in leaf after 60 DAS, in shoot after 90 DAS and in root after 75 DAS. The activity of the enzyme showed an increasing trend until the specific DAS when its activity was maximal and thereafter declined steadily. Uricase activity was greater in leaf and shoot of DS while a reverse effect was observed in DT. The overall picture revealed maximum uricase activity in leaf followed by shoot and then root.

Finger millet

Uricase activity was found to be greater in leaf and root of plants inoculated with FT326, while in shoot AZ204 treated plants exhibited maximal activity. All the treatments showed a significant increase over control with respect to

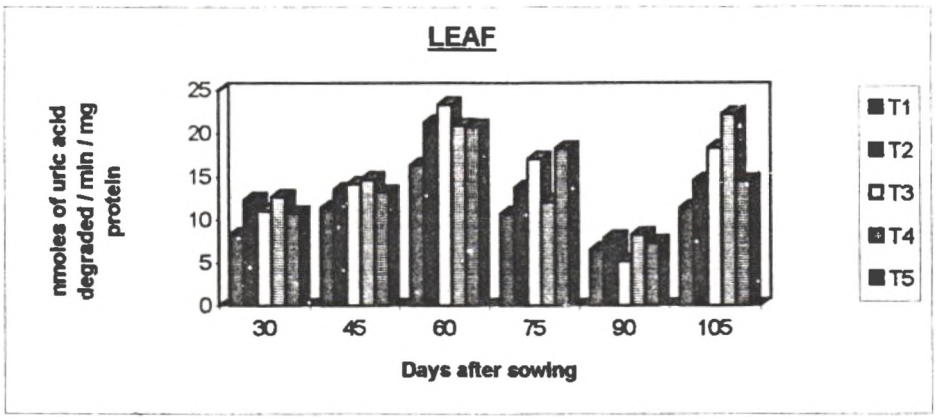
TABLE – XXII

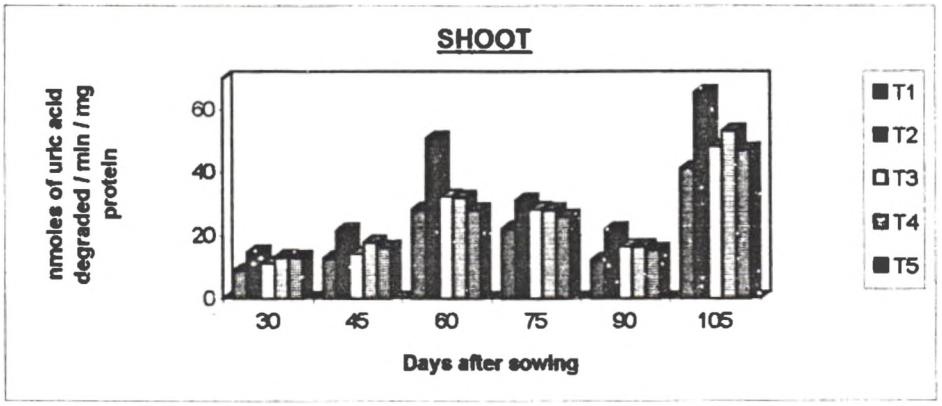
URICASE ACTIVITY (n moles of uric acid degraded/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	112.49	19.24	35.84	22.35	34.27	31.48	125.98	7.79	9.98	13.39	20.64	38.31	26.00	119.39	111.72	21.95	26.44	37.32	28.31	21.52	124.54
	DS	121.52	29.60	36.57	22.79	30.79	19.54	126.80	7.58	10.87	35.20	20.14	46.56	27.77	125.02	112.78	17.84	20.00	25.58	23.44	19.33	119.83
	MEAN	117.01	24.42	36.21	22.67	32.53	25.51	126.39	7.69	10.43	24.40	20.39	42.45	27.89	122.20	112.25	19.90	23.22	31.45	25.88	20.43	122.19
AZ204 (T2)	DT	118.01	22.98	46.01	38.24	56.78	44.63	137.78	111.60	14.27	26.75	36.48	54.82	47.87	131.97	116.25	32.80	28.58	48.43	37.51	23.78	131.23
	DS	137.37	42.68	57.53	33.32	43.98	31.30	141.03	119.17	29.52	54.32	48.32	60.22	51.84	143.90	114.52	31.60	38.12	59.00	40.22	33.24	136.12
	MEAN	127.69	32.83	51.77	35.78	50.38	37.97	139.40	115.39	21.90	40.54	42.40	57.52	49.86	137.93	115.39	32.20	33.35	53.72	38.87	28.51	133.67
AZ208 (T3)	DT	117.37	21.25	50.75	33.38	44.66	32.30	133.29	8.82	10.99	14.66	22.03	46.10	33.44	122.58	113.68	22.39	29.69	45.88	35.99	29.88	129.52
	DS	122.38	32.30	38.70	31.75	35.58	23.49	131.03	111.57	12.39	47.38	37.09	59.64	42.16	135.04	115.82	23.77	26.68	35.64	29.64	31.24	127.13
	MEAN	119.88	26.78	44.73	32.56	40.12	28.89	132.16	9.90	11.69	31.02	29.57	52.87	37.80	128.81	114.75	23.18	28.19	40.46	32.82	30.56	128.33
FT 326 (T4)	DT	115.92	29.84	41.66	32.09	37.89	35.97	132.23	112.29	14.55	16.82	28.85	44.41	47.01	130.89	119.14	29.07	40.34	42.48	39.11	33.04	133.86
	DS	133.08	40.08	58.78	29.42	53.80	47.69	143.81	114.13	22.99	46.28	38.18	61.81	43.58	138.08	115.31	28.68	37.17	44.83	41.17	35.29	133.74
	MEAN	124.50	34.96	50.22	30.76	45.85	41.83	138.02	113.21	18.77	31.25	33.52	63.86	46.30	134.48	117.23	28.88	38.76	43.66	40.14	34.17	133.80
AA/T (T5)	DT	117.66	23.65	38.92	25.80	38.54	36.26	130.14	110.10	13.65	25.78	28.92	48.50	38.34	127.35	115.32	26.30	32.93	41.62	30.26	24.48	128.52
	DS	129.73	33.18	56.34	29.16	41.18	33.32	137.15	111.89	20.39	45.06	22.93	58.31	29.60	131.23	116.34	22.34	25.14	32.78	28.22	33.93	128.13
	MEAN	123.70	28.42	47.63	27.48	39.86	34.79	133.65	110.99	17.12	35.42	25.93	53.41	33.47	129.39	115.83	24.32	29.04	37.20	34.24	29.31	128.32
	MEAN	122.55	29.48	46.11	29.85	41.75	33.80	133.92	111.43	15.98	32.52	30.36	54.02	39.06	130.56	115.09	25.69	30.51	41.30	34.39	28.59	129.86
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.03	0.03	0.04	0.06	0.08	0.02	0.02	0.03	0.03	0.04	0.04	0.08	0.02	0.01	0.02	0.03	0.03	0.05	0.07
	CD (5%)	0.05	0.03	0.05	0.07	0.07	0.12	0.16	0.05	0.03	0.05	0.07	0.07	0.12	0.17	0.04	0.03	0.04	0.06	0.06	0.10	0.14

Figure 7

URICASE ACTIVITY IN DTV OF FINGER MILLET





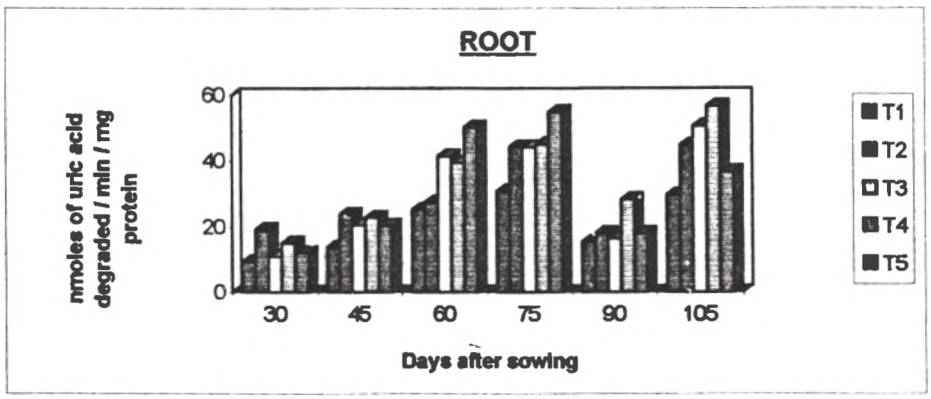


TABLE – XXIII

URICASE ACTIVITY (n moles of uric acid degraded / min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	8.27	11.42	16.34	10.71	6.53	11.38	10.78	8.79	12.77	28.09	22.06	12.14	41.03	20.81	9.41	13.73	25.18	30.84	15.34	29.67	20.70
	DS	8.15	13.22	15.34	16.30	13.76	11.60	13.06	9.22	17.36	22.36	31.27	43.87	29.96	25.67	7.53	12.33	23.79	34.67	43.87	25.96	24.73
	MEAN	8.21	12.32	15.84	13.51	10.15	11.49	11.92	9.01	15.07	25.23	26.67	28.01	35.50	23.24	8.47	13.14	24.49	32.76	29.61	27.82	22.71
AZ204 (T2)	DT	12.35	13.53	21.26	13.73	7.87	14.57	13.89	14.58	21.42	50.75	30.94	21.72	65.56	34.16	19.12	23.73	27.45	44.28	18.05	40.89	29.58
	DS	13.26	15.56	20.49	28.38	25.32	21.39	20.73	15.56	21.12	40.78	50.86	54.80	45.34	38.08	12.18	17.40	28.21	49.17	56.65	45.61	34.87
	MEAN	12.81	14.55	20.88	21.06	16.60	17.98	17.31	15.07	21.27	45.77	40.90	38.26	55.45	36.12	15.65	20.57	27.83	46.73	37.35	43.22	32.22
AZ208 (T3)	DT	11.02	14.18	23.34	17.05	5.15	18.32	14.84	11.13	14.18	32.25	28.24	16.47	47.99	25.04	10.99	20.59	41.48	44.13	16.39	50.54	30.69
	DS	9.12	13.56	17.63	23.79	18.17	15.87	16.36	10.11	11.96	31.66	39.26	58.71	38.70	31.73	9.98	13.30	25.79	39.02	50.52	33.34	28.66
	MEAN	10.07	13.87	20.49	20.42	11.66	17.10	15.60	10.62	13.07	31.96	33.75	37.59	43.35	28.39	10.49	16.95	33.64	41.58	33.46	41.94	29.67
T 326 (T4)	DT	12.68	14.75	20.92	12.13	8.28	22.25	15.17	13.15	17.63	31.82	27.91	16.39	53.13	26.67	15.11	22.79	39.51	45.07	28.34	56.62	34.57
	DS	14.12	19.77	21.29	29.98	19.45	18.89	20.58	12.35	29.13	37.07	48.46	70.46	41.95	39.90	14.62	26.74	35.69	45.46	64.54	43.47	38.42
	MEAN	13.40	17.26	21.11	21.06	13.87	20.57	17.88	12.75	23.38	34.45	38.19	43.43	47.54	33.29	14.87	24.77	37.60	45.27	46.44	50.05	36.50
AA/T (T5)	DT	10.79	13.23	20.84	18.30	7.34	14.48	14.16	12.90	15.98	27.85	25.84	15.14	47.03	24.12	12.14	20.44	50.19	34.93	18.01	36.79	32.08
	DS	11.86	15.14	18.71	29.62	23.92	11.74	18.50	18.47	19.37	26.24	52.00	60.22	42.38	36.45	11.19	21.86	40.56	47.88	50.22	35.13	34.47
	MEAN	11.33	14.19	19.78	23.96	15.63	13.11	16.33	15.69	17.68	27.05	38.92	37.68	44.71	30.29	11.67	21.15	45.38	51.41	34.12	35.96	33.28
	MEAN	11.16	14.44	19.62	20.00	13.58	16.05	15.81	12.63	18.09	32.89	35.68	36.99	45.31	30.86	12.23	19.31	33.79	43.55	36.19	40.80	30.88
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.09	0.03	0.04	0.06	0.02	0.01	0.02	0.02	0.03	0.04	0.06	0.11	0.07	0.12	0.15	0.17	0.26	0.37
	CD(5%)	0.04	0.02	0.04	0.05	0.05	0.09	0.12	0.03	0.02	0.04	0.05	0.05	0.08	0.12	0.22	0.14	0.24	0.30	0.33	0.53	0.75

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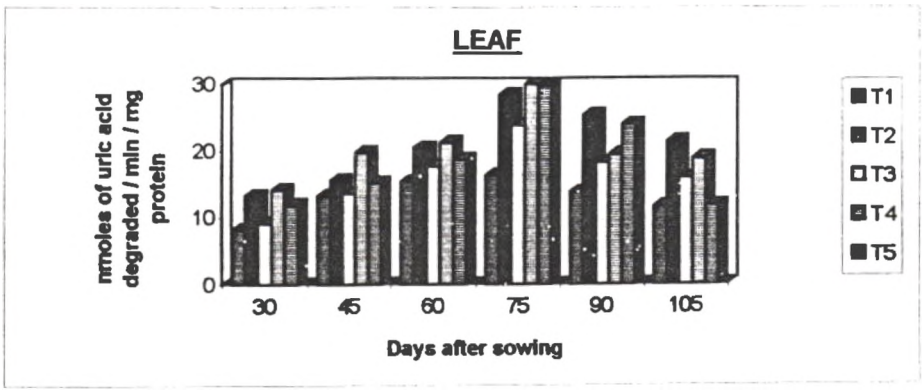
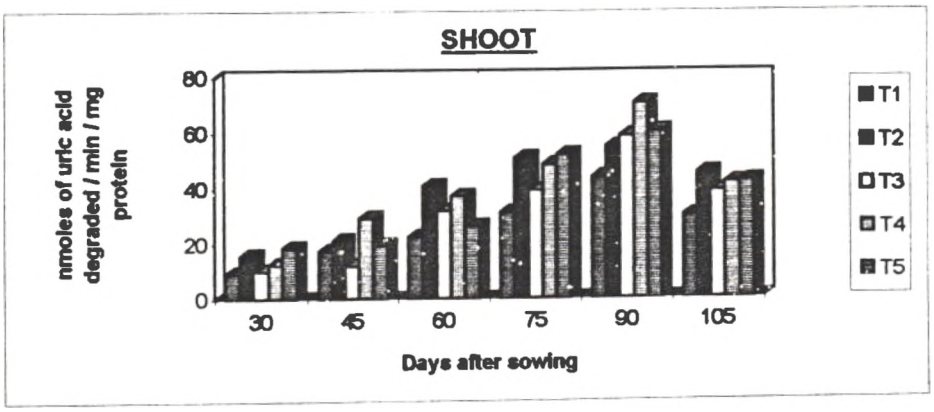
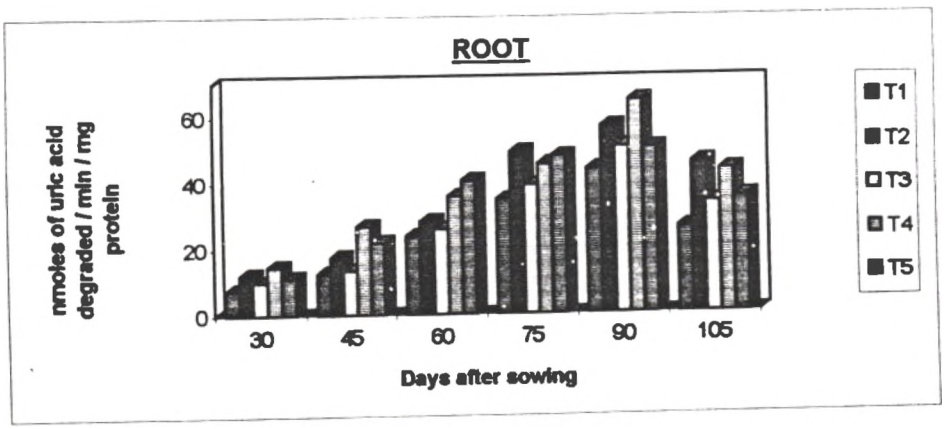
Figure - 8

URICASE ACTIVITY IN DSV OF FINGER MILLET







uricase activity. With regard to the stages, uricase activity was maximum in leaf and root at 75 DAS, whereas it peaked at 105 DAS in shoot.DS expressed greater activity than DT in all parts. Treatment and variety interaction in leaf showed maximum activity in FT326 applied plants. In shoot, maximal induction was seen by FT326 inoculation in DT and AZ204 inoculation in DS and this effect was reversed in root. Uricase activity was greater in root followed by shoot and leaf.In DT the activity increased until 60 DAS and then decreased at 75 and 90 DAS and again increased at 105 DAS.In DS the activity peaked at 90 DAS and then declined at 105 DAS.

Christensen and Jochimsen (1983) reported that among different parts of pea and soybean specific activity of uricase was maximum in roots. Next to root leaf showed maximum activity in soybean but it was stem which showed activity in pea. Our results also show increased activity in root of finger millet and leaf of sorghum.

Reynolds *et al.* (1982) reported that the uricase activity in soybean nodules increased from 11 to 17 days, declined at 19th day and then increased till 23rd day.

Polayes and Schubert (1984) studied the uricase activity in soybean seedlings from 0-18th day after inbibition and they too found a high level of uricase activity was found in the roots at a time when in the cotyledon it was declining. This suggested that roots are capable of synthesizing ureides.

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4.13.ALLANTOINASE

Effect of inoculation of different strains of *Azospirillum* and external application of IAA on the activity of Allantoinase in different parts of drought tolerant and susceptible varieties of sorghum and finger millet is given in Tables XXIV and XXV.

Sorghum

FT326 inoculated plants registered significantly higher activity in leaf, shoot and root than the rest of the treatment. In leaf, IAA treated and AZ204 inoculated plants were on par and in shoot AZ204 and AZ208 inoculated plants were on par with each other in allantoinase activity. When compared to the control all the treatments showed significantly increased activity. Allantoinase activity in each part peaked at flowering stage (75 DAS) and then declined continuously till maturity. DS exhibited increased activity in leaf and shoot but not in root. Maximum activity was observed in root followed by shoot and leaves.

Finger millet

It is found from the data presented that the allantoinase activity is significantly greater in FT326 treated plants in leaf and shoot, while the roots of plant treated with AZ204 had greater activity. AZ208 and AZ204 treatment were on par with each other in shoot and root. All the treatments differed significantly when compared to control. Enzyme activity peaked at 90 DAS in all the parts of the plant. In leaf DS showed more activity, but in shoot and

TABLE – XXIV

**ALLANTOINASE ACTIVITY (n moles of allantoic acid formed /min/mg prot .)
IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND
DROUGHT SUSCEPTIBLE (DS) VARIETIES OFSORGHUM**

		LEAF							SHOOT							ROOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	0.72	1.43	2.01	3.21	2.32	1.75	1.91	1.26	2.01	2.84	4.01	2.02	1.75	2.32	1.75	2.95	3.56	4.94	3.22	2.03	3.08
	DS	0.71	1.55	2.72	3.95	4.21	2.98	2.69	1.03	1.98	2.74	4.04	3.08	1.97	2.47	1.25	2.98	3.50	4.02	3.18	1.77	2.78
	MEAN	0.72	1.49	2.37	3.58	3.26	2.37	2.30	1.15	2.00	2.79	4.03	2.55	1.86	2.39	1.50	2.97	3.53	4.48	3.20	1.90	2.93
AZ204 (T2)	DT	0.92	2.11	2.47	3.94	3.44	2.40	2.55	1.71	2.44	3.25	4.83	2.18	2.00	2.74	1.92	3.37	4.12	5.29	3.70	2.35	3.46
	DS	1.00	1.80	3.27	4.15	4.53	3.18	2.99	1.24	2.33	3.27	4.94	3.23	2.21	2.87	1.61	3.50	4.01	4.97	3.50	2.50	3.35
	MEAN	0.96	1.96	2.87	4.05	3.98	2.79	2.77	1.48	2.39	3.26	4.89	2.71	2.11	2.80	1.77	3.44	4.07	5.13	3.60	2.43	3.40
AZ208 (T3)	DT	1.05	1.79	2.08	3.23	2.98	1.99	2.19	1.38	2.54	2.97	4.93	2.48	2.10	2.73	1.87	3.14	3.96	5.08	3.58	2.71	3.39
	DS	0.90	1.96	3.07	4.10	4.19	3.51	2.96	1.35	2.43	3.00	4.38	3.62	2.06	2.81	1.49	3.14	3.67	4.65	3.28	2.05	3.05
	MEAN	0.98	1.88	2.58	3.67	3.59	2.75	2.57	1.37	2.49	2.99	4.66	3.05	2.08	2.77	1.68	3.14	3.82	4.87	3.43	2.38	3.22
FT 326 (T4)	DT	0.81	2.09	2.96	3.96	3.15	2.43	2.57	1.60	2.37	3.63	4.25	2.98	2.28	2.85	2.00	3.84	4.37	5.51	3.55	2.95	3.70
	DS	1.12	1.75	3.45	4.58	4.92	3.59	3.24	1.59	2.48	3.45	4.33	3.76	2.61	3.04	1.76	3.29	3.97	5.07	3.82	2.11	3.34
	MEAN	0.97	1.92	3.21	4.27	4.04	3.01	2.90	1.60	2.43	3.54	4.29	3.37	2.44	2.94	1.88	3.57	4.17	5.29	3.69	2.53	3.52
IAA/T (T5)	DT	0.93	1.90	2.40	3.51	2.96	2.15	2.31	1.45	2.44	3.60	4.40	2.68	2.08	2.78	1.93	3.59	4.29	5.03	3.39	2.99	3.54
	DS	0.98	1.96	3.65	4.82	4.95	3.30	3.28	1.49	2.35	3.12	4.45	3.97	2.63	3.00	1.35	3.54	3.62	4.66	3.68	2.26	3.19
	MEAN	0.96	1.93	3.02	4.17	3.96	2.73	2.79	1.47	2.40	3.36	4.43	3.33	2.36	2.89	1.64	3.57	3.96	4.85	3.54	2.63	3.36
	MEAN	0.91	1.83	2.81	3.95	3.76	2.73	2.67	1.41	2.34	3.19	4.46	3.00	2.17	2.76	1.69	3.33	3.91	4.92	3.49	2.37	3.29
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.02	0.03	0.04	0.06	0.02	0.01	0.02	0.03	0.03	0.04	0.06	0.03	0.02	0.04	0.05	0.05	0.08	0.11
	CD(5%)	0.03	0.02	0.04	0.05	0.05	0.08	0.12	0.04	0.02	0.04	0.05	0.06	0.09	0.12	0.07	0.04	0.07	0.09	0.10	0.16	0.23

