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INTERACTION OF VESICULAR ARBUSCULAR MYCORRHIZAE, HORMONES AND DROUGHT IN SOYBEANS

by

SHAZIA ATIQUE KHAN

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2003

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Abstract

Interaction of Vesicular Arbuscular Mycorrhizae, hormones and drought in soybeans (*Glycine max*)

By

Shazia Atique Khan

Advisor: Dr. Jack Valdovinos (Professor Emeritus)

Vesicular Arbuscular Mycorrhizae (VAM) has been known to help plants in different types of environmental situations. The purpose of this study was to determine growth and yield response of drought stressed soybean *Glycine max* to two different species of vesicular arbuscular mycorrhizae (*Glomus intraradiesces* and *Glomus fasciculatum*) in interaction with exogenous hormonal application and to compare the levels of endogenous ABA in VAM and non-VAM droughted plants. Two separate experiments were performed in 2x2x3 full factorial design for study of each VAM species. In the greenhouse plants were with foliar application of 10^{-6} M ABA or IBA solutions one week before exposure of drought stress. In third set of experiments designed as 2x2 factorial with VAM and drought as the main factors, endogenous levels of ABA of the VAM and non-VAM plants were estimated by ELISA under droughted and non-droughted conditions. Plants phosphorus content was also determined to study the relationship between ABA levels of the plants and phosphorus content. Both species of VAM increased vegetative growth as well as grain yield, as compared to non-VAM plants under drought stress. Measurements showed that the VAM infected plants had higher phosphorus content (Mean = 2.443 mgs/gm of plant shoot) than non-VAM drought stressed plants (0.787 mgs/gm 0f plant shoot). VAM infected plants had lower level of

ABA (mean = 0.342 picomoles/gm of the fresh weight of shoot) as compared to no-VAM plants (mean = 0.429 picomoles/gm of the fresh weight of shoot). The highest levels of ABA were found in drought stressed non-VAM plants and the lowest in VAM, non-droughted plants. Total seed weight of the VAM plants was in direct correlation with the phosphorus content. The comparison of the both species of VAM used in the study showed that *Glomus fasciculatum* had significantly better influence on the drought stressed soybeans than *Glomus intraradieces.*

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Chapter 1

1. Introduction and History

Agricultural crop productivity results from a complex interaction of plants with their environment. There is limited productivity of crops because, in plants, drought is of worldwide occurrence. Drought is always accompanied by deficits of major nutrients (e.g., phosphorus and nitrogen). In the southern parts of the United States soybean productivity is reduced by drought stress. Irrigation is not usually an economically viable option for most U.S. farmland on which soybean is grown (Boyer, 1982). This situation is more serious in developing countries where, in addition to other problems, poor irrigation facilities multiply the problem. Phosphorus deficiency is a major factor, because most unfertilized soils do not release phosphorus at a rate sufficient for the requirements of most crops. Due to the increasing demand for food by the growing world population, there will be a 50% increase in the demand for P fertilizer in the next twenty years (Bumb and Banante, 1996). Attempts to manage these constraints by irrigation and fertilization are unaffordable by the farmers in undeveloped and developing parts of the world. Moreover, these constraints are also associated with social and ecological problems such as water pollution and undesirable changes in nutrient availability of soil.

In soil, microorganisms of different types are abundant in the plant rhizosphere. These microorganisms, which include many different species of bacteria and fungi, play important roles in the physiology of plants. This is due to their participation in saprotrophic, pathogenic, and symbiotic root activities.

There is a specific type of bitrophic symbiotic association between some fungi and roots of plants called "mycorrhiza." It is a mutualistic relationship in which both partners benefit. The mycorrhizal association is of two major categories:

i) Ectomycorrhiza.

ii) Endomycorrhiza.

An **ectomycorrhiza** is a root/fungus association in which the fungus grows as a "mantle" on the surface of the root, which it surrounds. The hyphae grow between the cells of the root cortex to produce a network, a characteristic structure known as **Harting net**.

Endomycorrhiza is a plant/fungus association where the fungus is present inside the cell of the root producing septate and aseptate hyphae (Brown and King, 1987). The endomycorrhizae have been divided into two groups on the basis of septation of fungal hyphae.

A: Endomycorrhizae caused by septate fungi.

According to Englander (1982) the endomycorrhizae caused by septate fungi are further divided in to three categories:

i) Arbutoid mycorrhizae. **ii)** Ericoid mycorrhizae. **iii)** Orchidaceous mycorrhizae.

B: Endomycorrhizae caused by aseptate fungi (VAM).

Vesicular Arbuscular Mycorrhizal (VAM) fungi have been recognized as obligate symbionts of a very wide range of plant species. The long-term compatible interaction is based on bi-directional nutrient transfer between the symbionts (Smith and Read 1997). This type of mycorrhiza is caused by fungi, of

the family Endogonaceae of Zygomycetes e.g. *Glomus*, *Sclerocystis*, *Acaulospora* and *Gigaspora*. They are distinguished by the morphology of their resting spores. The aseptate fungal hyphae are found with external mycelium on the root surface. From infected root surfaces, hyphae run either intercellularly or intracellularly inside the root cortex. The external mycelium is continuous with the internal one, thus forming one infection unit. External mycelia are dimorphic i.e. they have thick walled, yellow non-septate hyphae with unilateral angular projections. Many thin walled aseptate hyphae also develop as lateral branches from the thick walled hyphae, which are ephemeral in nature (Gerdemann, 1968). This type of endomycorrhizae is most abundant. It has been observed in the roots of over 1000 genera of plants from 200 families. Over 90 % of the 300,000 species of vascular plants in the world form VAM (Marx and Cordell, 1988). Soybean plants have this type of mycorrhizae.