TABLE–XXV

**ALLANTOINASE ACTIVITY (n moles of allantoic acid formed /min/mg prot .)
IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND
DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET**

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	0.50	0.81	1.66	2.46	3.68	1.78	1.82	1.98	2.47	3.71	4.38	5.48	4.02	3.67	3.01	4.49	5.05	5.96	5.14	4.41	4.68
	DS	0.60	2.13	2.85	3.60	2.42	1.75	2.23	1.30	2.49	3.28	4.18	4.79	3.95	3.33	2.00	2.79	3.98	4.95	5.75	4.06	3.92
	MEAN	0.55	1.47	2.26	3.03	3.05	1.77	2.02	1.64	2.48	3.50	4.28	5.14	3.99	3.50	2.51	3.64	4.52	5.46	5.45	4.24	4.30
AZ204 (T2)	DT	0.68	1.25	2.28	3.23	4.28	2.59	2.38	2.33	3.35	3.96	4.95	6.00	4.74	4.22	3.98	5.31	6.04	6.96	5.92	4.85	5.51
	DS	0.82	2.24	3.26	4.27	3.71	2.15	2.74	1.88	2.88	3.54	4.60	5.30	4.17	3.73	2.41	3.36	4.75	5.81	6.61	4.76	4.62
	MEAN	0.75	1.75	2.77	3.75	4.00	2.37	2.56	2.11	3.12	3.75	4.78	5.65	4.46	3.98	3.20	4.34	5.40	6.39	6.27	4.81	5.06
AZ208 (T3)	DT	0.59	0.91	1.84	2.58	3.98	1.85	1.96	2.60	3.32	4.10	4.95	5.71	4.87	4.26	3.21	4.91	5.75	6.26	6.24	4.56	5.16
	DS	0.76	2.14	3.76	4.87	2.78	1.94	2.71	1.62	2.81	3.36	4.57	5.28	4.63	3.71	2.30	3.09	4.03	5.38	6.85	4.29	4.32
	MEAN	0.68	1.53	2.80	3.73	3.38	1.90	2.33	2.11	3.07	3.73	4.76	5.50	4.75	3.99	2.76	4.00	4.89	5.82	6.55	4.43	4.74
FT 326 (T4)	DT	0.62	1.11	2.49	3.56	4.85	2.27	2.48	2.35	2.95	4.35	5.38	6.16	5.41	4.43	3.98	5.08	6.13	7.04	6.29	4.92	5.57
	DS	0.69	2.74	3.04	4.75	4.60	2.03	2.88	1.47	2.70	3.42	5.03	6.15	4.52	3.88	2.47	3.94	4.68	5.69	6.20	4.15	4.52
	MEAN	0.66	1.92	2.77	4.16	4.43	2.15	2.68	1.91	2.83	3.89	5.21	6.16	4.97	4.16	3.23	4.51	5.41	6.37	6.25	4.54	5.05
IAA/T (T5)	DT	0.74	1.10	1.98	2.87	4.54	2.00	2.20	2.44	2.86	3.85	5.01	5.69	4.59	4.07	3.56	4.92	5.92	6.75	5.71	4.62	5.25
	DS	0.70	2.42	3.06	4.76	3.56	2.00	2.75	1.58	2.50	3.66	4.30	5.05	4.00	3.52	2.58	3.90	4.23	5.61	6.10	4.15	4.43
	MEAN	0.72	1.76	2.52	3.82	4.05	2.00	2.48	2.01	2.68	3.76	4.66	5.37	4.30	3.79	3.07	4.41	5.08	6.18	5.91	4.39	4.84
	MEAN	0.67	1.68	2.62	3.69	3.78	2.04	2.41	1.96	2.83	3.72	4.74	5.56	4.49	3.88	2.95	4.18	5.06	6.04	6.08	4.48	4.80
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.05	0.07	0.10	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.03	0.03	0.05	0.07
	CD(5%)	0.06	0.04	0.06	0.08	0.09	0.14	0.20	0.04	0.03	0.05	0.06	0.06	0.10	0.14	0.04	0.03	0.05	0.06	0.06	0.10	0.14

root DT expressed more activity. The activity was found to be similar to that observed in sorghum, with root having the higher activity followed by shoot.

The above results agree with the findings of Tajima and Yamamoto(1975), who reported that allantoinase activity was present in all regions of the 4 week old soybean plants and that the specific activity was more in nodules followed by roots, leaves and stems. The distribution of these enzymes indicate that root might be the main site of purine degradation and that a substantial part of the allantoin produced in roots is translocated to shoot and leaves, where it is broken down and utilised via allantoinase. Polayes and Schubert (1984) studied purine synthesis and catabolism in soybean seedlings from 6 to 18 days of growth and reported that allantoinase activity was higher in the cotyledons during the early period but it was more in roots during later stages.

Luthra *et al.* (1983) reported that allantoinase activity in pigeon pea in each organ peaked at flowering stage and declined thereafter till maturity. The activity of allantoinase has been reported in pods and seeds of soybean and cowpea. (Herridge *et al.*,1978; Thomas and Schrader, 1981). The decline in the enzyme activity in different regions of the plant during maturity suggests that there may be increased activity in seeds, so that the ureides received by pods or seeds could be metabolized to urea and glyoxylate. The presence of significant amounts of urease, GS, GDH and allantoinase in various organs demonstrates that the plants

have the capacity to degrade allantoin and allantoic acid and further assimilate the same through the action of urease, GS and GDH.

4.14.UREASE

Tables XXVI and XXVII present the urease activity in different parts of sorghum and finger millet plants treated with the selected strains of *Azospirillum* and external application of IAA.

Sorghum

Control plants recorded the least activity. T4(FT326) plants exhibited significantly higher activity than the rest. It was followed by AZ204, AZ208 and IAA-T. Urease activity increased steadily in all parts of the plants upto 45 DAS, declined at 60 DAS and then peaked at stage S5 (90) and finally declined. However, the activity at the final stage was still higher than the initial value.

DS exhibited higher activity than DT in leaf and shoot, while the activity was more in the root of DT than in DS. A significant difference in the urease activity was observed when the interactions between treatments and stages, treatments and varieties and stages and varieties were considered.

Finger Millet

A similar trend of urease activity as found in sorghum was observed in finger millet also except that the final value in root was lower than that of the initial value.

TABLE – XXVI

UREASE ACTIVITY (n moles of ammonia liberated/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	8.01	10.10	16.21	11.01	36.69	23.67	117.62	118.01	31.26	17.64	28.65	49.55	27.09	128.70	127.01	31.58	20.95	30.68	50.68	25.04	130.99
	DS	10.36	12.77	27.27	15.73	34.42	41.35	123.65	121.77	33.44	12.60	35.77	50.14	30.83	130.76	118.81	22.14	16.76	28.96	41.23	18.57	124.41
	MEAN	9.19	11.44	21.74	13.37	35.56	32.51	120.63	119.89	32.35	15.12	32.21	49.85	28.96	129.73	122.91	26.86	18.86	29.82	45.96	21.81	127.70
AZ204 (T2)	DT	12.86	14.38	19.40	12.92	38.16	34.19	125.32	132.32	49.28	27.08	32.13	68.71	29.89	139.90	129.01	38.42	27.51	46.45	61.93	33.42	139.46
	DS	14.81	19.05	42.16	18.81	49.10	60.70	134.11	130.77	46.65	14.84	38.66	57.46	40.30	138.11	125.55	31.87	25.46	32.62	54.96	22.00	132.08
	MEAN	13.84	16.72	30.78	15.87	53.63	47.45	129.71	131.55	47.97	20.96	35.40	63.09	35.10	139.01	127.28	35.15	26.49	39.54	58.45	27.71	135.77
AZ208 (T3)	DT	10.15	12.13	18.43	15.13	52.91	32.19	123.49	123.80	37.35	17.69	29.88	51.79	37.71	133.07	130.35	40.39	26.01	33.40	50.50	38.88	136.59
	DS	13.28	14.04	32.54	17.25	47.99	54.72	129.97	125.40	40.57	14.64	49.82	58.19	31.46	136.68	124.41	29.28	20.84	38.26	45.57	19.55	129.65
	MEAN	11.72	13.09	25.49	16.19	50.45	43.46	126.73	124.60	39.06	16.17	39.85	54.99	34.59	134.88	127.38	34.84	23.43	35.83	48.03	29.21	133.12
IFT 326 (T4)	DT	14.36	19.36	25.48	17.60	42.32	27.35	124.58	135.05	57.35	23.57	38.42	57.07	34.73	141.03	140.30	53.88	37.31	56.03	53.49	45.95	147.83
	DS	14.07	20.90	47.89	23.45	53.14	65.49	137.49	129.44	39.52	27.15	50.09	60.43	35.82	143.74	121.54	27.42	21.96	40.91	52.89	20.75	130.91
	MEAN	14.22	20.13	37.19	20.52	47.73	46.42	131.03	132.25	48.44	25.36	44.26	58.75	45.28	142.39	130.92	40.65	29.64	48.47	53.19	33.35	139.37
IAA/T (T5)	DT	9.28	12.81	21.31	11.37	38.84	23.09	119.45	129.10	38.30	22.90	38.77	54.25	29.63	135.49	128.49	32.25	22.78	38.67	50.14	37.19	134.92
	DS	11.64	13.46	35.06	24.28	37.59	47.79	128.30	130.87	45.19	18.91	46.41	54.41	37.77	138.93	126.10	35.44	18.55	33.43	40.46	19.00	128.83
	MEAN	10.46	13.14	28.19	17.83	38.22	35.44	123.88	129.99	41.75	20.91	42.59	54.33	33.70	137.21	127.30	33.85	20.67	36.05	45.30	28.09	131.87
	MEAN	11.88	14.90	28.67	16.75	45.12	41.05	126.40	127.65	41.91	19.70	38.86	56.20	35.52	136.64	127.16	34.27	23.81	37.94	50.18	28.03	133.57
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.05	0.07	0.11	0.02	0.01	0.03	0.03	0.04	0.06	0.08	0.02	0.01	0.02	0.03	0.03	0.06	0.08
	CD(5%)	0.06	0.04	0.07	0.09	0.09	0.15	0.21	0.05	0.03	0.05	0.07	0.07	0.11	0.16	0.05	0.03	0.05	0.06	0.07	0.11	0.16

TABLE – XXVII

UREASE ACTIVITY (n moles of ammonia liberated/min/mg prot .) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	111.29	15.48	10.87	15.97	31.72	16.39	116.95	115.55	18.15	11.69	17.01	24.87	21.63	118.15	142.41	50.15	44.48	52.08	49.00	17.91	142.67
	DS	118.55	30.82	16.69	19.88	33.80	55.50	129.21	118.69	38.57	33.57	48.30	36.20	28.04	133.90	131.11	49.11	33.53	49.21	62.46	45.43	145.14
	MEAN	114.92	23.15	13.78	17.93	32.76	35.95	123.08	117.12	28.36	22.63	32.66	30.54	24.84	126.02	136.76	49.63	39.01	50.65	55.73	31.67	143.91
AZ204 (T2)	DT	116.61	18.61	15.61	29.11	34.81	29.11	123.98	121.67	27.56	15.70	24.51	31.51	26.00	124.49	154.00	61.54	53.80	74.74	59.49	20.87	154.07
	DS	123.09	41.52	25.96	26.21	47.47	78.50	140.46	126.11	46.07	40.40	77.53	57.52	33.36	146.83	140.18	60.65	39.43	60.88	74.53	62.42	156.35
	MEAN	119.85	30.07	20.79	27.66	41.14	53.81	132.22	123.89	36.82	28.05	51.02	44.52	29.68	135.66	147.09	61.10	46.62	67.81	67.01	41.65	155.21
AZ208 (T3)	DT	118.91	22.29	17.29	29.00	30.11	20.81	123.07	113.33	16.77	15.40	27.63	37.35	35.18	124.28	157.19	68.60	54.52	65.04	56.28	22.53	154.03
	DS	118.98	46.96	23.17	34.34	44.63	65.64	138.95	119.54	47.42	35.16	63.08	53.18	31.91	141.72	135.14	55.65	31.17	51.43	71.87	64.52	151.63
	MEAN	118.95	34.63	20.23	31.67	37.37	43.23	131.01	116.44	32.10	25.28	45.36	45.27	33.55	133.00	146.17	62.13	42.85	58.24	64.08	43.53	152.83
FT 326 (T4)	DT	117.92	28.38	19.03	33.40	44.14	18.86	126.95	119.54	23.90	17.42	21.71	42.33	32.54	126.24	160.11	63.09	45.06	75.04	62.43	29.94	155.95
	DS	127.66	42.77	19.51	24.00	39.20	69.73	137.14	128.83	56.24	45.71	72.00	53.89	26.98	147.27	142.19	59.95	41.47	59.76	85.74	53.08	157.03
	MEAN	122.79	35.57	19.27	28.70	41.67	44.30	132.05	124.19	40.07	31.57	46.85	48.11	29.76	136.76	151.15	61.52	43.27	67.40	74.09	41.51	156.49
AA/T (T5)	DT	119.35	22.39	11.92	20.81	37.24	22.01	122.32	121.01	27.11	13.43	19.37	27.24	21.71	121.65	132.01	59.41	47.28	70.19	57.48	17.93	150.72
	DS	139.05	50.53	24.33	29.16	45.75	66.75	139.26	122.03	45.70	39.39	55.63	45.81	28.25	139.47	138.45	54.07	44.69	62.13	76.45	51.08	154.48
	MEAN	119.30	36.46	18.13	24.99	41.50	44.38	130.79	121.52	36.41	26.41	37.50	36.53	24.98	130.56	145.23	56.74	45.99	66.16	66.97	34.51	152.60
	MEAN	119.16	31.97	18.44	26.19	38.89	44.33	129.83	120.63	34.75	26.79	42.68	40.99	28.56	132.40	145.28	58.22	43.54	62.05	65.57	38.57	152.21
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.04	0.06	0.09	0.03	0.02	0.03	0.04	0.04	0.06	0.09	0.03	0.02	0.03	0.04	0.04	0.07	0.10
	CD(5%)	0.05	0.03	0.06	0.07	0.08	0.13	0.18	0.05	0.03	0.05	0.07	0.08	0.12	0.17	0.06	0.04	0.06	0.08	0.09	0.14	0.20

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Also DS exhibited a significantly higher value than DT in all parts of the plant.

Our results are in agreement with the findings of Luthra *et al.* (1983). The specific activity of the enzyme showed a biphasic trend in shoots and nodules with peaks at days 60 and 120. The presence of urease at pod setting stage provides supporting evidence that ureides are metabolisesd to urea and further to ammonia and carbondioxide. The ammonia liberated in the urease reaction may be re-assimilated.

4.15.UREIDES

Tables XXVIII and XXIX and Figures - 9 and 10 represent the effect of inoculation of different strains of *Azospirillum* and external application of IAA on the ureide content in different parts of DT and DS of sorghum and finger millet.

Sorghum

The production of ureides in various parts of sorghum was more due to inoculation with *Azospirillum* strains than in control. In leaf AZ204 treated plants produced higher concentration and FT326 inoculated plants produced lower concentration of ureides. In shoot it was in the reverse order and in root AZ204 treated plants produced the highest and AZ208 inoculated plants produced the lowest ureide content. The values differed significantly from each other for all the treatments. Maximum ureide content was seen at 45 DAS in both leaf and root and at 75 DAS in shoot. Significant interaction

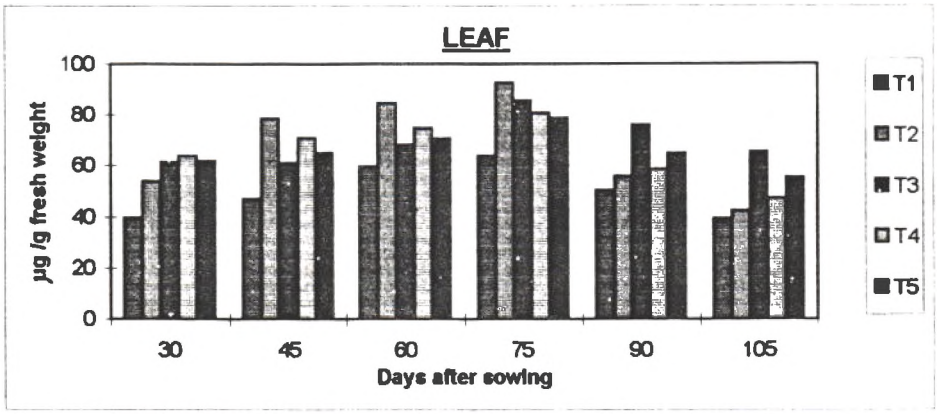
TABLE – XXVIII

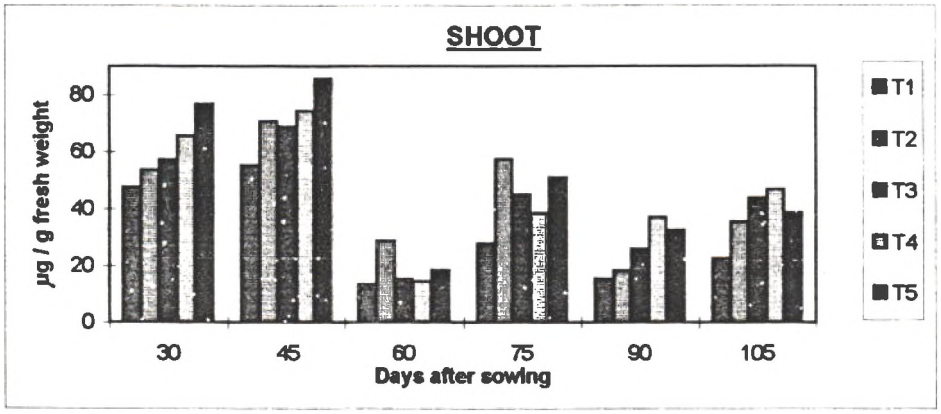
UREIDE CONTENT (µg/g f.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	ME	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	139.58	47.08	59.62	63.85	50.19	37.35	149.95	147.54	55.38	13.27	27.88	15.38	22.69	130.36	141.62	57.54	17.46	31.50	25.46	21.54	132.52
	DS	120.62	27.00	49.00	39.64	30.01	53.85	136.69	115.00	21.85	25.42	20.77	16.35	24.62	120.67	120.46	36.42	41.42	22.69	33.65	21.73	129.40
	MEAN	130.10	37.04	54.31	51.75	40.10	46.60	143.32	131.27	38.62	19.35	24.33	15.87	23.66	125.51	131.04	46.98	29.44	27.10	29.56	21.64	130.96
AZ204 (T2)	DT	153.69	78.46	84.62	92.77	55.77	42.31	167.94	153.85	70.81	28.85	57.69	18.27	35.58	144.18	149.58	60.38	45.19	49.12	37.50	34.62	146.07
	DS	128.81	30.77	69.23	66.35	48.35	76.92	153.41	121.13	29.95	41.35	32.35	25.85	29.23	129.98	131.44	37.50	78.08	36.54	41.73	22.12	141.24
	MEAN	141.25	54.62	76.93	79.56	52.06	59.62	160.67	137.49	50.38	35.10	45.02	22.06	32.41	137.08	140.51	48.94	61.64	42.83	39.62	28.37	143.65
AZ208 (T3)	DT	161.27	61.08	68.27	85.58	75.96	65.38	169.59	157.31	68.85	15.38	45.19	25.96	44.04	142.79	153.23	62.88	27.88	68.27	27.88	22.52	143.78
	DS	131.54	39.42	62.50	52.88	38.46	65.38	148.36	124.61	38.23	41.77	26.92	20.19	26.92	129.77	126.73	36.54	55.77	31.73	50.77	44.23	140.96
	MEAN	146.41	50.25	65.39	69.23	57.21	65.38	158.98	140.96	53.54	28.58	36.06	23.08	35.48	136.28	139.98	49.71	41.83	50.00	39.33	33.38	142.37
IFT 326 (T4)	DT	163.85	70.85	74.81	80.77	58.65	47.12	166.01	165.77	74.35	14.42	38.46	37.12	46.92	146.17	157.88	65.38	20.31	36.54	37.12	33.47	141.78
	DS	124.62	38.46	49.04	40.00	31.73	59.62	140.58	119.92	33.65	36.35	30.77	22.45	35.58	129.79	143.08	45.19	66.35	33.65	47.43	27.88	143.93
	MEAN	144.24	54.66	61.93	60.39	45.19	53.37	153.29	142.85	54.00	25.39	34.62	29.79	41.25	137.98	150.48	55.29	43.33	35.10	42.28	30.68	142.86
IAA/T (T5)	DT	161.92	65.23	70.85	78.85	65.00	55.19	166.17	176.92	85.58	18.65	51.15	32.69	38.85	150.64	162.71	78.08	28.85	57.31	40.38	35.37	150.45
	DS	125.38	47.69	52.31	51.47	30.77	54.04	143.61	116.58	26.92	29.85	21.42	28.65	23.45	124.48	133.65	38.85	43.65	29.11	43.46	30.00	136.45
	MEAN	143.65	56.46	61.58	65.16	47.89	54.62	154.89	146.75	56.25	24.25	36.29	30.67	31.15	137.56	148.18	58.47	36.25	43.21	41.92	32.69	143.45
	MEAN	141.13	50.60	64.03	65.22	48.49	55.92	154.23	139.86	50.56	26.53	35.26	24.29	32.79	134.88	142.04	51.88	42.50	39.65	38.54	29.35	140.66
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.02	0.01	0.02	0.03	0.04	0.06	0.08	0.03	0.02	0.03	0.04	0.04	0.07	0.10
	CD(5%)	0.08	0.05	0.08	0.11	0.12	0.19	0.27	0.05	0.03	0.05	0.06	0.07	0.11	0.16	0.06	0.03	0.06	0.08	0.09	0.14	0.19