1.1: General characteristics of VAM

After the intervention of VAM fungi inside the host, infection develops which possesses different structures such as extramatrical hyphae, arbuscules and vesicles (Bonfante-Fasolo, 1984). Roots infected with a VAM endophyte have an extensive, loose, and external hyphal network, which may extend deep into the soil. These are called **external mycelium/extraradical hyphae**. The hyphae become swollen at the point where they enter the root cortex; this structure is called an **apresorium** (Gerdemann, 1968). Once inside the cortical cells, fungal hyphae develop various structures, which are a part of **internal mycelium /intraradical hyphae**. Complex coils and loops are formed within the cells. The

hyphae remain aseptate when the fungus is growing and become septate as conditions become unfavorable (Bonfante-Fasolo, 1984).

Immediately after infection the fungus forms **arbuscules** within root cortical cells. Arbuscules are a type of haustoria. Usually they are terminal but in some hosts they are formed laterally on the hyphae where they develop by repeated dichotomous branching of the hyphae (Gerdemann, 1968). Arbuscules are important structures as they are considered the sites of fungus/plant metabolite exchange (Bonfante-Fasolo, 1986). The arbuscules are short lived and disintegrate into dense masses as reported by Cox and Tinker (1976). **Vesicles** are sac-like structures resembling balloons. They are actually swollen hyphal tips. With the enlargement of vesicles the primary cortex is sloughed off and shed out in the soil. Vesicles are formed after arbuscules and increase in number as the plant matures (Bonfante-Fasolo, 1984).

Mycorrhizal fungi probably increase the capability of the system to find water in drier soil. In this way mycorrhizae prevent the plants from the detrimental effects of water stress. This could be because of the ability of mycorrhizal roots to extract soil moisture more efficiently in dry soils (Faber *et al.* 1991). Duan *et al.* (1996) stated that mycorrhizae maintain host stomatal conductance during drought periods by influencing hydraulic and hormonal factors.

It is highly likely that many of the plant responses evoked by VAM infection such as enhanced growth, photosynthesis, increased water and phosphorus uptake may be regulated in part by phytohormone levels. The

production of abscisic acid (ABA) and auxins such as indole acetic acid (IAA), have been reported in different plants inoculated with VAM (Roullin et al., 1986; Branzanti et al., 1985; Danneberg et al., 1992).

The present research is a study of endogenous levels of these hormones in plants subjected to different treatments. The effects of exogenous applications of ABA and auxin on the growth of VAM inoculated or non-VAM soybean plants exposed to drought non-drought conditions were determined in various experiments.

1.2. Phytohormones and Mycorrhizae

1.2.1. Auxins and Mycorrhizae

Indole- 3- acetic acid (IAA) is the principal naturally occurring auxin. It is synthesized in leaf primordia and young leaves and in developing seeds. IAA is transported from cell to cell and the transport is unidirectional or polar in actively growing plants. Apical dominance, tropic responses, vascular tissue differentiation, promotion of cambial activity, induction of adventitious roots on cuttings, inhibition of leaf abscission, stimulation of ethylene synthesis, inhibition or promotion of flowering and stimulation of fruit development are among the roles induced by IAA in plants (Taiz and Zeiger, 1991).

Mycorrhizae involve a unique symbiotic association between plant roots and infecting fungi. This association often increases growth and yield in soybeans by enhanced nutrient uptake and resistance to drought (Busse and Ellis, 1985, Ruiz-Lozano et. al., 1987)). However, it has been suggested that phytohormones produced by mycorrhizal fungi also play some role in this

mutualistic relationship. The production of auxin by mycorrhizal fungi has often been reported. Slankis (1948,1951) was the first to demonstrate the production of auxins by mycorrhizal fungi. According to Ritter (1968), theoretically three ways might be possible to realize such high auxin concentration: 1) intensive and steady auxin synthesis by the mycorrhizal fungi. 2) induction of auxin formation in the roots by the fungi. 3) inactivation of IAA oxidase in the roots by inhibitors produced by the fungal symbionts. His experiments proved the third theory to be correct. Ek *et al.* (1983) investigated the production of IAA by 16 different mycorrhizae-forming fungi by gas chromatography-mass spectrometry. Results indicated that IAA was produced by all of these fungi although ability to produce this hormone varied greatly. The production of auxins has been reported by hundreds of ecto and endomycorrhizal fungi, but unfortunately *Glomus* is the least studied genus in this regard. Danneberg (1992) investigated the level of abscisic acid and auxin in plants infected with *Glomus*. He found no difference in auxin levels of mycorrhizal and non-mycorhizal plants. A possible reason for these results could be the use of bioassay only, which may not be sensitive enough to determine the differences in auxin content. Moreover, bioassays are unable to differentiate among various auxins, so it is possible that auxin metabolism in mycorrhizal roots may be different from those of nonmycorrhizal roots. Roullin *et al.* (1986) identified IAA in the culture medium of *Hebeloma hiemale* fungus and sugested that the amount of IAA accumulated in the filtrates represents the IAA synthesis by this fungus.