Figure - 9

TOTAL UREIDE LEVELS IN DTV OF SORGHUM





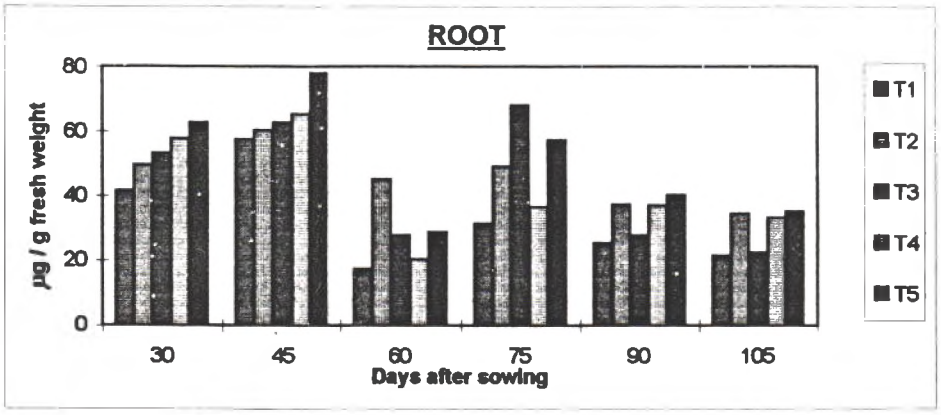


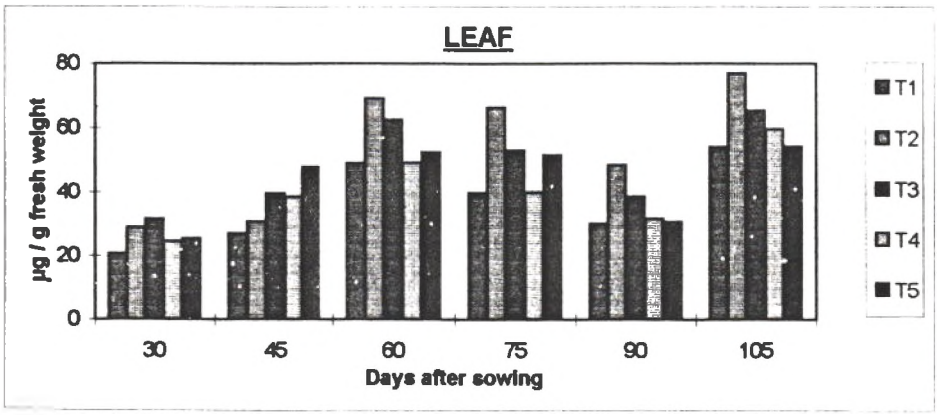
TABLE – XXIX

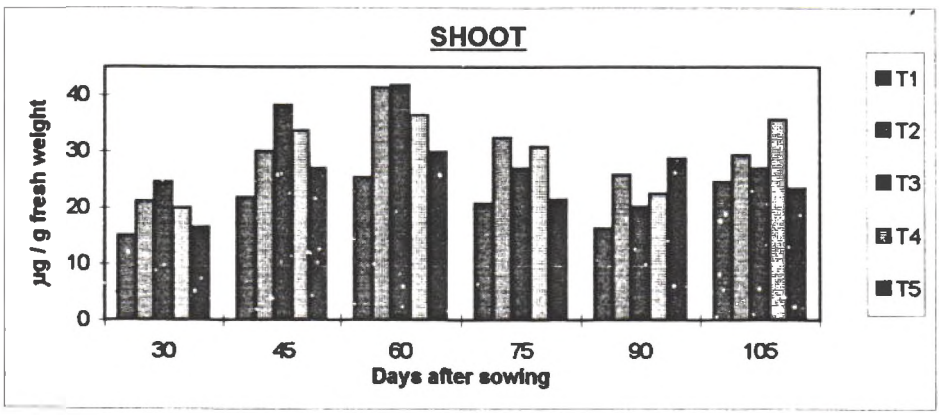
UREIDE CONTENT(µg/g f.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

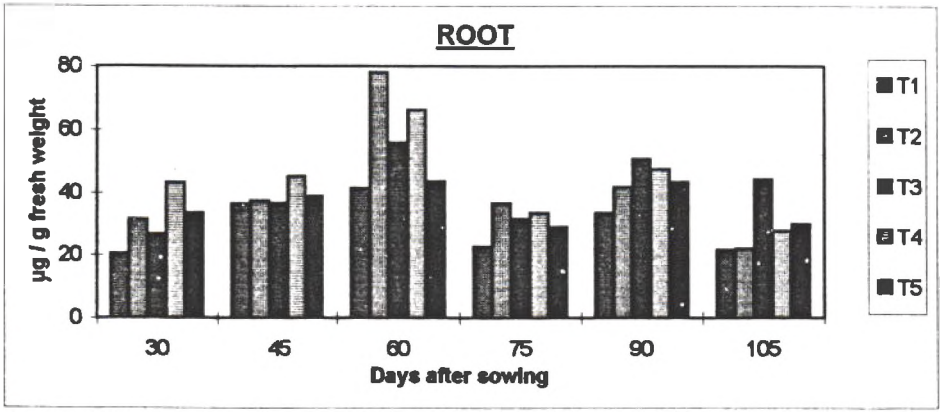
		LEAF							SHOOT							ROOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	118.46	24.73	14.42	15.46	25.96	21.96	120.17	113.46	20.46	14.04	7.77	14.04	7.19	112.83	120.65	29.42	9.23	12.58	13.46	11.35	116.12
	DS	117.65	19.31	52.50	40.35	26.69	35.33	131.97	114.03	18.15	28.08	10.88	28.77	12.38	118.72	120.19	24.50	41.04	11.31	23.76	20.81	123.60
	MEAN	118.06	22.02	33.46	27.91	26.33	28.65	126.07	113.76	19.31	21.06	9.33	21.41	9.79	115.77	120.42	26.96	25.14	11.95	18.61	16.08	119.86
AZ204 (T2)	DT	139.35	44.23	17.31	20.19	39.42	27.88	131.40	128.85	30.77	21.15	13.46	16.42	8.65	119.88	133.65	41.88	22.12	25.41	27.88	20.19	128.52
	DS	120.19	28.85	60.58	43.27	32.69	48.08	138.94	118.08	21.15	34.62	11.92	30.77	19.23	122.63	131.58	37.50	46.15	20.58	49.04	32.67	136.25
	MEAN	129.77	36.54	38.95	31.73	36.06	37.98	135.17	123.47	25.96	27.89	12.69	23.60	13.94	121.26	132.62	39.69	34.14	23.00	38.46	26.43	132.39
AZ208 (T3)	DT	130.72	45.19	21.73	24.04	30.77	24.73	129.53	122.12	28.08	14.42	13.46	17.31	8.65	117.34	121.96	24.04	10.58	21.15	22.12	16.17	119.34
	DS	123.77	28.85	76.92	36.54	31.42	39.42	139.49	115.38	21.15	41.35	16.31	31.73	17.31	123.87	122.22	26.92	50.96	13.46	26.92	24.04	127.42
	MEAN	127.25	37.02	49.33	30.29	31.10	32.08	134.51	118.75	24.62	27.89	14.89	24.52	12.98	120.61	122.09	25.48	30.77	17.31	24.52	20.11	123.38
IFT 326 (T4)	DT	124.31	33.08	18.27	30.77	42.31	33.65	130.40	115.38	23.08	16.35	11.54	28.12	20.19	119.11	130.78	50.00	14.42	18.27	20.19	18.04	125.28
	DS	121.15	27.88	56.73	37.50	30.44	41.35	135.84	120.19	22.12	33.65	15.77	35.58	14.42	123.62	121.73	26.92	50.00	25.96	47.12	30.77	133.75
	MEAN	122.73	30.48	37.50	34.14	36.38	37.50	133.12	117.79	22.60	25.00	13.66	31.85	17.31	121.37	126.26	38.46	32.21	22.12	33.66	24.41	129.52
IAA/T (T5)	DT	119.81	25.19	17.31	20.19	30.77	26.92	123.37	122.12	25.00	21.15	15.38	29.81	16.35	121.64	131.50	39.42	25.00	17.65	27.50	18.27	126.56
	DS	122.81	25.96	62.50	47.69	42.24	45.35	141.09	121.73	25.96	32.88	20.77	35.58	16.92	125.64	126.73	32.69	48.08	34.62	35.77	29.81	134.62
	MEAN	121.31	25.58	39.91	33.94	36.51	36.14	132.23	121.93	25.48	27.02	18.08	32.70	16.64	123.64	129.12	36.06	36.54	26.14	31.64	24.04	130.59
	MEAN	123.82	30.33	39.83	31.60	33.27	34.47	132.22	119.14	23.59	25.77	13.73	26.81	14.13	120.53	126.10	33.33	31.76	20.10	29.38	22.21	127.15
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.03	0.04	0.06	0.08	0.03	0.02	0.03	0.04	0.04	0.06	0.09	0.02	0.02	0.03	0.03	0.04	0.06	0.08
	CD(5%)	0.05	0.03	0.05	0.06	0.07	0.11	0.16	0.05	0.03	0.06	0.07	0.08	0.13	0.18	0.05	0.03	0.05	0.07	0.07	0.12	0.17

Figure - 10

TOTAL UREIDE LEVELS IN DSV OF SORGHUM







was found between treatments and stages. DT expressed higher activity than DS in all parts of the plant. The results indicated maximum accumulation of ureides in leaves followed by root and shoot in order. In DT, ureide content in each tissue increased progressively from 30 DAS attaining a peak at 75 DAS and then decreased till maturity, whereas in shoots it again increased at 105 DAS. In DS there was decline at 75 DAS and 90 DAS and increased at 105 DAS, except in root where an increase was observed at 90 DAS followed by a decrease at 105 DAS.

Finger millet

All treatments influenced leaf and root ureide content significantly with AZ204 application registering the highest concentration but in shoot of IAA treated plants had the maximum effect. It was found from the data obtained that ureide content increased gradually from 30 DAS. It was most at 60 DAS in leaf, 90 DAS in shoot and 45 DAS in root. Leaves were found to contain more ureides than the shoot and root DS registered higher ureide content than DT.

The above results agree with the findings of Luthra *et al.* (1983).They reported that ureide content in each organ increased progressively from 15 DAS, attaining a peak at flowering stage except in shoots, where it evinced maximum value at pod setting stage. However, in leaves ureide level, dropping after flowering increased at day 105. Decrease in the ureide content in the roots and increase in case of shoot and leaves at 105 DAS may be due to translocation of these ureides.

Examination of ureide levels in the roots and shoots however revealed that these tissues also accumulated ureides. The accumulation of ureides in different parts is probably due to the transport of these compounds from the root region. Tajima *et al.* (1977) reported that allantion is utilised mainly in the leaves after translocation. The ureide nitrogen was the predominant form of nitrogen translocated from nodulated root to shoots. Formation of ureides and their presence in xylem sap as dominant solutes is closely related to the presence of nitrogen fixing nodules (Matsumoto *et al.*, 1976; Atkins *et al.*, 1982).

Ureide concentration in various parts of pigeon pea decreased rapidly with seed filling (Fujihara et al., 1977). The decrease during seed filling could either be ascribed to the transport of these components from these organs (probably to the developing seeds which are dominant sinks at this stage) or to degradation and assimilation of ureides into other nitrogenous compounds which may then be translocated to the developing seeds.

Thomas *et al.* (1980) reported that the ureide content in bush beans increased gradually as the leaves developed and thereafter decreased during the later stages. In stem there was a decline inbetween stages. The same pattern was observed in the present study also. The observed increase in ureides within the plant could be associated with increase in nitrogen transport from the root via the xylem. The relative content of allantoin and allantoic acid in xylem sap or in particular

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tissue might reflect the current status of nitrogen fixation (Yoneyama *et al.*, 1985). The most likely explanation for the observed decrease could be that the plants have utilised all the reserves from the cotyledons. This is followed by an increase when the leaves might have started to export carbon assimilates to other parts of the plant such as the roots which have a lowered assimilatory capacity.

4.16.INDOLE ACETIC ACID

The amount of IAA content in sorghum and finger millet due to various treatments is given in Tables XXX and XXXI and Figures- 11 and 12.

Sorghum

Various treatments resulted in significantly higher IAA content in all parts of the plants than the control. Of all the treatments external application of IAA to the plant recorded the highest values of IAA in leaf and shoot.IAA treatment and FT326 application to tbe plant were on par. However in root AZ204 caused the maximum effect. In both the parts AZ208 exhibited the minimum effect.

The IAA content in both the parts increased steadily upto 75 DAS and then decreased at 90 and 105 DAS. Variety wise DS registered significantly higher value than DT. When treatements and varieties interaction was considered, IAA-T and FT326 exhibited higher levels of IAA in both leaf and shoot of DT and DS respectively. In root AZ204 and FT326 resulted in maximal IAA levels in DS and DT respectively.

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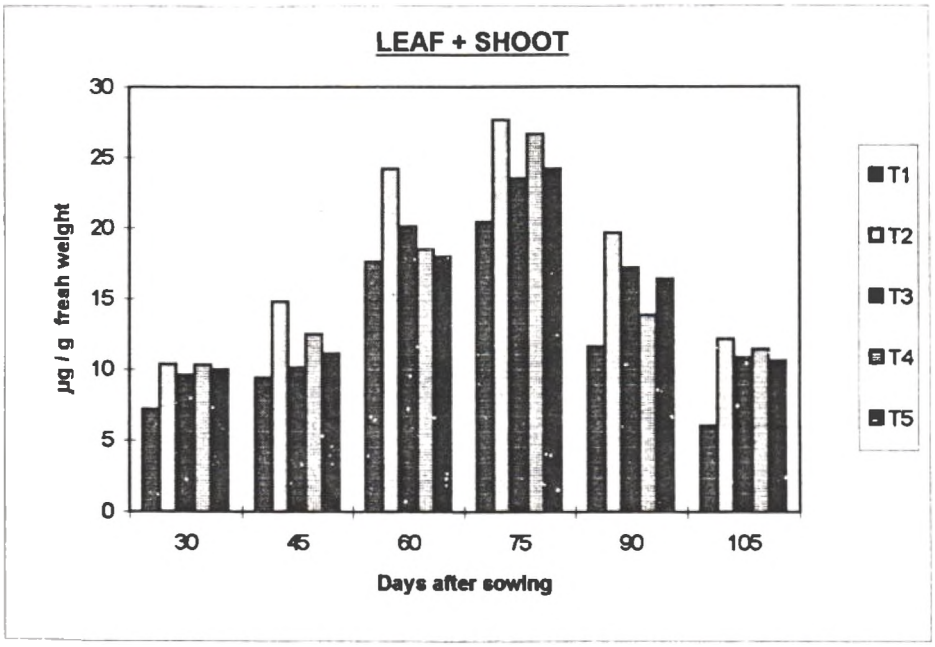
TABLE–XXX

INDOLE ACETIC ACID CONTENT (µg/g f.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

		LEAF/SHOOT							ROOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	5.88	8.19	10.65	12.77	9.12	7.63	9.04	12.63	17.70	20.50	23.21	17.93	11.88	17.31
	DS	8.40	10.56	14.56	18.56	16.86	11.89	13.47	14.25	17.01	19.06	16.42	13.25	7.19	14.53
	MEAN	7.14	9.38	12.61	15.67	12.99	9.76	11.26	13.44	17.36	19.78	19.82	15.59	9.54	15.92
AZ204 (T2)	DT	8.83	10.13	12.38	17.59	15.38	12.81	12.85	14.63	20.72	28.13	30.73	23.44	20.63	23.05
	DS	11.00	14.88	16.63	23.63	17.41	15.81	16.56	18.50	22.50	26.25	18.75	14.08	7.81	17.98
	MEAN	9.92	12.51	14.51	20.61	16.40	14.31	14.71	16.57	21.61	27.19	24.74	18.76	14.22	20.51
AZ208 (T3)	DT	7.50	11.09	13.13	16.66	10.08	8.28	11.12	18.13	21.97	24.01	26.03	18.65	12.66	20.24
	DS	12.57	15.56	18.59	21.59	17.59	12.72	16.44	15.36	20.23	21.59	20.65	14.34	8.45	16.77
	MEAN	10.04	13.33	15.86	19.13	13.84	10.50	13.78	16.75	21.10	22.80	23.34	16.50	10.56	18.51
IFT 326 (T4)	DT	9.50	12.56	13.92	14.52	12.59	10.25	12.22	16.69	18.53	22.44	25.14	23.02	15.98	20.30
	DS	13.75	18.56	20.57	22.25	16.97	14.33	17.74	17.23	21.56	24.09	22.44	13.38	11.02	18.29
	MEAN	11.63	15.56	17.25	18.39	14.78	12.29	14.98	16.96	20.05	23.27	23.79	18.20	13.50	19.29
IAA/T (T5)	DT	7.31	9.11	20.19	18.73	14.04	7.81	12.87	16.88	22.50	24.38	26.92	21.42	13.14	20.87
	DS	12.74	13.63	20.27	21.31	19.24	15.44	17.11	17.50	20.30	24.25	22.59	14.25	9.47	18.06
	MEAN	10.03	11.37	20.23	20.02	16.64	11.63	14.99	17.19	21.40	24.32	24.76	17.84	11.31	19.47
	MEAN	9.75	12.43	16.09	18.76	14.93	11.70	13.94	16.18	20.30	23.47	23.29	17.38	11.82	18.74
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.05	0.07	0.10	0.03	0.02	0.03	0.04	0.04	0.06	0.09
	CD(5%)	0.06	0.04	0.06	0.08	0.09	0.14	0.20	0.05	0.03	0.06	0.07	0.08	0.13	0.18

Figure - 11

IAA CONTENT IN DTV OF FINGER MILLET



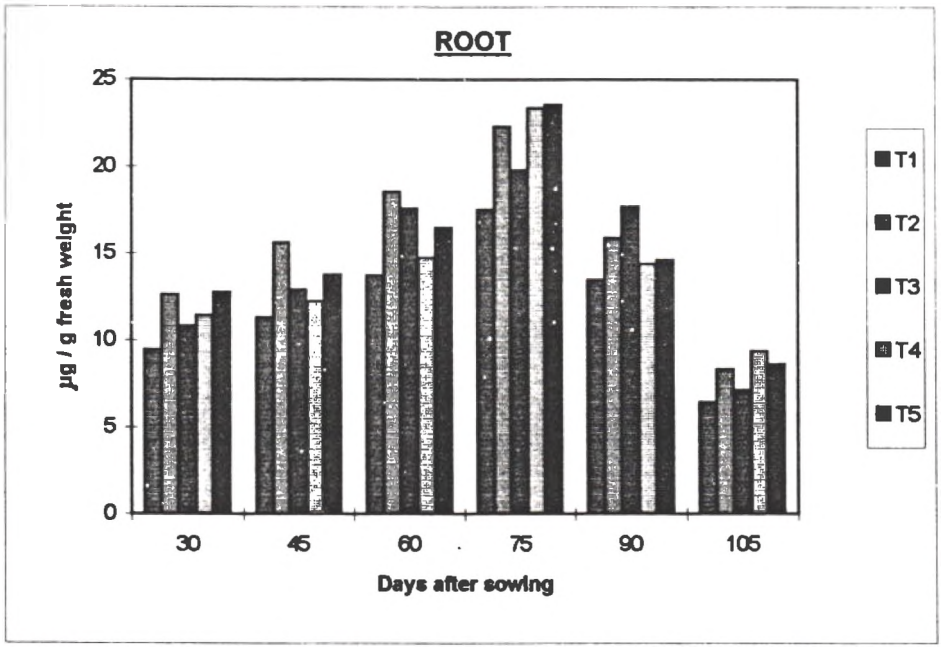


TABLE – XXXI

INDOLE ACETIC ACID CONTENT (µg/g f.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