The findings of the experiments done to study the role of exogenous auxin application as compared with the role of mycorrhizal inoculation also point out a relationship between the two. Branzanti *et al.* (1985) compared the effects produced by mycorrhizal fungi and application of auxin (IBA) on apple rootstock. Similar effects were produced by both of them, i.e., they promoted the rooting of these cuttings. This leads to the conclusion that these fungi produced some growth factors that interacted with the endogenous growth substances and produced the same results as those of exogenous application of auxins.

In a study done by Dutra *et al.* (1996), the application of auxin (IBA) to mycorrhizal seedlings significantly increased the mean root dry mass but had no effect on non-mycorrhizal seedlings. Improved root growth resulted in increased above-ground vegetative growth. According to the authors it could have been the result of interactive effects of auxin (IBA) and mycorrhiza. The results of another research showed that arbuscule development in VAM fungi increased with the application of indole acetic acid, in inoculated *Vigna unguiculata* plants (Gunze and Hennessy, 1980). The response of host plant to auxins in the presence of VAM implies an interaction between these two variables. The reports suggest hormonal interaction between mycorrhizal fungi and plants. However, further studies are needed to understand a clear relationship between auxins level of plants and mycorrhizal association (Frankenberger and Arshad, 1995).

1.2.2. Abscisic acid and mycorrhizae

Abscisic acid is synthesized in mature leaves in response to water stress as well as in seeds exposed to water stress. It is exported from leaves through the phloem. Some major roles performed by this hormone include stomatal closure, and induction of photosynthate transport from leaves to developing seeds. It also induces the synthesis of storage protein in seeds and during embryogenesis, which helps to avoid premature germination of seed. The breaking of dormancy in many seeds is correlated with declining ABA levels in seed.

Production of abscisic acid by mycorrhiza has not been demonstrated clearly although a few studies have investigated the alteration in ABA levels in mycorrhizal infected plants. Danneberg *et al.* (1992) determined the concentrations of ABA and other phytohormones in mycorrhizae (*Glomus* isolate T_6)-infected maize and in a non-mycorrhizal control. They found that concentrations of ABA, in both roots and shoots were higher in mycorrhizae-infected plants than in non-mycorrhizal plants throughout the growth period assayed. These findings were proposed to be the result of fungal colonization of the roots, and it was suggested that the long term effect of ABA could involve regulation of plant mycorrhizal symbiosis. According to Allen *et al.* (1980) and Dixon *et al.* (1988 a, b) ABA may be responsible for changes in the physiology of host plants. In contrast, the Coleman *et al.* (1990) study showed no relationship between altered ABA levels and morphological or physiological changes in ectomycorrhizal plants. Since phytohormones may interact with each other and Coleman *et al.* (1990) did not study the effects of auxins and gibberellins, further

research is needed to understand the possible role of these hormones. Bothe *et al.* (1994) observed much higher levels of ABA in mycorrhizal plants than non-mycorrhizal ones. The spores and hyphae of *Glomus* were found to have a higher concentration of ABA in hyphae than in corn roots in a study done by Esch *et al.* (1994). Their findings established that ABA synthesized and excreted by the fungi exerts metabolic control on the root cells. Since the primary role of ABA in stomatal closure is well established, its presence in VAM inoculated roots could be extremely important for plant growth under a water stressed environment such as arid and semiarid climates. Ebel *et al.* (1997) conducted a study to determine the relationship of mycorrhizal symbiosis, ABA concentration in xylem sap, and sensitivity of stomata to this symbiosis in water stressed *Vigna ungiculata.* The results of this study showed that the mycorrhizal plants had higher stomatal conductance and lower ABA concentration in xylem sap as compared with non-mycorrhizal at higher soil water content. This difference disappeared at low soil water content due to closure of most of the stomata. The authors concluded that mycorrhizal symbiosis changed the stomatal conductance of the plant nonhydraulically and the factor might be increased ABA concentration. It is possible that the fungal symbiont alters xylem ABA concentration in the host either directly, by producing ABA it self and transporting it to the host, or indirectly, by altering plant metabolism which results in altered production or redistribution of ABA.

Exogenous application of ABA also affects the plants physiology under water stress. Exogenous application of ABA to seeds of *Sorghum cultivars*

increased grain yield in drought stressed plants (Traore and Sullivan, 1990). In another study, application of 0.004-0.04 μM concentration of ABA increased the growth of the main axis from root cultures (Yamaguchi and Street, 1977). In my preliminary experiments the application of ABA along with VAM inoculation promoted plant growth. The purpose of the present study was to investigate further the vital aspect of plant-mycorrhizal interaction by observing the role of exogenously applied ABA to plants, and effects of ABA produced by VAM on growth of plants subjected to drought stress.

1.3. Drought and Mycorrhizae

Insufficient water known as "agricultural drought" (Van Bavel and Verlinden, 1956) is one of the major limitations to crop growth. In commonly used terminology "drought" is an environmental stress of sufficient duration to produce a plant water deficit or stress which in turn causes disturbances of physiological processes (Kramer, 1980). "Drought" can also be defined as absence of rainfall for a period of time long enough to cause depletion of soil moisture and damage to plants.