		LEAF							SHOOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	7.19	9.39	17.63	20.44	11.62	6.06	12.06	9.47	11.33	13.73	17.50	13.47	6.47	12.00
	DS	7.44	9.44	13.44	15.94	8.78	5.67	10.12	4.16	6.18	9.06	14.94	7.08	6.16	7.93
	MEAN	7.32	9.42	15.54	18.19	10.20	5.87	11.09	6.82	8.76	11.40	16.22	10.28	6.32	9.96
AZ204 (T2)	DT	10.38	14.84	24.25	27.69	19.66	12.19	18.17	12.60	15.63	18.54	22.31	15.86	8.86	15.63
	DS	9.73	14.84	16.94	18.59	13.02	9.94	13.84	5.58	9.47	11.88	16.91	11.33	6.25	10.24
	MEAN	10.06	14.84	20.60	23.14	16.34	11.07	16.01	9.09	12.55	15.21	19.61	13.60	7.56	12.94
AZ208 (T3)	DT	9.58	10.14	20.13	23.55	17.22	10.88	15.25	10.84	12.90	17.59	19.80	17.71	7.17	14.34
	DS	8.70	13.07	16.79	19.06	8.69	7.43	12.29	4.75	6.78	11.25	16.75	9.72	6.97	9.37
	MEAN	9.14	11.61	18.46	21.31	12.96	9.16	13.77	7.80	9.84	14.42	18.28	13.72	7.07	11.85
FT 326 (T4)	DT	10.31	12.50	18.50	26.69	13.88	11.44	15.55	11.43	12.25	14.73	23.38	14.38	9.38	14.26
	DS	11.77	16.77	18.88	19.31	9.04	7.97	13.96	5.36	7.17	13.46	16.10	7.60	7.33	9.50
	MEAN	11.04	14.64	18.69	23.00	11.46	9.71	14.76	8.40	9.71	14.10	19.74	10.99	8.36	11.88
IAA/T (T5)	DT	9.98	11.12	18.01	24.25	16.47	10.62	15.08	12.75	13.75	16.47	23.54	14.63	8.63	14.96
	DS	9.14	12.81	15.75	21.50	11.44	6.16	12.80	6.02	7.92	11.83	15.83	10.50	8.50	10.10
	MEAN	9.56	11.97	16.88	22.88	13.96	8.39	13.94	9.39	10.84	14.15	19.69	12.57	8.57	12.53
	MEAN	9.42	12.49	18.03	21.70	12.98	8.84	13.91	8.30	10.34	13.85	18.71	12.23	7.57	11.83
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.05	0.08	0.11	0.03	0.02	0.03	0.04	0.04	0.06	0.09
	CD(5%)	0.06	0.04	0.07	0.09	0.10	0.15	0.22	0.05	0.03	0.06	0.07	0.08	0.13	0.18

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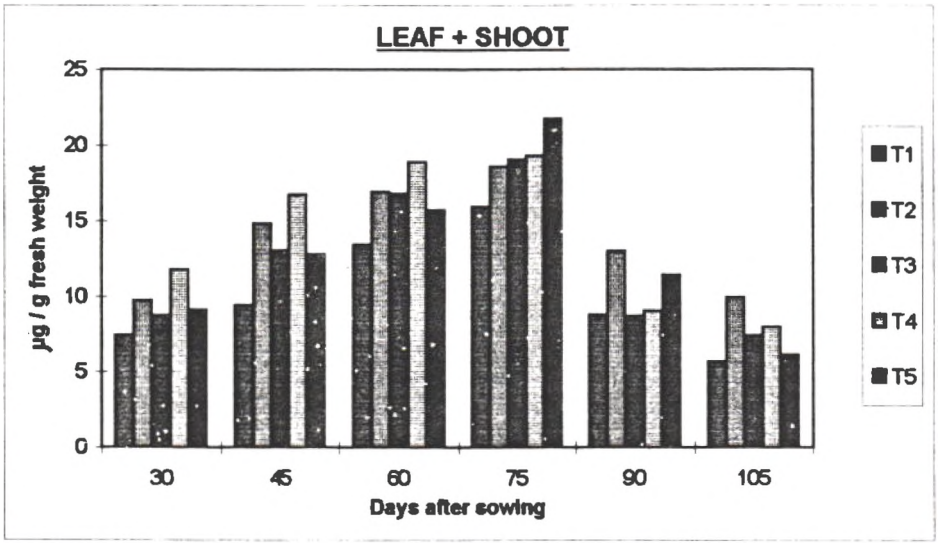
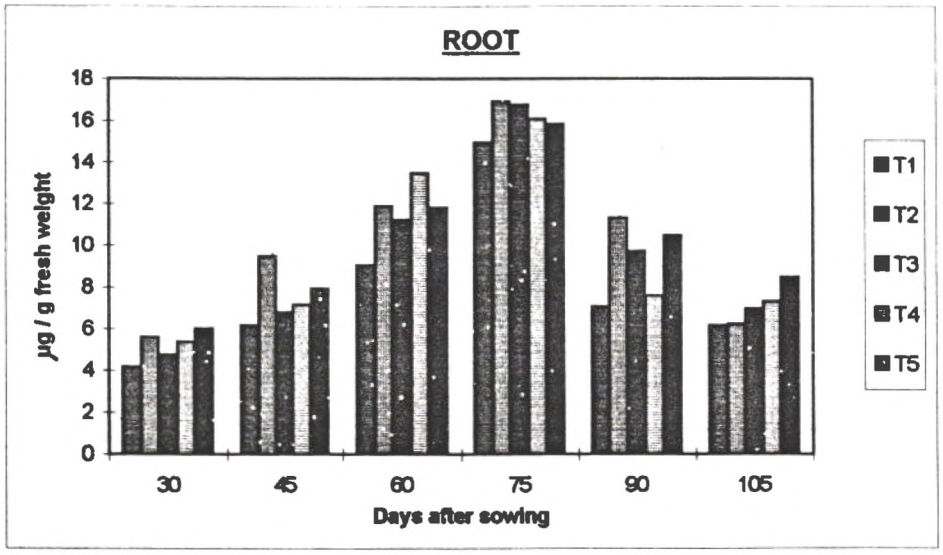
Figure - 12

IAA CONTENT IN DSV OF FINGER MILLET





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Finger millet

AZ204 inoculation registered highest IAA content and the lowest was recorded by AZ208 in all the parts. All the treatments resulted in a significantly higher content of IAA in all the parts. The trend of IAA content resulting from various treatments was similar to that in sorghum. DT expressed higher activity than DS.

4.17.GIBBERELLINS

Tables XXXII and XXXIII depict the amount of gibberellin content in sorghum and finger millet due to inoculation of *Azospirillum* strains and external application of IAA.

Sorghum

All the treatments resulted in a significantly higher content of gibberellins in all parts of the plants than in controls. AZ204 in shoot system and FT326 in root showed the maximum effect.The gibberellin content increased progressively upto 75 DAS and then declined. DS had a higher amount of gibberellins than DT.The gibberellin content of the root was more than that of the shoot system.

Finger millet

All the treatments differed significantly from the control. FT326 recorded the highest and AZ208 exhibited the lowest gibberellin content in all the parts. Gibberellin content steadily increased upto 75 DAS and then dropped. Similar to sorghum, root had greater hormone content than shoot. There was no significant difference in gibberellin

TABLE-XXXII

GIBBERELLIN CONTENT (µg/g f.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	LEAF/SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	2.08	2.76	4.20	5.06	3.95	2.98	3.50	2.68	3.63	5.01	6.65	4.80	2.78	4.26
	DS	2.62	3.84	4.40	5.01	3.95	2.84	3.78	3.56	4.87	5.90	6.50	4.00	3.00	4.64
	MEAN	2.35	3.30	4.30	5.03	3.95	2.91	3.64	3.12	4.25	5.45	6.58	4.40	2.89	4.45
AZ204 (T2)	DT	2.58	3.48	5.27	6.42	4.56	3.40	4.29	3.16	4.52	5.90	7.74	5.46	3.40	5.03
	DS	3.12	4.80	5.71	6.42	4.92	3.98	4.83	4.97	5.98	7.00	7.74	5.44	4.00	5.86
	MEAN	2.85	4.14	5.49	6.42	4.74	3.69	4.56	4.07	5.25	6.45	7.74	5.45	3.70	5.44
AZ208 (T3)	DT	2.37	3.08	4.98	5.94	4.06	3.29	3.95	3.41	4.66	5.75	7.06	5.63	3.12	4.94
	DS	2.98	3.98	5.00	5.84	4.06	3.28	4.19	4.14	5.00	6.85	7.37	4.98	3.56	5.32
	MEAN	2.68	3.53	4.99	5.89	4.06	3.29	4.07	3.78	4.83	6.30	7.22	5.31	3.34	5.13
IFT 326 (T4)	DT	2.18	3.18	4.96	5.84	4.72	3.14	4.00	3.75	4.89	6.50	7.88	5.98	3.48	5.41
	DS	3.60	4.59	5.20	5.93	4.72	3.14	4.53	5.00	6.12	7.80	8.88	5.86	4.34	6.33
	MEAN	2.89	3.88	5.08	5.89	4.72	3.14	4.27	4.38	5.51	7.15	8.38	5.92	3.91	5.87
IAA/T (T5)	DT	2.43	3.32	4.59	6.60	4.88	3.80	4.27	3.04	4.40	6.10	7.07	5.04	3.00	4.78
	DS	3.75	4.13	4.96	6.03	5.00	2.96	4.47	4.85	6.14	7.06	8.05	6.00	4.12	6.04
	MEAN	3.09	3.73	4.78	6.32	4.94	3.38	4.37	3.95	5.27	6.58	7.56	5.52	3.56	5.41
	MEAN	2.77	3.72	4.93	5.91	4.48	3.28	4.18	3.86	5.02	6.39	7.49	5.32	3.48	5.26
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.05	0.03	0.05	0.06	0.07	0.11	0.16	0.04	0.03	0.05	0.06	0.07	0.11	0.15
	CD(5%)	0.09	0.06	0.10	0.13	0.14	0.22	0.31	0.09	0.06	0.10	0.13	0.14	0.22	0.31

TABLE–XXXIII

GIBBERELLIN CONTENT (µg/g f.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT)AND DROUGHT SUSCEPTIBLE VARIETIES OF FINGER MILLET

		LEAF/SHOOT							ROOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	1.96	2.14	2.88	3.99	2.99	2.03	2.67	2.54	3.01	3.72	4.90	3.21	2.84	3.37
	DS	1.78	2.44	2.95	3.98	3.01	2.04	2.70	2.00	3.00	3.60	4.44	3.26	1.96	3.04
	MEAN	1.87	2.29	2.92	3.99	3.00	2.04	2.68	2.27	3.01	3.66	4.67	3.24	2.40	3.21
AZ204 (T2)	DT	2.39	2.99	3.64	4.62	3.40	2.72	3.29	3.17	4.00	4.75	5.25	4.01	3.01	4.03
	DS	2.38	3.15	3.76	4.84	4.00	2.98	3.52	2.85	3.98	4.25	5.05	4.10	2.80	3.84
	MEAN	2.39	3.07	3.70	4.73	3.70	2.85	3.41	3.01	3.99	4.50	5.15	4.06	2.91	3.94
AZ208 (T3)	DT	2.60	3.13	3.72	4.36	3.08	2.50	3.23	2.80	3.89	4.12	5.88	3.84	3.05	3.93
	DS	1.95	2.71	3.20	4.34	3.75	2.75	3.12	2.60	3.60	4.00	4.88	4.00	3.00	3.68
	MEAN	2.28	2.92	3.46	4.35	3.42	2.63	3.17	2.70	3.75	4.06	5.38	3.92	3.03	3.81
IFT 326 (T4)	DT	2.68	3.15	3.95	4.92	3.44	2.88	3.50	3.03	4.12	4.50	6.00	4.56	3.52	4.29
	DS	2.04	3.01	3.95	4.98	4.04	3.04	3.51	2.98	4.01	4.36	5.60	4.20	2.55	3.95
	MEAN	2.36	3.08	3.95	4.95	3.74	2.96	3.51	3.01	4.07	4.43	5.80	4.38	3.04	4.12
IAA/T (T5)	DT	2.70	3.05	3.63	4.84	3.40	2.40	3.34	2.74	3.64	4.08	5.83	4.25	3.12	3.94
	DS	2.25	2.85	3.18	5.01	4.00	2.82	3.35	3.01	3.75	4.01	5.26	3.98	2.78	3.80
	MEAN	2.48	2.95	3.41	4.93	3.70	2.61	3.34	2.88	3.70	4.05	5.55	4.12	2.95	3.87
	MEAN	2.27	2.86	3.49	4.59	3.51	2.62	3.22	2.77	3.70	4.14	5.31	3.94	2.86	3.79
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.04	0.05	0.05	0.08	0.11	0.03	0.02	0.03	0.04	0.04	0.07	0.10
	CD(5%)	0.07	0.04	0.07	0.09	0.10	0.16	0.23	0.06	0.04	0.06	0.08	0.09	0.14	0.20

content in the shoot system but significantly differed in the root, with DS having more amount than DT.

4.18.TOTAL PROTEIN

The amount of protein in sorghum and finger millet plants due to various treatments is given in Tables XXXIV and XXXV.

Sorghum

In leaf all the treatments resulted in a significantly higher content of protein than in the control with AZ204 application showing maximal activity. In shoot there was no significant difference between AZ204 and AZ208 and also between IAA and FT326 inoculation, although AZ204 treated plants recorded higher protein content. IAA-T registered maximum protein content in root. DT had higher protein than DS. The protein content was more in leaf followed by shoot and root. The protein content increased upto 75 DAS in leaf and root and upto 60 DAS in shoot and then declined during maturity.

Finger millet

The leaf and root protein content was higher in AZ204 treated plants. In leaf AZ208 and FT326, IAA-T and control were on par with each other in protein content. In shoot the difference between AZ208 and FT326, FT326 and IAA-T, AZ204 and IAA-T was not significant, although AZ208 had maximum protein content. Similar to sorghum DT recorded higher protein than DS. In leaf and shoot protein content inrcreased till 75DAS

TABLE – XXXIV

TOTAL PROTEIN CONTENT (mg/g f.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

		LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
	DT	3.41	3.56	4.08	4.57	3.98	3.13	3.79	1.75	2.02	2.20	1.62	1.12	1.03	1.62	1.12	1.48	1.32	1.00	1.38	1.03	1.22
CON	DS	3.09	3.36	3.75	4.86	3.18	1.85	3.35	1.37	1.56	2.01	1.16	1.01	0.87	1.33	1.49	2.25	1.46	1.51	1.43	0.75	1.48
(T1)	MEAN	3.25	3.46	3.92	4.72	3.58	2.49	3.57	1.56	1.79	2.11	1.39	1.07	0.95	1.48	1.31	1.87	1.39	1.26	1.41	0.89	1.35
AZ204	DT	4.56	4.69	5.55	5.98	4.85	4.01	4.94	2.06	2.42	2.88	2.48	1.36	1.08	2.05	1.78	1.95	1.70	1.24	1.52	1.30	1.58
(T2)	DS	4.46	4.97	5.19	6.02	4.54	2.25	4.57	1.56	1.74	2.34	2.12	1.93	1.52	1.87	1.75	2.32	1.74	1.88	1.71	0.90	1.72
	MEAN	4.51	4.83	5.37	6.00	4.70	3.13	4.76	1.81	2.08	2.61	2.30	1.65	1.30	1.96	1.77	2.14	1.72	1.56	1.62	1.10	1.65
AZ208	DT	4.06	4.32	4.72	5.36	4.54	3.24	4.37	2.00	2.62	3.07	2.11	1.44	1.35	2.10	1.65	2.05	1.74	1.47	1.59	1.32	1.64
(T3)	DS	4.01	4.15	4.66	5.22	4.28	2.30	4.10	1.66	2.29	2.44	1.72	1.59	1.33	1.84	1.36	2.13	1.60	1.66	1.20	0.88	1.47
	MEAN	4.04	4.24	4.69	5.29	4.41	2.77	4.24	1.83	2.46	2.76	1.92	1.52	1.34	1.97	1.51	2.09	1.67	1.57	1.40	1.10	1.55
FT 326	DT	4.00	4.49	4.96	5.68	5.12	4.83	4.85	2.16	2.20	2.33	2.78	2.00	1.73	2.20	1.96	2.11	1.75	1.35	1.70	1.51	1.73
(T4)	DS	3.91	4.38	4.82	5.58	3.49	2.68	4.14	1.64	1.83	2.13	1.19	1.07	0.90	1.46	1.62	2.80	1.67	2.12	1.44	0.95	1.77
	MEAN	3.96	4.44	4.89	5.63	4.31	3.76	4.50	1.90	2.02	2.23	1.99	1.54	1.32	1.83	1.79	2.46	1.71	1.74	1.57	1.23	1.75
IAA/T	DT	3.87	4.87	5.20	5.81	5.07	4.44	4.88	2.04	2.38	2.74	2.21	1.69	1.41	2.08	1.77	2.59	2.11	1.39	1.90	1.67	1.91
(T5)	DS	3.68	4.11	4.99	5.53	2.86	1.28	3.74	1.43	2.26	2.07	1.52	1.21	1.13	1.60	1.61	2.46	1.52	1.91	1.54	0.88	1.65
	MEAN	3.78	4.49	5.10	5.67	3.97	2.86	4.31	1.74	2.32	2.41	1.87	1.45	1.27	1.84	1.69	2.53	1.82	1.65	1.72	1.28	1.78
	MEAN	3.91	4.29	4.79	5.46	4.19	3.00	4.27	1.77	2.13	2.42	1.89	1.44	1.24	1.81	1.61	2.21	1.66	1.55	1.54	1.12	1.62
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.01	0.01	0.01	0.02	0.02	0.03	0.04
	CD(5%)	0.02	0.01	0.02	0.02	0.03	0.04	0.06	0.02	0.01	0.02	0.02	0.02	0.04	0.06	0.02	0.01	0.02	0.03	0.03	0.06	0.08

TABLE –XXXV

TOTAL PROTEIN CONTENT (mg/g f. wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND OF DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	4.01	4.52	4.93	5.20	3.38	3.10	4.19	1.89	2.15	1.71	1.59	0.91	0.56	1.47	1.48	1.62	1.55	0.85	1.45	0.76	1.28
	DS	4.07	4.35	4.57	4.70	4.16	3.83	4.28	1.73	2.01	1.82	0.95	0.80	0.45	1.29	1.24	1.67	1.58	0.64	0.87	0.79	1.13
	MEAN	4.04	4.44	4.75	4.95	3.77	3.47	4.24	1.81	2.08	1.77	1.27	0.86	0.51	1.38	1.36	1.64	1.57	0.75	1.16	0.78	1.21
AZ204 (T2)	DT	4.48	5.01	5.40	5.64	4.55	4.02	4.85	2.14	2.57	2.07	1.97	1.11	0.60	1.74	1.85	2.00	1.90	1.68	2.22	1.71	1.89
	DS	4.11	4.44	4.92	5.02	4.56	3.88	4.49	2.05	2.22	2.05	1.24	0.97	0.68	1.54	1.48	1.80	1.62	1.21	1.25	1.19	1.42
	MEAN	4.30	4.73	5.16	5.33	4.56	3.95	4.67	2.10	2.40	2.06	1.60	1.04	0.64	1.64	1.67	1.90	1.76	1.44	1.74	1.45	1.66
AZ208 (T3)	DT	4.13	4.71	5.15	5.56	4.38	3.86	4.63	2.36	2.65	2.36	2.10	0.94	0.54	1.83	1.67	2.12	2.02	1.97	2.13	0.94	1.81
	DS	4.23	4.45	4.88	5.28	4.31	4.03	4.53	1.91	2.20	2.01	1.38	0.99	0.88	1.56	1.35	2.05	1.95	0.88	1.09	0.91	1.37
	MEAN	4.18	4.58	5.01	5.43	4.35	3.94	4.58	2.14	2.43	2.19	1.74	0.97	0.71	1.69	1.51	2.09	1.99	1.43	1.61	0.93	1.59
FT 326 (T4)	DT	4.01	4.98	5.49	5.79	4.32	3.39	4.66	2.47	2.87	2.12	1.60	1.35	1.04	1.91	1.76	1.95	1.59	1.40	1.60	0.85	1.53
	DS	4.06	4.42	4.84	5.31	4.38	3.90	4.49	1.85	2.10	1.85	1.52	0.83	0.58	1.46	1.47	1.83	1.54	0.96	1.19	1.03	1.34
	MEAN	4.04	4.70	5.17	5.55	4.35	3.65	4.57	2.16	2.49	1.99	1.56	1.09	0.81	1.68	1.62	1.89	1.57	1.18	1.40	0.94	1.43
AA/T (T5)	DT	4.23	4.65	5.09	5.34	3.59	2.15	4.18	2.25	2.54	2.29	2.13	1.10	0.58	1.82	1.61	1.85	1.57	0.91	1.53	1.22	1.45
	DS	3.89	4.02	4.36	4.99	4.23	3.93	4.27	1.96	2.11	1.87	1.20	0.93	0.79	1.48	1.52	1.76	1.17	0.94	1.23	0.82	1.24
	MEAN	4.06	4.34	4.83	5.17	3.91	3.04	4.22	2.11	2.33	2.08	1.67	1.03	0.69	1.65	1.57	1.81	1.37	0.93	1.38	1.02	1.34
	MEAN	4.12	4.56	4.98	5.29	4.19	3.61	4.46	2.06	2.34	2.02	1.57	1.00	0.67	1.61	1.54	1.86	1.65	1.14	1.46	1.02	1.45
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.03	0.02	0.03	0.04	0.04	0.06	0.09	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.03	0.03	0.05	0.07
	CD(5%)	0.05	0.03	0.06	0.07	0.08	0.13	0.18	0.04	0.03	0.05	0.06	0.07	0.10	0.15	0.04	0.03	0.04	0.06	0.06	0.10	0.14

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and 45DAS respectively and then declined, but in root activity peaked at 45 and 90 DAS with a drop at 60^th and 75^th day.

4.19.TOTAL PHENOLS

Tables XXXVI AND XXXVII give the total phenol content of sorghum and finger millet as a result of the various treatments.

Sorghum

The total phenolic content of the *Asospirillum* treated plants were more than that of the controls and T5 (external IAA application). No significant difference among the treatments occurred. The total phenolic content of the plants increased over the time scale upto 75 DAS and at maturity stage got reduced. DS had more total phenolic content than DT.

Finger millet

The same trend as in sorghum was observed in this also.

The phenolic content in rice plants inoculated with *Azospirillum* showed progressive increase from 30-90 DAS (Siva Kumar,1993). The phenol and OD phenol content in shoot was reported to be higher than the root fraction. Mohan *et al.* (1987) also reported that the enhancement of phenolics by *Azospirillum* treatment may confer some sort of resistence to plants against shoot fly feeding. Mohan et al. (1988) reported that *Azospirillum* inoculation activates the enzyme phenylalanine ammonia lyase, implicated in the biosynthesis of phenolics resulting in increased phenolics in plants. Therefore *Azospirillum* inoculation could play an important

TABLE -XXXVI

TOTAL PHENOL CONTENT (mg/g f. wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIEITES OF SORGHUM

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	0.39	0.48	0.58	0.63	0.69	0.53	0.55	0.18	0.19	0.23	0.28	0.30	0.20	0.23	0.20	0.22	0.28	0.30	0.36	0.29	0.28
	DS	0.49	0.53	0.60	0.72	0.87	0.24	0.58	0.17	0.24	0.27	0.32	0.38	0.18	0.26	0.22	0.34	0.53	0.54	0.22	0.12	0.33
	MEAN	0.44	0.51	0.59	0.68	0.78	0.39	0.56	0.18	0.22	0.25	0.30	0.34	0.19	0.25	0.21	0.28	0.41	0.42	0.29	0.21	0.30
AZ204 (T2)	DT	0.50	0.62	0.68	0.83	0.88	0.78	0.71	0.21	0.23	0.27	0.33	0.36	0.24	0.27	0.30	0.34	0.37	0.40	0.43	0.38	0.37
	DS	0.70	0.90	1.02	1.15	1.20	0.31	0.88	0.24	0.31	0.36	0.48	0.52	0.29	0.37	0.32	0.51	0.63	0.67	0.26	0.14	0.42
	MEAN	0.60	0.76	0.85	0.99	1.04	0.52	0.79	0.23	0.27	0.32	0.41	0.44	0.27	0.32	0.31	0.43	0.50	0.54	0.35	0.26	0.40
AZ208 (T3)	DT	0.54	0.50	0.60	0.64	0.71	0.60	0.60	0.25	0.30	0.33	0.36	0.40	0.26	0.32	0.28	0.33	0.37	0.38	0.40	0.36	0.35
	DS	0.54	0.75	0.89	0.99	1.05	0.25	0.75	0.25	0.30	0.33	0.43	0.46	0.19	0.33	0.38	0.52	0.59	0.60	0.22	0.13	0.41
	MEAN	0.54	0.63	0.75	0.82	0.88	0.43	0.67	0.25	0.30	0.33	0.40	0.43	0.22	0.32	0.33	0.43	0.48	0.49	0.31	0.25	0.38
FT 326 (T4)	DT	0.42	0.55	0.61	0.82	1.04	0.69	0.69	0.23	0.34	0.38	0.42	0.44	0.28	0.35	0.27	0.30	0.31	0.34	0.44	0.38	0.34
	DS	0.62	0.69	0.76	1.17	1.23	0.43	0.82	0.23	0.28	0.40	0.44	0.46	0.21	0.34	0.31	0.44	0.68	0.71	0.23	0.16	0.42
	MEAN	0.52	0.62	0.69	1.00	1.14	0.56	0.75	0.23	0.31	0.39	0.43	0.45	0.25	0.34	0.29	0.37	0.49	0.52	0.34	0.27	0.38
IAA/T (T5)	DT	0.52	0.56	0.64	0.79	0.89	0.70	0.68	0.21	0.25	0.27	0.44	0.46	0.25	0.31	0.22	0.29	0.29	0.33	0.39	0.32	0.31
	DS	0.52	0.58	0.70	0.80	0.94	0.30	0.64	0.20	0.28	0.32	0.36	0.40	0.27	0.31	0.39	0.52	0.56	0.62	0.24	0.16	0.42
	MEAN	0.52	0.57	0.67	0.80	0.92	0.50	0.66	0.21	0.27	0.30	0.40	0.43	0.26	0.31	0.31	0.41	0.43	0.48	0.32	0.24	0.36
	MEAN	0.52	0.62	0.71	0.85	0.95	0.48	0.69	0.22	0.27	0.32	0.39	0.42	0.24	0.31	0.29	0.38	0.46	0.49	0.32	0.24	0.36
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.01	0.01	0.02	0.02	0.02	0.04	0.05	0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.01	0.01	0.01	0.02	0.02	0.03	0.04
	CD(5%)	0.03	0.02	0.03	0.04	0.05	0.07	0.10	0.02	0.01	0.03	0.08	0.04	0.06	0.09	0.02	0.02	0.03	0.03	0.04	0.06	0.08

TABLE XXXVII

TOTAL PHENOL CONTENT (mg/gf.wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

TREATMENT	VARIETY	LEAF							SHOOT							ROOT						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	0.22	0.24	0.29	0.35	0.45	0.20	0.29	0.09	0.11	0.17	0.19	0.24	0.13	0.16	0.11	0.14	0.15	0.18	0.22	0.10	0.15
	DS	0.19	0.32	0.41	0.52	0.57	0.39	0.40	0.09	0.12	0.14	0.16	0.19	0.07	0.13	0.08	0.10	0.12	0.16	0.21	0.09	0.13
	MEAN	0.21	0.28	0.35	0.44	0.51	0.30	0.35	0.09	0.12	0.16	0.18	0.21	0.10	0.14	0.10	0.12	0.14	0.17	0.22	0.10	0.14
AZ204 (T2)	DT	0.33	0.36	0.40	0.51	0.58	0.29	0.41	0.17	0.19	0.24	0.28	0.34	0.16	0.23	0.13	0.15	0.17	0.23	0.26	0.18	0.19
	DS	0.23	0.41	0.51	0.58	0.64	0.47	0.47	0.12	0.18	0.22	0.23	0.27	0.09	0.19	0.12	0.13	0.18	0.25	0.29	0.15	0.19
	MEAN	0.28	0.39	0.45	0.54	0.61	0.38	0.44	0.15	0.19	0.23	0.26	0.31	0.13	0.21	0.13	0.14	0.18	0.24	0.28	0.17	0.19
AZ208 (T3)	DT	0.30	0.38	0.42	0.50	0.50	0.32	0.40	0.14	0.18	0.20	0.22	0.29	0.16	0.20	0.11	0.18	0.20	0.22	0.26	0.17	0.19
	DS	0.24	0.38	0.52	0.58	0.68	0.43	0.47	0.11	0.14	0.15	0.19	0.21	0.10	0.15	0.09	0.12	0.19	0.23	0.26	0.10	0.17
	MEAN	0.27	0.38	0.47	0.54	0.59	0.38	0.44	0.13	0.16	0.18	0.21	0.25	0.13	0.17	0.10	0.15	0.20	0.23	0.26	0.14	0.18
FT 326 (T4)	DT	0.28	0.36	0.46	0.49	0.53	0.30	0.40	0.14	0.16	0.25	0.30	0.33	0.17	0.23	0.15	0.20	0.24	0.30	0.34	0.20	0.24
	DS	0.27	0.40	0.63	0.68	0.75	0.51	0.54	0.13	0.15	0.20	0.24	0.30	0.10	0.19	0.11	0.13	0.16	0.27	0.30	0.13	0.18
	MEAN	0.28	0.38	0.55	0.59	0.64	0.40	0.47	0.14	0.16	0.23	0.27	0.32	0.14	0.21	0.13	0.17	0.20	0.29	0.32	0.17	0.21
IAA/T (T5)	DT	0.25	0.26	0.33	0.40	0.49	0.22	0.33	0.13	0.16	0.19	0.25	0.29	0.14	0.19	0.12	0.18	0.20	0.23	0.30	0.12	0.19
	DS	0.21	0.37	0.50	0.55	0.65	0.43	0.45	0.11	0.17	0.19	0.23	0.28	0.11	0.18	0.11	0.13	0.15	0.22	0.27	0.12	0.17
	MEAN	0.23	0.32	0.42	0.48	0.57	0.33	0.39	0.12	0.17	0.19	0.24	0.29	0.13	0.19	0.12	0.16	0.18	0.23	0.29	0.12	0.18
	MEAN	0.25	0.35	0.45	0.52	0.58	0.36	0.42	0.12	0.16	0.20	0.23	0.27	0.12	0.18	0.11	0.15	0.18	0.23	0.27	0.14	0.18
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.01	0.01	0.02	0.02	0.02	0.04	0.05	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.02	0.03
	CD(5%)	0.03	0.02	0.03	0.04	0.05	0.07	0.10	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.03	0.03	0.05	0.07

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role in imparting resistance to the plants.

4.21.ORTHODIHYDRIC PHENOLS

The amount of orthodihydric phenols in sorghum and finger millet due to various treatments in given in Tables XXXVIII and XXXIX.

Sorghum

All the treatments resulted in a significantly higher content of orthodihydric phenols in all parts of the plants than in controls. AZ204 treated plants showed the maximum effect. However, no significant difference was noticed among the rest of the treatments. The orthodihydric phenol content increased steadily upto 90 DAS and then decreased. DS had higher amount of orthodihydric phenols than DT.

Finger millet

A similar trend of effect was noticed with regard to treatments and varieties. However, orthodihydric phenolic content increased upto 75 DAS and then started declining.

4.22.NITROGEN

As is evident from Table XL the influence of various treatments on the levels of nitrogen showed a similar trend in both sorghum and finger millet.

AZ204 inoculation caused the maximum increase in nitrogen content. No significant difference was observed between treatments AZ204 and FT326, and IAA and AZ208, though all the treatments caused a significant increase over control.The nitrogen content of the plants increased over the

TABLE -XXXVIII

ORTHO DIHYDRIC PHENOL CONTENT (mg/g f. wt) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

	VARIETY	LEAF							SHOOT							ROOT						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	0.33	0.39	0.49	0.61	0.74	0.52	0.51	0.12	0.16	0.22	0.24	0.15	0.08	0.16	0.08	0.12	0.12	0.15	0.18	0.12	0.13
	DS	0.28	0.41	0.53	0.61	0.52	0.27	0.44	0.11	0.14	0.18	0.23	0.28	0.20	0.19	0.09	0.12	0.14	0.20	0.12	0.09	0.13
	MEAN	0.31	0.40	0.51	0.61	0.63	0.40	0.47	0.12	0.15	0.20	0.24	0.22	0.14	0.18	0.09	0.12	0.13	0.18	0.15	0.11	0.13
AZ204 (T2)	DT	0.48	0.56	0.66	0.80	0.95	0.71	0.69	0.20	0.22	0.30	0.33	0.22	0.14	0.24	0.11	0.14	0.18	0.22	0.25	0.21	0.18
	DS	0.35	0.50	0.71	0.77	0.60	0.31	0.54	0.16	0.18	0.26	0.32	0.34	0.22	0.25	0.11	0.20	0.22	0.25	0.19	0.11	0.18
	MEAN	0.42	0.53	0.69	0.79	0.78	0.51	0.62	0.18	0.20	0.28	0.33	0.28	0.18	0.24	0.11	0.17	0.20	0.24	0.22	0.16	0.18
AZ208 (T3)	DT	0.43	0.50	0.60	0.71	0.88	0.63	0.63	0.18	0.21	0.24	0.28	0.18	0.09	0.20	0.09	0.11	0.16	0.19	0.22	0.16	0.16
	DS	0.31	0.54	0.64	0.70	0.55	0.34	0.51	0.12	0.16	0.22	0.25	0.29	0.21	0.21	0.12	0.16	0.18	0.21	0.15	0.10	0.15
	MEAN	0.37	0.52	0.62	0.71	0.72	0.49	0.57	0.15	0.19	0.23	0.27	0.24	0.15	0.20	0.11	0.14	0.17	0.20	0.19	0.13	0.15
IFT 326 (T4)	DT	0.40	0.49	0.61	0.73	0.91	0.65	0.63	0.16	0.22	0.29	0.32	0.20	0.11	0.22	0.11	0.15	0.18	0.24	0.26	0.17	0.19
	DS	0.40	0.58	0.65	0.70	0.62	0.45	0.57	0.14	0.17	0.26	0.30	0.34	0.28	0.25	0.13	0.16	0.22	0.25	0.14	0.11	0.17
	MEAN	0.40	0.54	0.63	0.72	0.76	0.55	0.60	0.15	0.20	0.28	0.31	0.27	0.20	0.23	0.12	0.16	0.20	0.25	0.20	0.14	0.18
IAA/T (T5)	DT	0.37	0.45	0.55	0.69	0.79	0.60	0.58	0.18	0.20	0.27	0.29	0.21	0.11	0.21	0.09	0.10	0.15	0.21	0.22	0.17	0.16
	DS	0.42	0.52	0.61	0.69	0.59	0.30	0.52	0.15	0.21	0.24	0.25	0.31	0.22	0.23	0.13	0.18	0.24	0.27	0.20	0.09	0.19
	MEAN	0.40	0.49	0.58	0.69	0.69	0.45	0.55	0.17	0.21	0.26	0.27	0.26	0.17	0.22	0.11	0.14	0.20	0.24	0.21	0.13	0.17
	MEAN	0.38	0.49	0.61	0.70	0.71	0.48	0.56	0.15	0.19	0.25	0.28	0.25	0.17	0.21	0.11	0.14	0.18	0.22	0.19	0.13	0.16
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.02	0.02	0.04	0.05	0.01	0.01	0.01	0.01	0.02	0.03	0.04	0.01	0.00	0.01	0.01	0.01	0.02	0.03
	CD(5%)	0.03	0.02	0.03	0.04	0.05	0.07	0.11	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.02	0.02	0.04	0.05

TABLE –XXXIX

ORTHODIHYDRIC PHENOL CONTENT (mg/g f. wt) IN DIFFREENT PARTS OF DDROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

		LEAF							SHOOT							ROOT						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	0.19	0.25	0.35	0.42	0.44	0.21	0.31	0.05	0.06	0.10	0.14	0.09	0.05	0.08	0.06	0.09	0.11	0.15	0.10	0.05	0.09
	DS	0.25	0.30	0.39	0.44	0.49	0.29	0.36	0.06	0.09	0.12	0.13	0.10	0.05	0.09	0.06	0.09	0.11	0.16	0.19	0.05	0.11
	MEAN	0.22	0.28	0.37	0.43	0.47	0.25	0.34	0.06	0.08	0.11	0.14	0.10	0.05	0.09	0.06	0.09	0.11	0.16	0.15	0.05	0.10
A2204 (T2)	DT	0.27	0.31	0.47	0.51	0.54	0.28	0.40	0.07	0.10	0.12	0.19	0.10	0.06	0.11	0.10	0.13	0.16	0.24	0.14	0.09	0.14
	DS	0.29	0.39	0.48	0.52	0.56	0.32	0.43	0.10	0.11	0.15	0.18	0.14	0.07	0.13	0.07	0.14	0.18	0.22	0.25	0.09	0.16
	MEAN	0.28	0.35	0.48	0.52	0.55	0.30	0.41	0.09	0.11	0.14	0.19	0.12	0.07	0.12	0.09	0.14	0.17	0.23	0.20	0.09	0.15
AZ208 (T3)	DT	0.22	0.43	0.42	0.47	0.50	0.31	0.39	0.05	0.07	0.14	0.16	0.11	0.06	0.10	0.08	0.11	0.14	0.17	0.15	0.07	0.12
	DS	0.32	0.39	0.47	0.50	0.51	0.39	0.43	0.08	0.12	0.14	0.17	0.11	0.07	0.12	0.06	0.13	0.16	0.19	0.21	0.07	0.14
	MEAN	0.27	0.41	0.45	0.49	0.51	0.35	0.41	0.07	0.10	0.14	0.17	0.11	0.07	0.11	0.07	0.12	0.15	0.18	0.18	0.07	0.13
FT 326 (T4)	DT	0.26	0.36	0.39	0.47	0.50	0.26	0.37	0.07	0.09	0.15	0.18	0.12	0.08	0.12	0.10	0.12	0.14	0.18	0.12	0.08	0.12
	DS	0.35	0.41	0.45	0.47	0.55	0.34	0.43	0.11	0.13	0.16	0.18	0.13	0.09	0.13	0.08	0.11	0.15	0.20	0.24	0.08	0.14
	MEAN	0.31	0.38	0.42	0.47	0.53	0.30	0.40	0.09	0.11	0.16	0.18	0.13	0.09	0.12	0.09	0.12	0.15	0.19	0.18	0.08	0.13
IAA/T (T5)	DT	0.26	0.31	0.42	0.51	0.56	0.29	0.39	0.06	0.07	0.13	0.19	0.10	0.08	0.11	0.09	0.11	0.12	0.20	0.12	0.06	0.12
	DS	0.31	0.36	0.45	0.49	0.53	0.38	0.42	0.09	0.13	0.13	0.15	0.11	0.08	0.12	0.08	0.10	0.14	0.19	0.23	0.06	0.13
	MEAN	0.29	0.34	0.44	0.50	0.55	0.34	0.41	0.08	0.10	0.13	0.17	0.11	0.08	0.11	0.09	0.11	0.13	0.20	0.18	0.06	0.13
	MEAN	0.27	0.35	0.43	0.48	0.52	0.31	0.39	0.07	0.10	0.13	0.17	0.11	0.07	0.11	0.08	0.11	0.14	0.19	0.18	0.07	0.13
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SEm	0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.01	0.00	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.01	0.01	0.01	0.02	0.03
	CD(5%)	0.02	0.02	0.03	0.03	0.04	0.06	0.08	0.01	0.01	0.01	0.02	0.02	0.03	0.05	0.02	0.01	0.02	0.02	0.02	0.04	0.05

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time scale upto 75 DAS and declined at maturity. DT had more nitrogen content than DS.

Several workers have reported considerable quantities of biological nitrogen fixation under natural conditions in cultivated plants due to inoculation with *A. lipoferum* (Amer,1982).The results therefore indicated that the increase in total nitrogen content due to inoculation with *Azospirillum* strains may be caused either by enhanced biological nitrogen fixation or increased uptake of soil nitrogen through high root proliferation. Sheela (1991) reported that the plants inoculated with *Azospirillum* isolate Y2 increased significantly the nitrogen content and total nitrogen assimilation.

4.23.PHOSPHORUS

Table XLI gives the phosphorus content of sorghum and finger millet as a result of various treatments.

Sorghum

AZ204 treated plants were found to contain a higher amount of phosphorus than the rest. However no significant difference was seen among the treatments in influencing the phosphorus content of the plant. Phosphorus content of plants increased till 75 DAS and then reduced. No significant difference in phosphorus content was seen between the varieties.