Drought lowers crop production through physiological and metabolic activities of the plants. According to a study done by Brumm and Hurburgh (1990) on soybean, drought stress created shriveled and wrinkled hypocotyl growth. Drought has been found to decrease the yield significantly. Drought stress imposed during early flowering in sorghum caused an 87% reduction in grain yield, which resulted from a higher incidence of pod abortion (Craufurd *et. al.* 1993). Plants have various responses to water stress, depending on the timing,

type and period of stress. The initial responses involve changes in internal water status. Subsequent responses include decreases in stomatal apertures, photosynthesis, less vegetative and reproductive growth. Hormones have been implicated as controlling factors in many of these processes. It was observed that under low water potentials the accumulation of ABA is more sensitive to changes in turgor, which takes place in the mature tissues of soybean seedlings (Creelman and Mullet 1991). Whereas, ABA production is believed to increase under water stress, the level of auxin is found to decline under water stress (Simpson, 1981).

Vesicular Arbuscular mycorrhizal fungi often have positive effects on growth and physiological processes of plants (Cooper, 1984; Smith and Gianinazzi-Pearson, 1988). Infection by VAM fungi may promote plant growth by enhancing the uptake of phosphate from the soil (Smith, 1980; Stribley, 1987). VAM association in plants contributes towards the development of resistance to water stress, the mechanism of which is not clear. According to Faber *et al.* (1991), it is the hyphae of the fungus that make considerable contribution towards the water uptake. Others have suggested that extraradical hyphae (Allen, 1991) or increased root branching (Kothari *et al.*, 1990) may allow mycorrhizal roots to more fully explore a particular soil volume, extending soil water depletion zones and giving a mycorrhizal root system more access to available water. This is so that the mycorrhizal root system may dry a particular soil volume more evenly. On the contrary at similar soil water contents, the non-mycorrhizal soil volume would have a drier rhizosphere, but more unexplored wet soil volumes than in mycorrhizal soils. Phosphorus delivery to non-mycorrhizal roots is limited under

prolonged periods of water stress. Under nutrient-limiting conditions the ability of mycorrhizal hyphae to maintain phosphorus delivery to roots at a low moisture level is the basis of improved drought tolerance in VAM infected plants (Nelson and Safir, 1982). In a study done by Busse and Ellis (1984) seed weight of VAM-infected soybean plants increased 10% as a result of reduced abortion of pods. Significant interactions were found in *Glomus fasciculatim* infected, drought stressed plants for total seed weight, pod number, seed number and root to shoot ratio. It showed the positive effect of VAM infection on drought stressed plants in comparison with non-VAM drought stressed plants (Bethlenfalvay *et al.*1988). The authors reported that drought decreased the growth of both VAM and non-VAM soybean plants. However VAM infected stressed plants had greater dry weight than non-VAM.

1.4. Objectives

The study of the interaction of ABA, auxin and VAM fungi in drought stress may generate some information helpful for increasing crop production in regions of the world where plants experience drought. In some reports it has been suggested that the effect of phytohormone and VAM on some plant tissues like roots seems to be parallel. For example, Branzanti et al. (1985) compared the effect of two, mycorrhizal fungal species and auxin (IBA) application on root initiation in hardwood cuttings of apple root stock. The effects of fungi and auxin application were found to be the same on root growth, i.e. root growth was promoted at least by one of the two fungal species studied. The results of some other studies (Dutra *et al.*, 1996) have shown that VAM inoculation along with

auxin application not only increases root development but also improves the above ground vegetative growth

The speculation that fungal phytohormones might have a role in the establishment of a symbiotic association and in the physiology of mycorrhizal plants is supported by two pieces of evidence. The first is the wide spread ability of mycorrhizal fungi to produce auxin in culture media (Ek *et al.*, 1983), and secondly the induction of increased growth in plants in response to exogenous applications of auxin or as a result of VAM inoculation (Branzanti *et al.*, 1985). The physiological effects of auxins released by fungal symbiont and the role of this metabolite in symbiotic association are still not fully understood. It is known that root systems are sensitive to very low auxin concentrations. A low but continuous amount of the hormone released in the direct vicinity of host cells might alter their metabolism.

The phyohormone abscisic acid has been shown to regulate several biochemical and physiological processes within plants (Zeevart and Creelman, 1988). It has primary roles at the whole plant level especially in response to stress. But it is also involved at the cellular level in membrane transport (Farkas *et al.* 1985).

There are mixed findings regarding the ABA level in the VAM infected plants versus non-VAM plants. Some suggest lower levels of ABA in the plant tissues of VAM infected plants. Because the VAM plants never really experience that degree of drought stress which is necessary to stimulate of production of higher level of ABA in these plants due to the VAM's ability to scavenge even

harder-to-reach water in the soil. Other reports suggest higher levels of ABA found in VAM infected plants as compared to non-VAM plants. In the present study the role of VAM in the amelioration of adverse effects of drought on the host plant was evaluated through the examination of growth levels of the plant and the level of endogenous ABA in drought stressed VAM and non- VAM plants.

Specific hypotheses of this study included: Vesicular- Arbuscular Mycorrhizae improve the growth of drought stressed plants because:

1) They improve the phosphorus uptake by soybean plants

2) They increase, the drought tolerance of soybean plants by increasing the endogenous level of ABA

3) Exogenously applied ABA and Auxin along with VAM help improve the plant growth and yield of drought stressed soybeans.

This study may have an agricultural implication in terms of plant productivity particularly in tropical arid or semiarid regions of world. Increased understanding of the hormonal interaction between mycorrhizae and plants under drought stress could be of great benefit to the agriculture industry. The following criteria were used to test the hypothesis.

I). Yield was determined as a measure of reproductive growth in terms of number of pods, weight of seeds/pod and total seed weight/plant in plants of all treatments.

II). Dry weights of roots and shoots were determined after harvest as a measure of vegetative growth of plants. Root-shoot ratio was also used to

determine the effect of VAM colonization on the root system of soybean plants under drought stress. The results helped test the hypotheses that mycorrhizae and/ or exogenous application of hormones improved the plant growth under water stress.

III). Endogenous level of ABA in VAM/non-VAM, droughted /non droughted plants were estimated using ELISA.

1.5. Materials and Methods

1.5.1. Experimental design

The experiment 1 and 2 were designed as fully crossed factorial (2x2x3). VAM, Drought and Hormone application, were the main factors. There were twelve treatments with N=10 in experiments 1 & 2. The Control group in this experiment was no VAM, no drought and no phytohormones. The experiment # 3 was 2x2 factorial design with VAM and drought as the main factors and carried N=15 per combination of treatments.

1.6. Treatment combinations

1.6.1. Experiment # 1 and # 2

These experiments were repeated twice. The first one with *Glomus intraradieces* and the second one with *G. fasciculatum*

1: VAM

i) Drought

a) ABA. b) IBA. c) No hormone.

ii) No Drought.

a) ABA. **b)** IBA. **c)** No hormone.

2: No VAM

i) Drought

a) ABA. **b)** IBA. **c)** No hormones.

ii) No drought

a)ABA. **b)** IBA. **c)** No hormone

1.6.2. Experiment # 3

This experiment was done with *Glomus fasciculatum* and the plants were used to determine the endogenous levels of ABA.

1. VAM

i) Drought **ii)** No Drough

2. No VAM

i) Drought **ii)** No Drought

1.6.3. Plant growth conditions

Soybean (*Glycine maxima*) was used as the host (autosymbiont) plant, because it is an important crop as a food source and has a medicinal value. Seeds of D120, which is a mid-group II variety of soybean were obtained from Garst seed company. Plants were grown in 38 cm plastic pots inside the greenhouse in a medium, containing top soil. Temperature was maintained between 26-27° C with 14-16 hour day length in the green house. Autoclaved garden soil was used as the potting medium and was found to be free of any indiginous microorganisms. All plants received an inoculum wash, free of VAM fungal propagules, to equalize the microbiota of VAM and non-VAM treatments.

During the course of the study, VAM inoculua of *Glomus,* was obtained from two different companies, "INVAM", a Division of Plant and Soil Sciences West Virginia University. Two years later, a fresh inoculum was needed and was purchased from another company. The inocula were placed in pots at least 4 cm below the upper surface of the medium to prevent splashing and contamination of the non-VAM plants. This is a perfect depth for placing the inoculum because when the seedlings grow their roots are very close to the inoculum, which ensures the maximum infection of the roots by the fungi. One reason for that could be the root exudates, which stimulate the growth of the fungal spores. The sowing was done directly in the pots because preliminary trials in which seedlings were transplanted to ensure that all the plants had the same weight in the beginning resulted in massive failure of the transplanted seedlings. To minimize the effects, because of the difference in the size of plants in the beginning of the experiments, about five seeds were planted. Then at the 10^{th} day the plants with newly formed leaves with same height and size of cotyledons were left in the pots and others were taken out. Hoagland's nutrient solution was supplied twice a week. Plants were exposed to two 8-day water drought cycles before and after flowering. Exogenous (foliar) application of ABA and auxin (IBA was used because it is known to be more stable than IAA) in 10^{-6} M concentration were made a week prior to the induction of first water stress. To minimize the effects of greenhouse hetrerogeneity, the plants of different treatment combinations were rotated every other day.

1.7. Assays

Assays of plant physiological responses and whole plant growth and development were performed as follows.

1.7.1. Estimation of colonization using Light Microscopy.

Root samples were prepared by the method of Gemma and Koske (1989). The root samples were collected at two different phases of plant growth, (i) four days after the application of hormones and befor nthe induction of first drought cycle and (ii) at the time of harvest. Root samples from plants of each treatment were collected, washed with water and fixed in 95% methanol. Fixed roots were cut into pieces of 1cm length and cleared in 5% KOH for about 20-25 minutes in a water bath, under fume hood. Fully cleared roots were stained in glycerol-aniline blue by heating in water bath for 10-15 minutes. The roots were then observed under a light microscope to determine mean percentage infection for each sample using magnified intersection method. The % hyphal, arbuscular and vesicular colonization were determined by a method proposed by McGonigle *et al.* (1990).

1.7.2. Plant phosphorus content

Usually, studies that involve phytohormone changes do not consider the phosphorus levels of the plant tissue. Because there is a large body of evidence, relating mycorrhizae to increased plant phosphorus nutrition, its estimation seemed logical in this study. The results can help shed light on the old belief that mycorrhizae help absorb nutrients especially phosphorus. The improved

phosphorus absorption is known to increase the overall biomass of the plant. Safir *et al.* (1971, 1972) study supported that improved phosphorus uptake by VAM infected soybeans indirectly affected the water relations of the plants. The phosphorus content of VAM and non-VAM plant was analyzed by a method proposed by Kiston and Mellon (1944) with the steps given below.