Finger millet

The influence of various treatments revealed the same

TABLE–XL

NITROGEN CONTENT (g%)
IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM AND FINGER MILLET

		SORGHUM							FINGER MILLET						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	1.25	1.43	1.68	1.95	1.76	1.65	2.01	1.69	1.92	2.15	2.24	2.10	1.94	2.01
	DS	1.44	1.64	1.78	1.90	1.68	1.51	1.85	1.51	1.72	1.98	2.21	1.97	1.71	1.85
	MEAN	1.35	1.54	1.73	1.92	1.72	1.58	1.93	1.60	1.82	2.07	2.23	2.04	1.83	1.93
AZ204 (T2)	DT	1.42	1.75	1.90	2.21	2.04	1.94	2.37	1.80	2.14	2.54	2.80	2.61	2.35	2.37
	DS	1.60	1.75	1.96	2.06	1.84	1.61	2.08	1.71	2.04	2.30	2.58	2.08	1.79	2.08
	MEAN	1.51	1.75	1.93	2.14	1.94	1.78	2.23	1.76	2.09	2.42	2.69	2.35	2.07	2.23
AZ208 (T3)	DT	1.46	1.55	1.92	2.09	1.96	1.77	2.21	1.73	2.02	2.30	2.66	2.52	2.02	2.21
	DS	1.54	1.65	1.90	1.98	1.71	1.57	2.03	1.65	1.94	2.20	2.34	2.22	1.86	2.03
	MEAN	1.50	1.60	1.91	2.04	1.84	1.67	2.12	1.69	1.98	2.25	2.50	2.37	1.94	2.12
IFT 326 (T4)	DT	1.35	1.58	1.86	2.34	2.08	1.90	2.31	1.72	2.18	2.45	2.72	2.58	2.23	2.31
	DS	1.47	1.74	2.02	2.14	1.83	1.69	2.12	1.77	2.00	2.25	2.55	2.24	1.92	2.12
	MEAN	1.41	1.66	1.94	2.24	1.96	1.80	2.22	1.75	2.09	2.35	2.64	2.41	2.08	2.22
IAA/T (T5)	DT	1.32	1.60	1.77	2.16	1.99	1.81	2.17	1.82	2.01	2.25	2.42	2.39	2.11	2.17
	DS	1.52	1.80	1.88	2.03	1.75	1.56	2.05	1.69	1.85	2.04	2.46	2.31	1.95	2.05
	MEAN	1.42	1.70	1.83	2.10	1.87	1.69	2.11	1.76	1.93	2.15	2.44	2.35	2.03	2.11
	MEAN	1.44	1.65	1.87	2.09	1.86	1.70	2.12	1.71	1.98	2.25	2.50	2.30	1.99	2.12
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.03	0.03	0.05	0.06	0.02	0.01	0.02	0.03	0.03	0.04	0.06
	CD(5%)	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.04	0.02	0.04	0.05	0.06	0.09	0.13

TABLE—XLI

PHOSPHORUS CONTENT (g%) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM AND FINGER MILLET

	VARIETY	SORGHUM							FINGER-MILLET						
TREATMENT		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
	DT	0.06	0.10	0.13	0.15	0.11	0.06	0.10	0.05	0.08	0.12	0.17	0.11	0.06	0.10
CON	DS	0.05	0.10	0.12	0.17	0.11	0.06	0.10	0.05	0.07	0.11	0.16	0.20	0.10	0.12
(T1)	MEAN	0.06	0.10	0.13	0.16	0.11	0.06	0.10	0.05	0.08	0.12	0.17	0.16	0.08	0.11
AZ204	DT	0.10	0.13	0.17	0.22	0.18	0.11	0.15	0.10	0.13	0.19	0.24	0.18	0.09	0.16
(T2)	DS	0.09	0.14	0.17	0.21	0.15	0.08	0.14	0.09	0.11	0.19	0.23	0.27	0.17	0.18
	MEAN	0.10	0.14	0.17	0.22	0.17	0.10	0.15	0.10	0.12	0.19	0.24	0.23	0.13	0.17
AZ208	DT	0.08	0.11	0.14	0.17	0.17	0.08	0.12	0.08	0.12	0.17	0.21	0.13	0.09	0.13
(T3)	DS	0.08	0.12	0.15	0.19	0.14	0.07	0.13	0.09	0.11	0.14	0.21	0.26	0.12	0.16
	MEAN	0.08	0.12	0.15	0.18	0.15	0.08	0.12	0.09	0.12	0.16	0.21	0.20	0.11	0.14
FT 326	DT	0.09	0.12	0.16	0.19	0.15	0.09	0.13	0.09	0.11	0.16	0.23	0.16	0.08	0.14
(T4)	DS	0.09	0.16	0.18	0.21	0.13	0.07	0.14	0.08	0.12	0.15	0.19	0.23	0.14	0.15
	MEAN	0.09	0.14	0.17	0.20	0.14	0.08	0.14	0.09	0.12	0.16	0.21	0.20	0.11	0.15
IAA/T	DT	0.08	0.14	0.14	0.19	0.14	0.07	0.13	0.08	0.09	0.17	0.20	0.16	0.07	0.13
(T5)	DS	0.07	0.13	0.16	0.17	0.13	0.08	0.12	0.07	0.09	0.14	0.19	0.22	0.13	0.14
	MEAN	0.08	0.14	0.15	0.18	0.14	0.08	0.13	0.08	0.09	0.16	0.20	0.19	0.10	0.13
	MEAN	0.08	0.13	0.15	0.19	0.14	0.08	0.13	0.08	0.10	0.15	0.20	0.19	0.11	0.14
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.01	0.00	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.01	0.01	0.01	0.02	0.02
	CD(5%)	0.01	0.01	0.01	0.02	0.02	0.03	0.05	0.01	0.01	0.01	0.02	0.02	0.03	0.04

TABLE -XLII

POTASSIUM CONTENT (g%) IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM AND FINGER MILLET

		SORGHUM							FINGER-MILLET						
TREATMENT	VARIETY	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
	DT	0.59	0.71	0.81	1.08	0.62	0.49	0.72	0.55	0.77	0.98	1.01	0.80	0.50	0.77
CON	DS	0.60	0.78	0.84	0.99	0.66	0.50	0.73	0.62	0.73	0.83	0.88	0.71	0.69	0.74
(T1)	MEAN	0.60	0.75	0.83	1.04	0.64	0.50	0.72	0.59	0.75	0.91	0.95	0.76	0.60	0.76
AZ204	DT	0.70	0.85	0.96	1.23	0.68	0.54	0.83	0.72	0.94	1.05	1.10	1.01	0.61	0.91
(T2)	DS	0.72	0.85	0.92	1.18	0.81	0.66	0.86	0.71	0.83	0.98	1.07	0.88	0.83	0.88
	MEAN	0.71	0.85	0.94	1.21	0.75	0.60	0.84	0.72	0.89	1.02	1.09	0.95	0.72	0.89
AZ208	DT	0.63	0.79	0.88	1.13	0.71	0.49	0.77	0.57	0.80	1.03	1.06	0.81	0.66	0.82
(T3)	DS	0.68	0.95	0.89	1.08	0.75	0.55	0.80	0.63	0.74	0.88	0.93	0.76	0.70	0.77
	MEAN	0.66	0.82	0.89	1.11	0.73	0.52	0.79	0.60	0.77	0.96	1.00	0.79	0.68	0.80
FT 326	DT	0.68	0.83	0.96	1.18	0.63	0.59	0.81	0.61	0.86	1.01	1.11	0.77	0.59	0.83
(T4)	DS	0.71	0.82	0.91	1.03	0.71	0.61	0.80	0.72	0.85	0.95	1.05	0.81	0.75	0.86
	MEAN	0.70	0.83	0.94	1.11	0.67	0.60	0.81	0.67	0.86	0.98	1.08	0.79	0.67	0.84
IAA/T	DT	0.63	0.80	0.91	1.17	0.64	0.50	0.78	0.63	0.87	1.03	1.04	0.82	0.63	0.84
(T5)	DS	0.75	0.87	0.85	0.99	0.76	0.58	0.80	0.68	0.87	0.93	1.03	0.91	0.85	0.88
	MEAN	0.69	0.84	0.88	1.08	0.70	0.54	0.79	0.66	0.87	0.98	1.04	0.87	0.74	0.86
	MEAN	0.67	0.82	0.89	1.11	0.70	0.55	0.79	0.64	0.83	0.97	1.03	0.83	0.68	0.83
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.02	0.02	0.04	0.05	0.02	0.01	0.02	0.02	0.03	0.04	0.06
	CD(5%)	0.03	0.02	0.03	0.04	0.05	0.08	0.11	0.03	0.02	0.04	0.05	0.05	0.08	0.11

pattern as found in sorghum.

Veerasamy *et al.* (1992) reported that inoculation of sorghum with *A.lipoferum* resulted in enhanced uptake of nitrogen and phosphorus.

4.24.POTASSIUM

Effect of inoculation of different *Azospirillum* strains and external application of IAA on potassium levels in selected varieties of sorghum and finger millet is presented in Table XLII.

Sorghum

It is found from the data presented that inoculation of AZ204 exhibited maximum potassium content. However, no significant difference between the varieties was observed. The potassium content increased over time upto 75 DAS and declined thereafter.

Finger Millet

Similar to sorghum AZ204 application registered maximum potassium content. All the treatments differed significantly from control. No significant difference between the varieties was seen. Similar to sorghum potassium content increased upto 75 DAS and then declined.

4.24.BIOMETRIC OBSERVATIONS

Effect of inoculation of different strains of *Azospirillum* and external application of IAA on selected varieties of sorghum and finger millet is presented in Tables XLIII and XLIV and plates I and II.

TABLE-XLIII

BIOMETRIC OBSERVATIONS IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF SORGHUM

TREATMENT	VARIETY	SHOOT LENGTH (cm)							ROOT LENGTH (cm)							PLANT FRESH WEIGHT g/p						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON (T1)	DT	118.26	27.08	41.20	50.30	57.10	67.08	143.50	8.22	13.15	16.30	19.50	22.90	27.41	117.91	1.08	1.51	2.90	4.70	5.80	7.20	3.87
	DS	114.55	20.40	27.36	39.90	48.13	57.38	134.62	7.34	9.28	11.94	14.86	19.40	21.88	114.12	0.86	1.50	2.81	4.20	5.90	7.00	3.71
	MEAN	116.41	23.74	34.28	45.10	52.61	62.23	139.06	7.78	11.22	14.12	17.18	21.15	24.64	116.01	0.97	1.51	2.86	4.45	5.85	7.10	3.79
AZ204 (T2)	DT	124.04	35.85	48.30	56.20	65.10	74.82	150.72	111.65	16.22	18.50	22.90	26.28	31.00	121.09	1.35	2.10	3.70	5.54	6.90	8.80	4.73
	DS	118.80	28.25	32.74	44.86	57.45	65.66	141.29	9.98	11.20	14.00	17.31	21.16	24.30	116.33	1.30	1.87	3.11	5.00	6.40	8.30	4.33
	MEAN	121.42	32.05	40.52	50.53	61.28	70.24	146.01	110.82	13.71	16.25	20.11	23.72	27.65	118.71	1.32	1.99	3.41	5.27	6.65	8.55	4.53
AZ208 (T3)	DT	119.67	29.90	44.20	53.25	60.28	69.37	146.11	9.23	14.63	17.40	20.90	24.42	28.13	119.12	1.18	1.98	3.00	5.10	6.20	8.20	4.28
	DS	116.24	21.26	30.26	41.75	52.87	61.30	137.28	8.90	10.84	13.56	16.42	20.26	22.94	115.49	0.99	1.68	2.94	4.80	6.20	7.90	4.09
	MEAN	117.95	25.58	37.23	47.50	56.57	65.33	141.69	9.07	12.74	15.48	18.66	22.34	25.54	117.30	1.09	1.83	2.97	4.95	6.20	8.05	4.18
MT 326 (T4)	DT	121.00	36.30	50.40	58.40	68.30	73.15	151.26	112.56	15.36	19.42	24.03	27.18	27.07	120.94	1.23	1.95	3.50	5.59	7.11	9.10	4.75
	DS	120.40	25.60	33.40	42.80	54.70	66.15	140.51	8.89	12.44	15.13	18.36	21.06	20.06	115.99	1.14	1.75	3.40	5.08	6.40	8.10	4.31
	MEAN	120.70	30.95	41.90	50.60	61.50	69.65	145.88	110.73	13.90	17.27	21.20	24.12	23.57	118.46	1.19	1.85	3.45	5.33	6.75	8.60	4.53
AA/T (T5)	DT	123.15	23.17	45.60	55.45	62.50	70.44	146.72	111.24	15.56	18.14	23.32	27.07	30.65	121.00	1.31	1.83	3.30	4.93	6.80	8.60	4.46
	DS	116.40	22.40	31.60	42.60	52.50	61.96	137.91	9.04	12.23	14.84	17.45	20.11	23.55	116.20	1.10	1.65	3.32	4.70	6.20	7.80	4.13
	MEAN	119.78	22.78	38.60	49.03	57.50	66.20	142.31	110.14	13.90	16.49	20.38	23.59	27.10	118.60	1.21	1.74	3.31	4.81	6.50	8.20	4.29
	MEAN	119.25	27.02	38.51	48.55	57.89	66.73	142.99	9.71	13.09	15.92	19.50	22.98	25.70	117.82	1.15	1.78	3.20	4.96	6.39	8.10	4.26
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.01	0.02	0.02	0.03	0.04	0.06
	CD(5%)	0.04	0.03	0.05	0.06	0.07	0.10	0.15	0.04	0.02	0.04	0.05	0.06	0.09	0.13	0.03	0.02	0.04	0.05	0.05	0.09	0.12

TABLE-XLIV

BIOMETRIC OBSERVATIONS IN DIFFERENT PARTS OF DROUGHT TOLERANT (DT) AND DROUGHT SUSCEPTIBLE (DS) VARIETIES OF FINGER MILLET

TREATMENT	VARIETY	SHOOT LENGTH (CM)							ROOT LENGTH (CM)							PLANT FRESH WEIGHT (g/p)						
		30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN	30	45	60	75	90	105	MEAN
CON	DT	12.40	17.80	21.10	28.15	40.44	48.18	128.01	8.10	10.03	13.40	17.34	20.18	24.80	15.68	1.90	2.93	3.95	5.00	7.50	9.86	5.19
(T1)	DS	10.18	13.20	21.00	26.14	39.24	46.11	125.98	7.01	9.86	12.01	15.19	18.41	24.15	14.44	1.85	2.96	3.50	4.95	6.85	8.86	4.83
	MEAN	11.29	15.50	21.05	27.15	39.84	47.15	127.00	7.56	9.95	12.71	16.27	19.30	24.48	15.06	1.88	2.94	3.72	4.98	7.18	9.37	5.01
AZ204	DT	16.84	20.42	26.32	32.89	46.20	52.64	132.55	10.06	12.00	15.16	20.23	24.66	28.42	18.42	2.70	3.70	5.00	6.00	8.30	11.90	6.27
(T2)	DS	12.48	16.85	23.67	29.23	42.46	49.44	129.02	9.23	11.44	13.56	18.23	21.98	23.98	16.40	2.31	3.25	4.35	5.95	8.01	10.11	5.66
	MEAN	14.66	18.64	25.00	31.06	44.33	51.04	130.79	9.65	11.72	14.36	19.23	23.32	26.20	17.41	2.51	3.48	4.67	5.98	8.16	11.01	5.96
AZ208	DT	14.32	19.25	23.45	30.16	43.40	50.24	130.14	9.55	11.00	14.97	18.44	23.53	26.24	17.29	2.40	3.20	3.97	5.55	7.90	10.50	5.59
(T3)	DS	13.24	15.14	22.08	27.15	44.27	47.55	128.24	8.40	10.95	13.07	16.23	21.25	23.06	15.49	2.04	3.02	3.98	5.34	7.75	9.30	5.24
	MEAN	13.78	17.20	22.77	28.66	43.84	48.90	129.19	8.98	10.98	14.02	17.34	22.39	24.65	16.39	2.22	3.11	3.98	5.44	7.83	9.90	5.41
FT 326	DT	15.18	19.95	27.65	34.20	47.27	51.57	132.64	10.41	12.00	16.12	19.89	24.35	27.40	18.36	2.90	3.60	4.42	5.90	8.50	11.13	6.07
(T4)	DS	12.93	17.14	25.00	30.75	43.25	53.27	130.39	8.00	12.12	14.84	18.14	22.83	24.56	16.75	1.99	3.49	4.70	6.02	8.62	10.56	5.90
	MEAN	14.06	18.55	26.33	32.48	45.26	52.42	131.51	9.20	12.06	15.48	19.01	23.59	25.98	17.55	2.45	3.55	4.56	5.96	8.56	10.84	5.99
AA/T	DT	14.21	18.41	26.75	31.00	46.35	43.18	131.65	9.23	11.08	15.19	18.22	23.46	27.00	17.36	2.33	3.30	4.03	5.75	8.16	10.90	5.74
(T5)	DS	13.14	15.20	22.96	31.25	39.33	51.01	128.82	8.23	11.19	13.96	17.24	21.41	24.12	16.03	2.10	3.01	4.00	6.23	7.40	9.99	5.46
	MEAN	13.68	16.81	24.86	31.13	42.84	52.09	130.23	8.73	11.13	14.58	17.73	22.44	25.56	16.69	2.21	3.16	4.02	5.99	7.78	10.45	5.60
	MEAN	13.49	17.34	24.00	30.09	43.22	50.32	129.74	8.82	11.17	14.23	17.93	22.21	25.37	16.62	2.25	3.25	4.19	5.67	7.90	10.31	5.59
		T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS	T	V	S	TV	VS	TS	TVS
	SED	0.14	0.09	0.15	0.20	0.21	0.34	0.48	0.02	0.01	0.02	0.03	0.03	0.05	0.07	0.02	0.02	0.03	0.03	0.04	0.06	0.08
	CD(5%)	0.28	0.17	0.30	0.39	0.43	0.68	0.96	0.04	0.03	0.04	0.06	0.06	0.10	0.14	0.05	0.03	0.05	0.07	0.08	0.12	0.17

SORGHUM (DT)

SORGHUM (DS)

Plate I - BIOMETRIC OBSERVATIONS (30 DAS)

Sorghum

AZ204 application recorded a higher value of shoot length, root length and plant fresh weight. FT326 and AZ204 treated plants were on par with each other in influencing the shoot length. All the treatments differed significantly over the control. Biometric observations showed a gradual increase over time. DT registered a higher value of all the observations made than DS.

Finger Millet

Inoculation of FT326 registered increased shoot length, root length and plant fresh weight. FT326 and AZ204 inoculation were on par with each other in influencing the plant fresh weight. As compared to control all treatments differed significantly. Variety wise DT registered a significantly higher value than DS in all the biometric observations .

Gamo (1991) reported an increase in plant height, shoot and root dry weight in maize inoculated with *Azospirillum*. Inoculation of sorghum and pearl millet with *Azospirillum* resulted in increased shoot and root mass when compared to the control (Ghonsikar *et al.*, 1986; Veeraswamy *et al.*, 1992).

Azospirillum species by improving root development, increased the rate of water and mineral uptake thus improved yields of cereal crops (Wani and Konde,1986).In the present study, the effect of nitrogen fixation and production of

FINGER MILLET (DT)

SORGHUM (DT)

Plate II - ROOT DEVELOPMENT (60 DAS)

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growth promoting substances together have probably contributed to increased shoot length, root length and biomass.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present investigation entitled "Effect of *Azospirillum* inoculation on the growth and nitrogen assimilation of sorghum (*Sorghum bicolor* (C_4)) and finger millet (*Eleusine coracana* (C_3))" was undertaken with the main objective of studying the growth, process of nitrogen assimilation, the mode of transport of the fixed nitrogen, variation in hormone levels, total protein, total phenol and ortho dihydric phenol contents under the influence of dinitrogen fixing microaerophilic microbe *Azospirillum*. The impact of external application of IAA was also studied alongside.

C_4 and C_3 plants of two different varieties, Drought Tolerant (DT) and Drought Susceptible (DS) were chosen for the study. The seeds of the selected plants were treated with three different *Azospirillum* strains, as detailed below.

T1 - Uninoculated control

T2 - Inoculation with A.*lipoferum* (AZ204)

T3 - Inoculation with A. *halopraeferens*(AZ208)

T4 - Inoculation with A.*brasilense* (FT326)

T5 - IAA - T

Plants were carefully uprooted from the pots at 15 days interval till the harvesting period (105) days, starting from the 30th day. They were washed and then observed for the physiological parameters and analysed for biochemical changes in leaf, shoot and root.

The changes observed in the various parameters studied are summarized below:

FT326 strain recorded higher indole acetic acid production than the other strains.

In sorghum maximum activity of GS in leaf was found in FT326 inoculated plants. In shoot and root, AZ204 treated plants showed the maximum activity. Of the three parts studied the activity peaked in shoots. DT recorded higher activity of GS. In finger millet, FT326 inoculation exhibited maximum effect in leaf and root, but it was AZ204 in shoot. DS showed increased activity.

FT326 treated plants exhibited maximum GOGAT activity in all the parts of the plant in both sorghum and finger millet. Maximal GOGAT activity was observed in roots. DS registered higher activity than DT.

The different treatments of the sorghum plant evinced increase in the GDH activity of all the tissues as compared to control. FT326 application resulted in higher activity of GDH in all the tissues studied in sorghum, while in finger millet this treatment caused maximum increase only in shoot and root. In leaf of finger millet plants external application of IAA produced maximum effect. Root showed higher GDH activity than other parts.

In the present study GS activity was more than the GOGAT and GDH. GOGAT was more than the GDH activity in both

the varieties considered. Higher activity of GS and GOGAT than GDH suggested that ammonia assimilation was carried out in the plant cytoplasm by the coupled enzyme system of the two enzymes GS/GOGAT.

Increased activity of GDH on 30 DAS may be due to the presence of high levels of ammonia produced by *Azospirillum*. The GDH system might have been turned on actively in the initial stages of nitrogen fixation. However due to the transport of ammonia to other parts and due to utilisation, ammonia concentration might have decreased at later stages and hence GS-GOGAT system probably started functioning at low concentration of ammonia effectively. This might probably be the reason for the lower activity of GDH at later stages.

The higher ammonia in the shoot, rather than in the underground parts, may be more beneficial to the plants because of the availability of photochemically produced energy and reducing power for ammonia assimilation.

AZ204 in shoot and root of sorghum and FT326 in all parts of finger millet induced maximal AAT activity in both the plants. DS exhibited maximal activity. Shoot had better activity than root and leaf.

Maximum AS activity was noticed in all the parts of AZ204 treated plants of sorghum as well as finger millet. In leaf and shoot DS expressed higher activity and in root it was vice versa. Among the different parts root showed increased

activity. However, asparagine synthetase activity was relatively less when compared to the activities of other ammonia assimilating enzymes.

Inoculation of FT326 induced asparaginase activity to a maximal extent in leaf. In shoot and root AZ204 application exhibited higher activity in sorghum. The activity was greater in DT than in DS. In finger millet AZ204 inoculation registered higher activity in leaf whereas it was FT326 inoculation which induced maximum activity in shoot and root. In sorghum DT expressed higher activity and in finger millet the effect was reversed.

AZ204 showed maximum glutaminase activity in leaf and FT326 in shoot and root of sorghum. DT registered higher value than DS in sorghum. In finger millet IAA treated plant had the maximum activity in leaf, and FT326 in shoot and root. Contrary to sorghum DS of finger millet recorded higher activity in leaf and shoot. But maximum activity was seen in root.

AZ204 treated plants were found to be superior in influencing nitrate reductase activity in all parts of sorghum plants. Similar trend was observed in finger millet except it was FT326 inoculation which showed maximum activity in root. DT showed maximum effect in sorghum while it was the reverse in finger millet. Leaves contained maximum amount of this enzyme.

In the case of sorghum, leaf nitrite reductase was highest in AZ208 treated plants, and in shoot and root activity was highest in FT326 and AZ204 inoculated plants. DT expressed higher activity than DS. In leaf of finger millet enzyme activity ranked highest in IAA-T plants and in shoot and root, it was AZ204 which recorded maximum activity. Higher activity was found in root than in shoot and leaves.

AZ204 treatment caused maximal increase of XDH activity in different parts of sorghum and finger millet except it was FT326 which showed maximum increase in finger millet shoot. DS registered increased activity in leaf while in shoot and root DT recorded higher activity.

In leaf and shoot of sorghum plants and in shoot of finger millet maximal uricase activity was induced by AZ204. FT326 registered higher activity in root of sorghum and in leaf and root of finger millet. Uricase activity was greater in DS than DT.

FT326 inoculated plants registered higher allantoinase activity in leaf, shoot and root in sorghum. In finger millet allantoinase activity was greater in FT326 treated plants in leaf and shoot, while the root of plants treated with AZ204 had greater activity. In both the plants maximum activity was observed in root.