1.7.3. Ignition of plant material.

Exactly 1 gram of plant material (shoots) was processed for ignition in 6ml of magnesium nitrate solution (50 % Mg $(NO_3) \cdot 6H_2O$) and sufficient water to wet the sample. Then the tissue was heated at low heat on hot plate and temperature was increased gradually, until the sample was dried. The tissue was then ashed in a muffle furnace at 550° C for approximately two hours. The ash was dissolved in 5ml of 5N nitric acid solution. 5ml of 5N nitric acid per 50 ml of final volume are sufficient to give optimum acidity. Some water was added and filtered through an acid washed filter paper into a volumetric flask to make a 50ml solution. A yellow color developed in 30 minutes and remained stable for 2 to 8 weeks depending on the concentration of the phosphorus in the sample.

1.7.4. Reagents

Reagents used by this procedure were:

i) Ammonium molybedate-ammonium vanadate in nitric acid,

Vanadate, molybdate and orthophosphates react to give a yellow color, which is the end point for this test. In nitric acid the color does not develop if the acidity is less than 0.2 N and develops very slowly if it is grater than 1.6 N. It has

been established that 5ml of 5N nitric acid/50 ml of final volume are sufficient to give the optimum acidity.

ii) Phosphate standards.

phosphorus standards were prepared by taking 0,5,10,15, 20 and 25 ml aliquotes of 50 PPP phosphorus solution and diluted to 50 ml in volumetic flasks.

1.7.5. Procedure for phosphorus quantification

An aliquot containing 0.1 to 1.0 mg of standard phosphorus was transferred to a 50 ml volumetric flask. 10 ml of ammonium molybdate - ammonium vanadate solution was added, mixed and diluted to a 50 ml volume and mixed again. After 30 minutes, color density was read at 470 mμ using a spectrophotometer. phosphorus contents were determined from a curve made from standards. For samples 10 ml of the sample solution were taken, 10 ml of molybdate - ammonium vanadate added and left for 30 min before reading the color density.

1.7.6. Yield determination

The plants used for this assay were dried at 40°C, until constant dry weight was achieved.

I). Yield was determined as a measure of reproductive growth in terms of number of pods, weight of seeds/pod and total seed wt/plant in plants of all treatments.

II). Dry weight of roots and shoots were determined after harvest as a measure of vegetative growth of plants. Root-shoot ratio was also used to determine the effect of VAM colonization on the root system of soybean plants

under drought stress. The results helped test the hypotheses that mycorrhizae and/ or exogenous application of hormones improved the plant growth under water stress.

1.7.7. Estimation of Endogenous abscisic acid in plant shoots

1.7.8. Extraction procedure

The plant shoots were weighed immediately after the harvest, wrapped in aluminum foil and placed in –20 ° C freezer until extracted. All steps were carried out under minimal light to avoid photo-oxidation of the hormones. For extraction, 2gms of the plant material were ground under liquid nitrogen, followed by 30 ml of acidified methanol (methanol/acetic acid, 99:1) with 10 mg of butylated hydroxy toluene per liter added as an anti-oxidant. The extract was centrifuged at 7600 g for 10 min at 4 °C. The supernatant was collected and vacum dried 35 °C in a speed Vac concentrator. The residue was placed in 10 ml of 10 % methanol in water and centrifuged at 15600 g. The supernatant was separated and stored at – 80 °C in a freezer until analyzed.

1.7.9. Estimation of ABA by using Enzyme Linked Immunosorbent assay

Competitive Enzyme Linked Immunoassay became the procedure of choice, for several reasons. First of all the extraction procedure was simple as compared to the preparation of samples for HPLC and Gas chromatography. It did not have multiple steps so there was less chance of hormonal breakdown due to procedural complexity. Secondly, HPLC and GC were not available. Immunoassays are becoming increasingly popular because of the simplicity and sensitivity of the procedure, and reproducibility of the results. The materials used

to perform the ELISA for ABA was purchased from Sigma-Aldrich. The abscisic acid test utilizes a monoclonal antibody to ABA and is sensitive to the range of 0.16-100 picomoles ABA /ml. The assay principal uses the competitive antibody binding method to measure concentrations of ABA in plant extracts. ABA is labeled with alkaline phosphatase (tracer) and then added along with plant extracts to antibody coated, micro-wells. A competitive binding reaction is set up between a constant amount of tracer, a limited amount of antibody and the unknown sample containing ABA.

The hormone in the sample competes with the tracer for antibody binding sites. A positive reaction was the appearance of a yellow color, the amount of which was determined photometricaly by using a vertical light path photometer and is inversely proportional to the amount of hormone in the sample. The intensity of color was related to the sample ABA concentration by means of a standard curve.

1.8. Data analysis

The data was subjected to three-wayanalysis of variance using JMP (SAS) version 4. VAM, drought and hormonal application were the main factors.

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Chapter 2

Experiment 1

"Effect of *Glomus intraradices* on growth and phosphorus uptake of water-stressed soybeans in combination with exogenous application of ABA/auxin."

2.1. Results

The independent variables in this experiment were VAM, Drought and exogenous hormonal application (ABA/Auxin). The VAM species used was *Glomus intraradices*. The experiment was repeated twice and the results were same. The data were subjected to three-way ANOVA using JMP and Statview (SAS).

2.1.1. Shoot dry weight (gms) /plant

The results of three-way ANOVA for shoot dry weight are given in Table 2.1 and show that some of the treatments altered shoot dry weight significantly. The means are shown in Fig 2.1.a and Fig 2.1.b. The treatments were responsible for 62% of the variability in shoot dry weight/plant. The most significant treatment was that of VAM x Hormone interaction, with an R-sq of 12.4 %. The treatments involving hormonal application were responsible for 11.3 % variability in shoot dry weight. Hormonal application increased the shoot dry weight by about 5% over the control (no hormone). However the auxin application increased the shoot dry weight by 6.5 % over ABA application. VAM x Drought interaction was responsible for 9.9% of the variability in shoot dry weight/plant due to treatment effects. The treatment involving VAM x Hormone x Drought interaction had an R-sq of only about 2 %. Fig 2.13- Fig 2.15 show, effect of different treatments on the growth of plants of different treatments as compared with control.

2.1.2. Root dry weight (gms) plant

The VAM was responsible for 5% of variation in root dry weight (Table 2.2) with P = 0.005. Drought alone caused no significant differences in root dry mass of plant as compared to non-droughted plants (R-sq = 0.5%). The hormonal application alone caused, 6% of the variation in root dry mass. The root dry weights of the plant groups that received exogenous hormonal application were significantly higher than the control group (P <0.001). Furthermore, the root dry mass, of plants that received exogenous application of auxins was statistically significantly different from ABA treated plants (P <0.001). VAM x Hormone interaction was responsible for 5.6 % variability (P = 0.01) and, Hormone x Drought had and R-sq of 4 % (P = 0.04). The treatment involving the interaction of VAM x Hormone x Drought was responsible for 8% increase in the root dry mass (P = 0.002). The mean values are presented in Fig 2.2.a and 2.2.b.

2.1.3. Length of 2nd internode (cm)

The results given in Table 2.3 show how different treatments affected the length of the 2nd inter-node from the top. It turned out that the presence of VAM increased the length of the 2nd internode by 34.5 %, which was statistically significant (P<0.001). Among other treatments the one involving the VAM x Hormone increased the length of the 2nd inter-node statistically significantly (P <0.001) with an R-sq of 16.9%. The interaction of VAM x Hormone x Drought (AxBxC) had an R-sq of 4.3 % and P = 0.02 which is still a significant influence. Fig 2.3.a and 2.3.b represent the mean values for the length of second internode.

2.1.4. Number of pods/plant

The results given in Table 2.4 reflect that VAM significantly increased the number of pods per plant ($P<0.001$) with an R-sq of 2.3%. Similarly exogenous hormonal application also increased the number of pods significantly ($P <0.001$) R-sq in this case is 14.3%. The orthogonal comparisons of hormonal treatments show that the difference was significant between the treatments involving hormonal applications and that of control (no hormone). The second orthogonal comparison showed that the outcome of ABA application was significantly, different from auxin application ($P <0.001$). Fig 2.4.a and Fig 2.4.b represent the mean values for number of pods/plant. The mean values also given in Table 2.4 show that the number of pods/plant was higher due to ABA application rather than auxin. The treatments involving drought did not show a statistically significant outcome over that of non-droughted plants.

As far as the interaction treatments were concerned the VAM x Hormone interaction increased the number of pods / plant significantly as compared to VAM x Drought and Drought x Hormone. The treatments involving the interaction of all three factors (AxBxC) also increased the number of pods significantly ($P <0.001$). As far as the overall ANOVA is concerned, the hormonal application (B) was responsible for 14% of the variation followed by, AxBxC interaction (7.3%). The VAM x Hormone (AxB) interaction had an R-sq of 5.8 % and $P = 0.001$ which is statistically significant as well.

2.1.5. Dry seed weight (gms)/pod

Table 2.5 presents the results ofanalysis of variance for the seed weight /pod in the plants of different treatment combinations. The treatment involving hormonal application, were responsible for 17.7 % of the variability (P <0.001) in dry seed weight (gms)/pod. In the orthogonal comparison, no significant difference was found, due to the effects of ABA versus Auxin. The treatment involving VAM caused 13 % difference in seed dry weight/pod, significantly different from the non-VAM treatment group (P <0.001).

VAM x Drought had an R-sq of 5.7% and the interaction with all the three factors, VAM x Hormone x Drought, with 5.9 % R-sq caused significant variation in the seed weight /pod, again statistically significant (P<0.001). The greatest influence was that of interaction (AxB) VAM x Hormone, which was responsible for 18% of the variability. At P <0.001 it is a statistically significant result. Drought did not caused a statistically significant difference as compared to non-droughted plant groups (means of seed weight/pod given in Table 2.5 and prersented in Fig 2.5.a and 2.5.b)

2.1.6. Total seed weight (gms)/plant.

Table 2.6 describes the results of three way- ANOVA for total seed weight /plant. The treatments involving hormonal application were responsible for about 22.7 % of the variation in total seed weight (gms)/plant caused by this treatments (P<0.001). The results of orthogonal comparison given in Table 2.6 show that there was a significant difference between the influence of ABA and auxin on this variable. That was followed by, treatment combinations involving VAM, a total of 17.4% (P <0.001). VAM and Hormone interaction (AxB) together however, was responsible for 8.6 % of the variation,

still statistically significant (P<0.001). Drought was responsible for 3 % of the variation (P = 0.009), statistically significant though. None of the other interactions caused any significant variation. Fig 2.6.a and 2.6.b represent the mean values for total seed weight /plant.