FT326 plants exhibited higher urease activity in all parts of the plant in sorghum and finger millet. In both the cases DS expressed higher activity. It was shoot in sorghum and root in finger millet which exhibited maximum activity.

The presence of urease at pod setting stage provides supporting evidence that ureides are metabolised to urea and further to ammonia and carbondioxide. The ammonia liberted in the urease reaction may be re-assimilated.

The distribution of these enzymes indicate that root might be the main site of purine degradation and that a substantial part of the allantoin produced in roots is translocated to shoot and leaves, where it is broken down and utilised via allantoinase.

Generally, the activity of the enzymes increased in vegetative and flowering stage and decreased during maturity. However, fluctuation in the enzyme activity was seen inbetween the stages as a result of some treatements.

The decline in the enzyme activity in different regions of the plant during maturity suggests that there may be increased activity in seeds, so that the ureides received by pods or seeds could be metabolised to urea and glyoxylate. The presence of significant amounts of urease, GS, GDH and allantoinase in various organs suggests that the plants have the capacity to degrade allantoin and allantoic acid and further assimilate the same through the action of urease, GS and GDH.

The ureide production in various parts of sorghum and finger millet was enhanced due to inoculation with AZ204. Maximum ureide content was observed in leaf. DT of sorghum expressed higher activity while it was high in DS of finger millet.

The presence of ureides in all the parts however revealed that these tissues accumulated ureides. This accumulation of ureides in different parts is probably due to the transport of these compounds from the root region. This observed increase in ureides within the plant could be associated with increase in nitrogen transport from the root via the xylem. The relative content of ureides in particular tissue might reflect the current status of nitrogen fixation.

In leaf/shoot external application of IAA and in root AZ204, inoculation caused maximum IAA production in sorghum but in finger millet AZ204 application registered higher IAA content in both the plant parts.The reason for such an observation is not known, especially because FT326 was found to produce maximum amount of IAA among the three strains tested.

AZ204 in shoot system and FT326 in root showed maximum gibberellin content in sorghum. In finger millet FT326 exhibited maximal effect. The hormone production was more in root than in shoot system.

Both in sorghum and finger millet AZ204 application showed increased protein content. DT had higher protein than DS. Protein content was more in leaf than in other parts. There was no significant difference in protein content between the treatments.

The total phenol and orthodihydric phenol contents was maximum in FT326 and AZ204 treated plants. There was no

significant difference in phenol and OD phenol content between the treatments. Leaf showed increased phenol and OD phenol contents.

Both in sorghum and finger millet AZ204 application showed increased nitrogen, phosphorus and potassium content. No significant difference in all the nutrient contents was seen between treatments.

The higher nitrogen content in *Azospirillum* treated plants compared to controls might be reflective of higher protein content of these plants. In general, the increase in the levels of nitrogen, phosphorus and potassium could reflect better uptake from the roots as a result of *Azospirillum* treatment.

Biometric observations made, revealed that AZ204 inoculation in sorghum and FT326 inoculation in finger millet showed maximum effect. It can be concluded that the effect of nitrogen fixation and production of growth promoting substances together might have contributed to maximum shoot and root length and the biomass.

In conclusion, among the *Azospirillum* strains selected for the study, AZ204 and FT326 proved to be the best microbial inoculants respectively for sorghum and finger millet as indicated by their influence on various biometric and biochemical parameters. External application of IAA was not as effective as that of *Azospirillum* inoculation. This could be because of a steady release of IAA by the microbes which is better utilized by the plants as against three concentrated

treatments which might not have been absorbed effectively by the roots. The nitrogen fixed might be transported through the ureide pathway in both the plants. DT recorded better biometric observations than DS. But regarding the rest of the parameters assesssed no definite conclusion could be made since consistent difference between the varities was not realised. This needs further investigation by creating drought conditions. Variation in the enzyme activities could be attributed due to environmental factors which requires a detailed investigation.

Use of biofertilizers in agriculture is an area with great potential. The elucidation of ideal conditions of biofertilizer exposure on growth and biochemical parameters of agriculturally and ecologically important plants would be highly advantageous. Therefore, further studies to evaluate the optimum conditions should be conducted. This would definitley help an agriculture based nation like India take giant strides forward.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. Studies with temperate and tropical plants can be conducted to trace the pathway of nitrogen fixation.

2. Environmental factors such as pH, temperature, water stress, salt stress and high metal toxicity on the enzymes involved in nitrogen assimilation can be studied.

3. The strains may be genetically modified for higher nitrogen fixation and for growth hormone production.

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APPENDIX

APPENDIX-I

ESTIMATION OF GLUTAMINE SYNTHETASE
(Shapiro and Stadtman,1970)

PRINCIPLE

Glutamine synthetase catalyses the γ-glutamyl transfer reaction. It can be assayed by measuring the production of γ-glutamyl hydroxamate. This is then made to react with ferric chloride to produce brown colour in acidic medium.

REAGENTS

Prepared the following reagents in 20mM Tris-HCI buffer (pH 8.0). The concentration of stock solution is indicated in parentheses.

L-Glutamine - 0.2M (700mg/ 12ml)

Sodium Arsenate - 20mM (500mg/10ml)

MnCl2 - 3mM(83mg/10ml)

Hydroxylamine - 50mM(278mg/10ml)

Adenosine diphosphate - 1mM(40mg/10ml)

Ferric chloride reagent- Dissolved 10g trichloro-acetic acid and 8g ferric chloride in 250ml of 0.5N hydrochloric acid.

γ-Glutamyl hydroxamate - 10mM or 5mM

Enzyme Extract-Extracted 1g plant material in 5ml of 50mM imidazole-acetate buffer (pH 7.8) containing 0.5mM EDTA, 1mM dithiothreitol, 2mM MnCl2 and 20% glycerol at 4 C.

Centrifuged at 10,000g for 30min. Whenever purification was required, the enzyme was precipated with ammonium sulphate at 60% saturation. Resuspended the precipitate in extraction buffer. Desalted over sephadex G25.

PROCEDURE

Pipetted out the reagents as mentioned in the following order: glutamine 2.0ml + sodium arsenate 0.5ml + MnCl2 0.3ml + hydroxylamine 0.5ml + ADP 0.5ml + Enzyme extract 0.2ml to the experimental tubes.For the blank, added 2ml of 20mM Tris-HCI buffer instead of glutamine.Incubated the reaction mixture for 30min at 37°C. Stopped the reaction by adding 1ml of ferric chloride reagent.Measured the brown colour developed at 540nm.

Prepared a range of standards containing 100-500ug ɣ-glutamyl hydroxamte in 4ml buffer solution and developed the colour by adding 1 ml of ferric chloride reagent.

CALCULATION

The amount of ɣ-glutamyl hydroxamate formed in the reaction was found using the standard graph. Expressed the enzyme activity as nanomole ɣ-glutamyl hydroxamate formed per min per mg protein.

APPENDIX -II

ESTIMATION OF GLUTAMATE SYNTHASE
(Tempest et al., 1970)

PRINCIPLE

Glutamate synthase was assayed spectrophotometrically by

recording the rate of oxidation of NADPH or NADH as indicated by a change in absorbance at 340 nm following the addition of enzyme extract.

REAGENTS

50mM Tris Hcl buffer (pH 7.6)

Prepared the following reagents in Tris HCl buffer .

Glutamine - 5mM (36.5 mg/10 ml), 2-oxoglutarate - 5mM (36.5 mg/10 ml), NADH- 0.25mM (10 mg/ml).

Enzyme extract: Extracted 1g of the plant material with 5ml of 100mM phosphate buffer pH 7.5 containing 1mM disodium EDTA, 1mM dithioerythritol and 1% poly vinyl pyrolidine (PVP) and centrifuged at 10,000 g for 30 minutes at 4°C. Collected the supernatant and used it for enzyme assay.

PROCEDURE

Glutamine 1.0ml + 2-oxoglutarate 1.0ml+ NADH 1.0ml + enzyme extract 0.2ml + buffer 1.8ml was added to the experimental tubes. Omitted 2-oxoglutarate in the blank, instead added 1.0ml buffer.Incubated for 15 - 60 minutes at 37°C. Recorded the change in absorbance at 340nm.

CALCULATION

The amount of NADH oxidised was calculated from the molar extinction co-efficient. Activities were expressed as nanomole of NADH oxidised per min/mg protein.

Nanomole of NADH oxidised / min / mg protein

$$= \frac{A340 \times \text{Volume of assay solution} \times 1000}{6.22 \times \text{time of incubation (min)} \times \text{mg of protein in enzyme extract.}}$$

APPENDIX- III

ESTIMATION OF GLUTAMATE DEHYDROGENASE
(Doherty, 1970)

PRINCIPLE

Glutamate dehydrogenase was assayed by following the oxidation of the reduced co-enzyme NADH (or) NADPH. These reduced co-enzymes absorb light at 340nm, which in most biological systems is uniquely uncluttered with interfering absorption by other compounds. Thus even in crude extracts the absorption of NADH at 340 nm can be easily detected. The molar extinction coefficient of NADH at 340nm is 6.22×10^3, or one micromole NADH per ml has an absorbance of 6.22.

REAGENTS

0.1M Potassium phosphate buffer (pH 7.5)

0.1M 2 - oxoglutarate

0.1M Ammonium chloride

NADH, 10 mg/ml

Enzyme extract:Followed the method as given under GOGAT.

PROCEDURE

Phosphate buffer 1.0ml + 2-oxoglutarate 0.3ml +ammonium chloride 0.5ml + NADH 0.12ml + enzyme extract 0.2ml and water 8.0ml were added to the experimental tubes. Added 0.3 ml of water in the blank instead of 2-oxoglutarate. Incubated the reaction mixture at 37 C for 15-30 min.Recorded the change in absorbance at 340 nm.

CALCULATION

The amount of NADH oxidised was calculated from the molar extinction co-efficient. Activities are expressed as n mole NADH oxidised / min / mg protein.

Nanomole of NADH oxidised / min / mg protein

$$= \frac{A340 \times \text{Volume of assay solution} \times 1000}{6.22 \times \text{Time of incubation (min)} \times \text{mg protein in enzyme extract used.}}$$

APPENDIX-IV

ESTIMATION OF ASPARTATE AMINO TRANSFERASE
(Bergmeyer and Bernt, 1974)

PRINCIPLE

Aspartate aminotransferase catalyses the reversible interconversions between glutamate and asparatate and their 2-oxo analogues. The oxaloacetic acid is measured colori metrically by reaction with 2,4- dintrophenylhydrazine giving a brown-coloured hydrazone after the addition of 0.4 N sodium hydroxide.

REAGENTS

Phosphate buffer, pH 7.4: Added 11.3g dry anhydrous disodium hydrogen phosphate and 2.7g dry anhydrous potassium dihydrogen phosphate in one litre volumetric flask and made up to the mark with water. Checked the pH and stored at 4 °C.

Substrate Solution: Dissolved 13.3g DL-aspartic acid in minimum amount of 1N sodium hyroxide and prepared a solution

with pH 7.4(about 90ml is required). Added 0.146g 2-oxoglutarate and dissolved it by adding a little more sodium hydroxide solution. Adjusted to pH 7.4 and then made upto to 500ml with phosphate buffer. Divided into 10ml portions and stored frozen at-15°C.

Pyruvate Standard (2mM): Dissolved 22mg sodium pyruvate in 100ml water in a standard flask.

2,4-Dinitrophenyl Hydrazine (DNPH) : Dissolved 19.8mg dinitrophenylhydrazine in 10 ml concentrated hydrochloric acid and made upto 100ml with water. Stored it in an amber bottle at room temperature.

Sodium Hydroxide 0.4N: Dissolved 16g sodium hydroxide in one litre water.

Enzyme Extract: Prepared the crude extract by grinding the plant tissue in 0.2M potassium phosphate buffer, pH 7.5 in a homogenizer for 2min. Passed the slurry through eight layers of cheese cloth and then centrifuged at 25,000g for 15 min to get the enzyme fraction.

PROCEDURE

Warmed 0.5 ml of substrate solution in a waterbath at 37°C for 3 min. Added 0.2 ml enzyme extract and mixed gently. Incubated for 60 min at 37°C. Removed the tubes from the bath and immediately added 0.5ml dinitrophenyl hydrazine solution and mixed well. Mixed 0.5ml substrate with 0.5ml DNPH solution and then added 0.1ml of enzyme extract for control. Allowed the DNPH to react for 20min at room temperature. Added 5ml of 0.4N sodium hydroxide, mixed well and left for further 10

min. Recorded the absorbance at 510nm. Pipetted out pyruvate standard 0.05 to 0.20ml and made up to 0.2ml. Added 0.5ml susbstrate and 0.5ml DNPH solution. For blank mixed 0.5 ml susbstrate , 0.2ml water and 0.5ml DNPH solution. Then developed the colour as above.

CALCULATION

The pyruvate formed by enzyme is responsible for the absorbance difference between test and control. The pyruvate in standard produces the difference between standard and blank. Express the enzyme activity as micromole of pyruvate formed per min per mg protein.

APPENDIX-V

ESTIMATION OF ASPARAGINE SYNTHETASE
(Ravel, 1970)

PRINCIPLE

Aspartate reacts with hydroxylamine in the presence of ATP and Mg to form aspartyl hydroxamic acid. This is then determined colorimetrically with ferric chloride reagent.

REAGENTS

Tris-NH_2OH-$MnCl_2$ Solution: Weighed 2.4g tris base (0.25M), 11.1g hydroxylamine hydrochloride (2M) and 476mg $MnCl_2.4H_2O$ and dissolved in about 40ml water. Adjusted to pH 6.4 by the addition of 8N KOH. Made up the final volume to 80ml. Prepared this reagent fresh.

ATP, 0.1M: Dissolved 551mg ATP disodium salt in 10ml water and neutralised it. Prepared fresh solution.

L-Aspartic Acid,0.1M,pH 6.4: Dissolve 133mg of L-Aspartic acid in 10ml water and adjusted to pH 6.4.

Ferric chloride Reagent: Mixed 125ml of 20% TCA, 50g $FeCl_3.6H_2O$ and 28ml of Conc.HCl and made up to a final volume of 500ml with water. This is a stable reagent.

Enzyme Extract: Homogenized 1g chilled plant materials in 10ml of 100mM Tris-HCl buffer pH 8.5 containing 15% (by vol) glycerol, 56mM mercaptoethanol and 4mM KCN. Passed the homogenate through four layers of cheese cloth and centrifuged the filtrate at 15,000g for 30min.

PROCEDURE

Pipetted out 0.4ml of the Tris-NH_2OH-$MnCl_2$ solution, 0.1ml of ATP, 0.2ml of enzyme extract, 0.2ml of L-Aspartic acid and 0.1ml distilled water in the order given. Incubated at 37°C for 10 min. Stopped the reaction by the addition of 3ml ferric chloride reagent. Centrifuged and removed the supernatant solution for measurement. Measured the absorbance of the supernatant solution at 540nm. Used 1ml water and 3ml of ferric chloride reagent for zero adjustment. Set up a control in which aspartate was omitted from the reaction mixture. Prepared a standard curve by taking 0-2.5 micromoles of β-aspartyl hydroxamate.

CALCULATION

Expressed the enzyme activity as micromoles of β-aspartyl hydroxamate formed per mg protein per min. The concentration of β-aspartyl hydroxamate can also be obtained by multiplying the assay absorbance value by a factor of 6.1, if the synthetic one is not available for standardization.

APPENDIX VI

ESTIMATION OF NITRATE REDUCTASE
(Hageman and Reed, 1980)

PRINCIPLE

Nitrate reductase (NR) is capable of utilizing the reduced form of pyridine nucleotides, flavins or benzyl viologen as electron donors for reduction of nitrate to nitrite. NADH - dependent nitrate reductase is most prevalent in plants. NR activity is commonly measured by colorimetric determination of nitrite produced.

REAGENTS

Potassium Phosphate Buffer 0.1M (PH 7.5)

Potassium Nitrate 0.1M.

NADH 2 mM.

Sulphanilamide, 1% (w/v).

N-(1-naphthyl)- ethylenediamine dihydrochloride, 0.02%.

Potassium nitrite Standard Solution (0.01M): Dissolved 851mg pure nitrite in 100ml water in a standard flask. Diluted 10ml of this solution to 100ml and used as working standard solution.

Enzyme Extract : Homogenized a weighed quantity of the plant material in a known volume of medium (6ml for 1g fresh tissue) containing 1mM EDTA, 1-25mM cysteine and 25mM potassium phosphate adjusted to a final pH 8.8 with KOH. Filtered through four layers of cheese cloth and centrifuged for 15 min at 30,000g. Decanted the supernatant through glass wool and used for assays. Extracted under ice-cold conditions.

PROCEDURE

Pipetted out 0.5 ml phosphate buffer (pH 7.5) in a test tube. Added 0.2ml potassium nitrate solution,0.4ml NADH solution and 0.7ml water. Initiated the reaction by the addition of 0.2ml enzyme extract. Set up a control in the same way but with water instead of enzyme extract. Incubated at 30°C for 15min. Terminated the reaction by the rapid addition of 1ml sulphanilamide followed by 1ml napthyl ethylenediamine reagent. Waited for 30min. Measured the absorbance at 540nm. Prepared a standard graph with sodium nitrite. Pipetted out different known aliquots of potassium nitrite standard solution into a series of test tubes and made up the volume in each tube to 2ml by adding water. Developed the colour and read in a colorimeter at 540nm.

CALCULATION

Activity is expressed as nanomole nitrite produced per min per mg protein (or per g fresh tissue).

APPENDIX-VII

ESTIMATION OF NITRITE REDUCTASE
(Vega et al.,1980)

PRINCIPLE

Nitrite is reduced to ammonia without the liberation of free intermediates by nitrite reductase. The disappearance of nitrite is measured in the reaction. Reduced methyl viologen or NADH is used as electron donor.

REAGENTS

Tris-HCI Buffer 0.5M (pH7.5)

Sodium Nitrite Solution: Dissolved 43.2mg $NaNO_2$ in 20ml distilled water.

Methyl Viologen Solution: Dissolved 60.1mg methyl viologen in 20mL water.

Sodium Dithionite-Bicarbonate Solution: Dissolved 250mg each of sodium bicarbonate and sodium dithionite in 10ml water.

Enzyme Extract: Homogenized the leaf tissue (10g/100mL) with Tris-HCI buffer (pH 7.5) in a Waring blender at high speed for 3min and forced the homogenate to flow through eight layers of cheese cloth at 4°C. Used the filtrate as enzyme source.

PROCEDURE

Prepared a reaction mixture by mixing 6.25ml of Tris- HCl buffer, 2ml of sodium nitrite solution, 2ml methyl viologen solution and 14.75ml water. Pipetted out 1.5ml reaction mixture and 0.3ml of enzyme preparation into a test tube. Set up a blank without the enzyme. Started the reaction by adding 0.2ml of freshly prepared dithionite-sodium bicarbonate solution.

Incubated for 15min at 30°C. Stopped the reaction by vigorous shaking (vortex mixer) until blue colour disappears. Used a 20ml aliquot for nitrite determination. Added 1ml of sulphanilamide followed by 1ml napthyl ethylenediamine reagent. Waited for 30 min. Measured the absorbance at 540nm.

Prepared a standard graph with sodium nitrite. Estimated the amount of nitrite disappeared using blank as reference.

CALCULATION

The enzyme activity is expressed as the amount of nitrite (nM) reduced per min per mg protein.

APPENDIX VIII

ESTIMATION OF ASPARAGINASE

Nesslerization method
(Farnden and Robertson, 1980)

PRINCIPLE

Enzyme activity was measured by determination of the rate of ammonia liberation.

REAGENTS

L-Asparagine-100mM

Potassium phosphate buffer-50mM,pH 8.0.

Trichloro acetic acid-20% w/v.

Nessler's reagent-Weighed 100g of mercuric iodide and 70g of potassium iodide in a 1L volumetric flask, added about 400ml of water, and stirred until dissolved. Dissolved 100g of NaOH in about 500ml water, cooled, and added it with constant stirring to the mixture in the flask, then diluted with water to 1L mark. Allowed any sediment formed to settle and decanted the supernatant for use.

PROCEDURE

Reaction mixture contained 0.5ml of 100mM L-asparagine, 50mM phosphate buffer (pH8.0), and enzyme in phosphate buffer

to give a final volume of 1.5ml. Mixtures were incubated for various times (0-30min) and the reaction was stopped by the addition of 20% trichloro acetic acid (0.1ml). The samples were centrifuged (6000g for 5 min) and the supernatant solution was added to 6.0ml of distilled water. Nessler's reagent (1 ml) was added and the absorbance read at 480nm after 10 min. Absorbances were converted into micromoles of ammonia from a calibration graph prepared using 80-400µg of 2mM ammonium sulphate. Activity is expressed as nanomoles of ammonia produced per min per mg protein.

APPENDIX- IX

ESTIMATION OF GLUTAMINASE

Nesslerization method
(Farnden and Robertson, 1980)

PRINCIPLE

Enzyme activity was measured by determining the rate of ammonia liberation.

REAGENTS

L-Glutamine-100mM

Potassium phosphate buffer-50mM, pH 8.0

Trichloro acetic acid-20% W/V

Nessler's reagent: (As in Asparaginase assay).

PROCEDURE

Reaction mixture contained 0.5ml of 100mM L-glutamine, 50mM phosphate buffer,and enzyme in phosphate buffer to give a final volume of 1.5ml. Mixtures were incubated for various

times (0-30min) and the reaction was stopped by the addition of 20% trichloro acetic acid (0.1ml). The samples are centrifuged (6000g for 5 min) and the supernatant solution was added to 6ml of distilled water. Nessler's reagent (1.0ml) was added and the absorbance read at 480nm exactly after 10min to avoid non-enzymatic release of ammonia from glutamine. Absorbances were converted into micromoles of ammonia from a calibration graph prepared using 80-400µg of ammonium sulphate. Activity is expressed as nanomoles of ammonia produced per min per mg protein.

APPENDIX-X

ESTIMATION OF UREASE

Nesslerisation method (Sumner,1955)

PRINCIPLE

Urease catalyses the hydrolysis of urea to carbondioxide and ammonia. This is then treated with Nessler's reagent and the yellow colour developed read at 495nm in a colorimeter.

REAGENTS

30mM urea

10% TCA

Nessler's reagent:(As in Asparaginase assay).

0.2M Phosphate buffer

PROCEDURE

Incubated 0.5ml of buffer,0.1ml substrate and 0.1ml enzyme for 15 min at 37°C. The reaction was stopped by the

addition of 0.2ml of 10% TCA. Along with these, controls were also set up to which the enzyme was added at the end of the incubation period. Allowed to stand for 30min and then centrifuged.0.5ml of the supernatant from each tube was transferred to other tubes.

1.0ml of Nesslers reagent was added and the volume made upto 10.0ml with distilled water. The colour developed was read at 495nm after 5 min.

APPENDIX- XI

ESTIMATION OF XANTHINE DEHYDROGENASE
(Boland,1981)

PRINCIPLE

XDH activity was assayed spectrophotometrically at 340nm as the xanthine dependent NAD occurs.

REAGENTS

Tris-HCl buffer-100mM

Xanthine-0.3mM

NAD-1mM

DTT-1mM

PROCEDURE

The assay mixture contained 0.4ml of Tris buffer, 0.2ml of DTT,0.3ml of NAD,0.5ml of xanthine and 0.1ml of enzyme.The mixture was incubated for 15 min at 30° C. Boiled enzyme was added to the control.Recorded the change in absorbance at 340nm.

APPENDIX-XII

ESTIMATION OF ALLANTOINASE
(Vogels and van der Drift, 1970)

PRINCIPLE

Allantoinase catalyses the hydrolysis of allantoin to allantoate. Allantoate released was determined as glyoxylate, following acid hydrolysis of allantoate released. Glyoxylate reacts with phenylhydrazine hydrochloride to form phenylhydrazone. This hydrazone on reaction with potassium ferricyanide produces a red colour which is measured at 520nm.

REAGENTS

Tris buffer 0.1M

Allantoin 15mM

Phenylhydrazine hydrochloride 0.3%

Potassium ferricyanide 1.6 %

Enzyme extract: Plant tissue was homogenized in the presence of about five times its weight of 0.1M potassium phosphate huffer, pH 7.5 containing 1mM EDTA and 1mM dithiothreitol in a glass homogenizer. The homogenate was squeezed through four layers of gauze. The filtrate was centrifuged at 12,000g for 30 min and the supernatant was used for enzyme assays.

PROCEDURE

23.7mg of allantoin substrate and 9ml of 0.1M Tris buffer were adjusted to pH 7.5 with HCl. With the addition of 1ml of sample to be assayed, this yielded a final substrate

concentration of 15mM and did not alter the pH of the mixture. Reaction temperature was 25 °C and incubation was usually for not more than 30min. After specified reaction times, 2ml aliquots were removed by pipette and transferred immediately into tubes containing one drop of conc.HCl to stop the reaction. 1ml of 0.3% phenylhydrazine hydrochloride was added to each tube; the tubes were placed in boiling water for 2 min, then plunged into ice-water. To each tube was added 1.2ml of conc.HCl and 1.0ml of 1.6% potassium ferricyanide; the tubes were mixed well and filtered. The clear, red filtrate was read at 540 nm after 15min. Controls for color due to endogenous glyoxylate and other non-specific reactions were obtained by boiling homogenate for 10 min prior to analysis. Allantoinase activity was determined by measuring the glyoxylic acid freed from enzymatically derived allantoic acid using a standard curve prepared from known quantities of glyoxylate. Specific activity is expressed as nanomoles of glyoxylic acid formed per mg protein per min. Protein was determined by Bradford's method.

APPENDIX-XIII

ESTIMATION OF URICASE
(Muller and Moller,1969)

PRINCIPLE

Uricase activity was assayed by determining the decrease in absorbance at 292nm due to enzymatic oxidation of uric acid.

REAGENTS

Tricine-KOH buffer - 0.3mM

Uric acid-0.3uM

PROCEDURE

The assay mixture contained 0.3mM of tricine-KOH buffer (pH 7.0), 0.3µM of urate (prepared daily by dissolving uric acid in a minimal amount of 0.02M KOH and then adjusting the volume of the solution with 0.1M tricine buffer),and 100 to 400 µg of protein, in a total volume of 3.0ml.After incubating the protein with buffer for 3 to 5 min, the reaction was started by adding urate and followed for at least 10min at 30°C. The molecular extinction coefficient of uric acid at 292nm was taken as 1.22×10^3 .

One unit of enzyme was defined as the amount causing decomposition of one umole of substrate per minute under the above conditions at 30°C. Protein was determined by the method of Bradford.

APPENDIX-XIV

ESTIMATION OF UREIDES
(Young and Conway, 1942)

PRINCIPLE

Allantoin in the presence of alkali forms allantoic acid. In turn allantoic acid in acidic medium forms glyoxylic acid and urea. Glyoxylic acid reacts with phenylhydrazine hydrochloride to from phenylhydrazone. This hydrazone on reaction with potassium ferricyanide produces a red colour which is measured at 530nm.

REAGENTS

Sodium hydroxide, 0.5N

Hydrochloric acid, 0.65N

Phenylhydrazine hydrochloride, 0.33%

Concentrated hydrochloric acid, 10N.

Allantoin Stock Standard - Dissolved 50mg allantoin in 100ml distilled water.

Working Standard - Diluted 1ml to 10ml (50µg/mL)

Phosphate buffer, 0.05M, pH 7.5

Potassium ferricyanide, 1.67%

PROCEDURE

Excised the particular tissue of the plant in which allantoin has to be measured. Ground with 10ml of 0.05M phosphate buffer (pH 7.5) and 0.05g polyclar AT per gram of tissue in a glass homogenizer immersed in boiling water. Centrifuged the homogenate at 10,000g for 5 min and collected the supernatant. Pipetted out 0.5ml of the supernatant into a test tube. Diluted to 2.5ml with distilled water. Added 0.5ml of 0.5 N sodium hydroxide. Placed the tube in vigorously boiling water for 7 min. Removed the tube and brought to room temperature by placing in a waterbath. Added 0.5ml of 0.65N hydrochloric acid. Then added 0.5ml of phenylhydrazine solution. Shook well and placed the tubes in a boiling water bath for exactly 2 min. Immediately plunged the tube into an ice bath and chilled it for 20 min. Removed from the bath, added 2 ml of already chilled 10N hydrochloric acid and 0.5ml of potassium ferricyanide solution. Mixed the contents thoroughly. After 30

min measured the absorbance in each tube at 530nm in a spectrophotometer. Prepared a standard graph with 0 to 40µg concentration of allantoin.

CALCULATION

Calculated the amount of ureides (as allantoin) in the unknown samples using the standard curve andexpressed as mg ureides/g f.wt.

APPENDIX-XV

ESTIMATION OF IAA IN PLANT TISSUE
(Gorden and Paleg,1957)

REAGENTS

HCl IN, 6N

Sodium bicarbonate

Ethyl ether

Methyl alcohol

Salper's reagent:Mixed 1ml of 0.5M FeCl3 in 50ml of 35% HClO4. Prepared fresh.

Extraction of IAA from plant tissues.

Frozen the tissue (200-250g) immediately after collection. Cut into pieces of 1-2 cm and homogenized with prechilled absolute alcohol (alternatively either methanol or ethyl acetate may be used) in a blendor. Filtered through cheese cloth and repeated the extraction with the residue. Reduced the volume of the pooled filtrate on a rotary evaporator to about 50ml with reduced pressure at 40-45°C. Adjusted the pH of the aqueous residue to 3 with 1 N HCl and extracted three to four times with equal volumes of

peroxide free ether over a period of an hour using a separating funnel. Combined the ether extracts and partitioned with 4x100ml of sodium bicarbonate solution to separate acidic and neutral auxins. The ether layer contains the neutral auxins while the bicarbonate fraction contains acidic substances. Acidified the alkaline fraction to pH 3 with 6 N HCl and extracted with 4x100ml of peroxide free ether. Pooled the ether extract which contains acidic auxins. Evaporated both the ether extracts containing neutral and acidic auxins separately on a rotary evaporator to dryness. Dissolved the residue in minimum quantity of methanol.

PROCEDURE

To 1ml of extract containing IAA, added 2 ml of Salper reagent. Added the reagent dropwise but rapidly with continuous agitation. Incubated the samples in the dark (35 min for diethyl ether dissolved IAA;60 min for methanol sample). The pink colour is stable. Measured its absorbance at 535nm against a solvent reagent blank. If the intensity is deep, diluted the reaction mixture with the solvent. From a standard curve drawn from known concentrations of IAA, find out the quantity of IAA in the extract.

APPENDIX-XVI

ESTIMATION OF GIBBERELLINS IN PLANT TISSUES
(Graham and Henderson,1961)

PRINCIPLE

Gibberellins reacts with phosphomolybdic acid and the latter is reduced to molybdenum blue and the absorbance measured at 780nm.

REAGENTS

Methanol

Ethylacetate

Hcl, 1.6 and 3.2 M

Sodium bicarbonate, 0.48M

Phosphomolybdic acid reagent: Dissolved 35g of molybdic acid and 5g of sodium tungstate in 200ml of 10% sodium hydroxide solution contained in a beaker, and then added 200 ml of distilled water. Boiled the contents vigorously for 40 min to remove any ammonia present in the molybdic acid. Cooled the mixture to room tempreature and diluted to 350ml with distilled water. Added 125ml of conc(85%) phosphoric acid to the mixture and made up the volume to 500ml with distilled water.

Extraction of gibberellins from plant tissues:

Homogenized 250g of shoot portions in blendor at 4°C with suitable aliquot of 80 per cent pre-chilled methanol(10ml/g fresh tissue). Transferred the homogenate to a beaker and allowed it to stand overnight at 4°C. Filtered through Buchner funnel using Whatman No.41 filter paper. Reextracted the residue twice with ethanol in cold. Washed the residue after the final extraction with methanol until the residue is colourless. Combined the methanol extracts and evaporated the methanol at 40°C using a rotary evaporator. The water phase of the extract which was frozen was thawed it to the room temperature and filtered through whatman No.41 filter paper. This removed chlorophyll and precipitated proteins. Stirred the aqueous phase of the extract with PVP,

acidified it with 3.2 M HCl to pH 2.5 and extracted thrice with equal volumes of ethyl acetate using separating funnel. Saved the aqueous phase for the extraction of bound gibberellins. Combined the ethyl acetate extracts and partitioned it with 0.48 M sodium bicarbonate. Adjusted the pH of the bicarbonate phase to 2.5 with 1.6M HCl and partitioned 2 or 3 times again with ethyl acetate. Combined the ethyl acetate fractions and evaporated to dryness in a rotary evaporator at 40°C. Dissolved the residue in minimum quantity of methanol. This methanol extract contains 'free' acidic fractions of gibberellin-like substances.

PROCEDURE

Pipetted 1ml of the gibberellin extract into 25ml volumeric flasks and added 15ml of phosphomolybdic acid reagent to it. Mixed the contents of the flask thoroughly and place the flasks in a boiling water bath for 1 hr. After 1hr, removed the flasks and rapidly immersed them in an ice water bath. After cooling to room temperature, made up the volume to 25ml with distilled water. Measured the absorbance at 780nm in a colorimeter using distilled water blank. Maintained reagent blank and subtracted the absorbance of the reagent blank from the values obtained with samples.

APPENDIX - XVII

ESTIMATION OF TOTAL PROTEINS
(Bradford, 1976)

REAGENTS

Bradford's reagent: 100mg coomassie brilliant blue G-250 + 50ml of 95% ethanol + 100ml of concentrated o-phosphoric acid. Dye was mixed in ethanol and phosphoric

acid and the volume was made upto 200 ml with water and stored at 4°C.

PROCEDURE

The protein extract of volume 0.1 ml was taken and 5.0 ml of Bradford's reagent was added and mixed well. The blue colour developed was read in a spectrophotometer at 595 nm. The sample protein concentration was found out by using the standard graph prepared with Bovine serum albumin. The protein contents were expressed in mg/g.

APPENDIX-XVIII

ESTIMATION OF TOTAL PHENOLS

Folin- Ciocalteu method (Bray and Thorpe, 1954)

PRINCIPLE

Estimation of phenols with Folin-Ciocalteu reagent is based on the reaction between phenols and an oxidizing agent phosphomolybdate which results in the formation of a blue complex. The intensity of the colour is measured in colorimeter.

REAGENTS

20% Sodium carbonate

Folin-Ciocalteu reagent: Dissolved 100 g sodium tungstate and 25 g sodium molybdate in 700ml water in 1 L flask. Added 50ml 85% ortho phosphoric acid and 100ml conc HCl and boil and refluxed gently for 10 hr. Cooled and added 250 g lithium sulphate dissolved in 50ml water and 4-5 drops of liquid bromine. Boiled the mixture without condenser for

15 min to remove the excess bromine. Cooled, diluted to volume with water and filtered. The reagent should be golden yellow in colour, stored in brown bottles. It is stable for many months. Just before use, diluted 1 volume of this stock solutions with 2 volumes of water.

PROCEDURE

Pipetted 1 ml of the extract in a graduated test tube, added 1 ml of folin-Ciocalteu reagent followed by 2 ml of sodium carbonate solution. Shook the tube and heated in a boiling water bath for exactly 1 min. Cooled under a running tap. Diluted the blue solution to 25 ml with water and measured its absorbance at 650 nm in a colorimeter. (If any precipitate occurred, filtered or centrifuged the solution before measuring its absorbance.) Read the unknowns from a standard curve made from different concentrations of catechol or caffeic acid. A blank containing all the reagents minus plant extract was used to adjust the absorbance to zero.

APPENDIX - XIX

ESTIMATION OF ORTHO DIHYDRIC PHENOL
Arnow's Method (Mahadevan, 1966)

PRINCIPLE

Arnow's reagent specifically reacts with ortho dihydric phenols by producing a pink coloured complex the intensity of which is measured in a colorimeter at 515nm.

REAGENTS

Arnow's reagent: Dissolved 10 g of sodium nitrite and 10g of sodium molybdate in 100 ml of distilled water. Stored

the reagents in a brown bottle. The reagent is stable for a year.

HCl, 0.5 N

NaOH, 1 N

PROCEDURE

Pipetted out 1 ml of the alcolhol extract of plant tissues into a test tube, added 1 ml of 0.5 N HCl,1 ml of Arnow's reagent, 10 mL of distilled water and 2 ml of 1 N NaOH. Soon after the addition of the alkali, pink colour appeared. Maintained a reagent blank without the extract. If the colour intensity was high, diluted to 25 ml and read the absorbance of the solution at 515 nm. Calculated the OD phenols present in the samples from a standard curve prepared with catechol.

APPENDIX - XX

ESTIMATION OF TOTAL NITROGEN

Microkjeldahl method (Humphries, 1956)

PRINCIPLE

Total nitrogen is the sum of ammonia nitrogen and organic nitrogen. This does not include nitrite nitrogen and nitrate nitrogen. Nitrogen of organic matter is converted to ammonium sulphate when treated with sulphuric acid. An excess of alkali is then added to liberate ammonia and distilled. The distillate is titrated with standard sulphuric acid after absorption in boric acid solution.

REAGENTS

1.Diacid - 4:1 ratio of sulphuric acid and perchloric acid.

2.Mixed indicator - Dissolved 0.5 g bromocresol green and 1g of methylred in 100ml ethylalcohol.

3.N/50 sulphuric acid

4.40% Sodium hydroxide

5.2% Boric acid

PROCEDURE

Ground the dried plant sample and made it a fine powder. Took 0.5 g of sample in a mircrokjeldahl flask with 12ml of diacid. Digested the sample over a sand bath. Made up the volume to 100 ml with distilled water. Pipetted out 10ml of the aliquot into a microkjeldahl distillation apparatus. Kept at the delivery end, 10 ml of 2 per cent boric acid with mixed indicator in a 100 ml beaker. Added 10 ml of 40 per cent sodium hydroxide into the microkjeldahl di stillation apparatus and steamed the distillate until a blue colour was reached. After distillation, titrated against N/50 sulphuric acid until a red wine colour was got.

CALCULATION

$$\text{Nitrogen content (g \%)} = \frac{0.00028 \times \text{titre value} \times 100 \times 100}{10 \times 0.5}$$

APPENDIX - XXI

ESTIMATION OF PHOSPHORUS
(Jackson, 1973)

PRINCIPLE

Phosphorus reacts with ammonium molybdate and ammonium

metavanadate in acidic medium to give a yellow coloured product. The intensity of the yellow colour is directly related to the concentration of phosphorus and is read at 420 nm in a colorimeter.

REAGENTS

1. Triple acid mixture: Concentrated nitric acid- perchloric acid - concentrated sulphuric acid (3:2:1).
2. Barteu Reagent

   Solution A: 25 g of Ammonium molybdate/400ml of warm water.

   Solution B: 1.25 g of Ammonium metavanadate/300ml boiling water.

   Solution A was added to solution B and the volume was made upto 1000ml.

3. Phosphorus standard- 0.2195g of potassium dihydrogen phosphate with 25ml of 7N sulphuric acid made upto a litre with water. This consists of 50ppm phosphorus which was diluted to give 5 ppm working standard solution.

PROCEDURE

5.o g of the finely powdered sample was taken in a 100 ml kjeldahl flask. Added 25ml of the triple acid mixture and heated for four hours. Cooled and made up the solution to 100 ml with distilled water. From the acid extract pipetted out 5.0 ml of the aliquot into a 25ml volumetric flask. Introduced a bit of red litmus paper. Neutralised with ammonia solution until litmus paper turned blue. Again acidified it with concentrated nitric acid until litmus paper turned red. Added 5.0 ml of the Barteu reagent. Made up the

volume to 25 ml with distilled water. After 30 minutes the intensity of yellow colour developed was read at 420 nm in a colorimeter. Using the standard phosphorus curve constructed with the 50 ppm stock phosphorus solution, the phosphorus concentration in the sample was read.

CALCULATION

Phosphorus content

$$(g\ \%) = \frac{\text{Phosphorus concentration in ppm} \times 25 \times 100}{10 \times 5 \times 0.5}$$

APPENDIX - XXII

ESTIMATION OF POTASSIUM
(Jackson, 1973)

PRINCIPLE

In flame photometry, the solution under test is passed under carefully controlled conditions as a very fine spray in the air supply to a burner. In the flame, the solution evaporates and the salt dissociates to give neutral atoms. A very small proportion of these move into a higher energy state. When these excited atoms fall back to the ground state, the light emitted of characteristic wavelength is measured. Potassium is estimated at 770nm.

REAGENTS

1. Triple acid mixture: As in Phosphorus

2. Potassium stock solution : 1.907g of potassium chloride was dissolved in 100ml of distilled water to get a 1000 ppm solution of potassium.

3.Working standard solution : 100ml of 1000 ppm potassium solution was diluted to 1 litre to get a 100 ppm solution of potassium.

PROCEDURE

5.0 g of the sample was taken in a 100ml kjeldahl flask. Added 25ml of the triple acid mixture and heated for four hours. Cooled and made up the solution to 100ml with distilled water. 5.0 ml of the acid extract was pipetted out into a vial and the transmission was read in a flame photometer.

From the working standard, a series of potassium standards ranging from 0-100ppm were prepared and the percentage transmission was read in a flame photometer to construct a standard curve. Using the standard curve, the concentration of potassium was determined in the sample.

CALCULATION

$$\text{Potassium content (g \%)} = \frac{\text{Potassium concentration in ppm} \times 100 \times 100}{10 \times 5 \times 0.5}$$

APPENDIX-XXIII

ESTIMATION OF IAA FROM CULTURE FILTRATE
(Gorden and Paleg, 1957)

REAGENTS

HCl-1 N,6N.

Sodium bicarbonate- 5%

Ethyl ether

Methyl alcohol

Extraction of IAA from culture filtrate:

Centrifuged 100ml of the culture filtrate at 2,000 g

for 30 min to remove mycelial fragments and spores. Acidified the supernatant to pH 3 with 1 N HCl employing a pH meter and extracted with 100ml of ethyl ether in a separating funnel. Repeated the extraction with ether three more times, using 100ml for each extraction. Complete extraction was obtained when ether with the filtrate was kept at 4°C for at least 1 hr between each solvent change. Pooled the ether extracts and reduced the volume to about 25 ml either by flash evaporation or on a rotary evaporator at 35-40°C.

Partitioned the ether fraction with 25ml of sodium bicarbonate. Repeated this process 3 more times each time with 25 ml of bicarbonate. Acidified the bicarbonate fraction to pH 3 with 6 N HCl using a pH meter and extracted with 50 ml of ethyl ether. Repeated the extraction with 3x50 ml of ether. Evaporated the ether **in vacuo** and dissolved the residue in 2 ml of methanol. Stored in a vial at -5 to -10°C.

Salper's reagent: (Refer to Appendix XV).

PROCEDURE

To 1 ml of extract containing IAA, added 2 ml of Salper reagent. Added the reagent dropwise but rapidly with continuous agitaion. Incubated the samples in the dark (35 min for diethyl ether dissolved IAA;60 min for methanol sample). The pink colour is stable. Measured its absorbance at 535nm against a solvent reagent blank. When the intensity was deep, diluted the reaction mixture with the solvent. From a standard curve drawn from known concentrations of IAA, the quantity of IAA in the extract was found.