2.1.7. Phosphorus concentration mgs/gm of plant

The results of ANOVA showed statistically significant effects of different treatments on the phosphorus content of the plants (Table 2.7, means are illustrated in Fig 2.7.a and 2.7.b). The VAM treatments were responsible for about 46.8 % of the variation in the phosphorus content of plants. The improved plant growth can be due, in part, to increased up take of phosphorus (Aguilera *et al.* 1999). The hormonal application did not cause any statistically significant differences in this regard. Plants subjected to drought had significantly lower phosphorus than plants that were non-droughted (P<0.001).

All interactions had statistically significant effects on the plant phosphorus content. VAM x Hormone (AxB) was responsible for 3.8 % of the variation in the phosphorus content of the plants (P <0.001). VAM x Drought (AxC) caused 3% of the variations (P<0.001). 7% variability was due to the interaction of Drought x hormone (BxC). The three factors interaction VAM x Hormone x Drought was responsible for 1.6 % of the variation (P <0.001).

2.1.8. Root /shoot dry weight ratio

The results for root/shoot dry weight ratio are given in Table 2.8 and the means for different treatments are given in Fig. 2.8.a and 2.8.b. The VAM did not cause any significant influence on the root/shoot dry weight ratio of soybean plants. Hormonal application had a significant effect, while the effect of ABA was not statistically different

from that of auxin treated plants. Drought also significantly altered the root /shoot dry weight ratio of the plants. All the interactions except the VAM x Hormone x drought caused a significant variation in the root/shoot dry weight ratio of the soybean plants.

2.1.9. Percent colonization of roots (hyphal, arbuscular + vesicular before stress)

The results described in Table # 2.9 and 2.10 represent the % hyphal colonization and % arbuscular colonization respectively. VAM treatment was responsible for major part of the variation in hyphal colonization (R-sq 84.5 %) and arbuscular + vesicular colonization R-sq 88.2 %. There was a significant dufference between the ABA and auxin treated plants in terms of hyphal as well as arbuscular and vesicular colonization. Same was the case with VAM x Hormone interaction which caused 0.8 % variation in the hyphal and 0.6 % variation in the arbuscular + vesicular colonization.

2.1.10. % colonization of roots (hyphal, arbuscular+vesicular) at harvest

The three-wayanalysis of variance described in Table 2.11 reveals that highest hyphal colonization was observed in the treatments involving VAM only (79.8 % R-sq). Presence or absence of drought caused a statistically significant change in the percentage hyphal colonization of the rootsv (P =0.003). The plants that received hormonal application had significantly less arbuscular + vesicular colonization as compared to the control (Table 2.12). There was a statistically significant difference in hyphal as well as arbuscular + vesicular VAM colonization of roots due to ABA vs Auxin treatment as shown in Table 2.11 and 2.12. Fig 2.11.a, 2.11.b as well as 2.12.a and 2.12.b represent he means. Fig 2.16 shows characteristics of *Glomus intraradieces* inside the roots.

2.2. Discussion.

Mycorrhizae are believed to have been involved with the vascular plants since the Paleozoic times (Taylor 1990). The availability of water and nutrients such as P and N has always been a constraint in the growth and development of most plant communities. It is believed that Carbon is the limiting factor for the growth of fungi. Obviously, mutually beneficial relationships between the plants and the fungi have co-evolved by the process of natural selection. The results of the present study shed some light on different aspects of plant growth that can be affected by this mutually symbiotic association.

2.2.1. Shoot dry weight (gms)/plant

The results of this study show that the plants that were subjected to drought and were inoculated with VAM (VAM x Drought) had 10% more shoot dry weight than the drought stressed plants that did not have VAM. This finding is coherent with the previous reports. It is a known fact that water stress is one of the most important factors. One of the reasons that drought stressed mycorrhizal plants did better than the drought stressed non-mycorrhizal plants was improved nutrition in addition to availability of ambient water, because these plants were grown in pots with a limited supply of nutrients. Improved nutrient uptake can increase the drought resistance of plants (Nelson and Safir 1982). The plants that are subjected to drought stress receive a non-hydraulic chemical signal from the dehydrating roots. This chemical signal is believed to be a hormone, possibly abscisic acid. (Davies and Zhang, 1991; Hartung and Slovik, 1991). The response of the plant to that non-hydraulic signal is decreased stomatal conducatnce that leads to decrease in vegetative growth of plants (Auge *et al.* 1986a ; Davies and Zhang 1991). In the case of mycorrhizal plants the response of plants to this root-shoot signal is

altered by eliminating the response of the leaves. According to Auge *et al.* 1986a the leaf response is controlled by phosphorus deficiency, which always accompanies drought, hence improved phosphorus uptake by VAM infected plants eliminates that response. The extraradical fungal hyphae increase the absorptive surface area of the roots (Hampp *et al.* 2000). This reduces the resistance to water uptake (Allen 1982). Mycorrhizae have been found to reduce significantly the resistance to water uptake in the host plants (Safir *et al.* 1972.). *Glomus* species were found to increase the water uptake in rose plants (Auge *et al.* 1986a).

The other treatment that increased the shoot dry weight in this study is the exogenous application of the hormones. This is in accordance with a previous report, which suggested that, the physiological and growth responses of the plants could be controlled by exogenously applied ABA in drought stressed plants (Traore and Sullivan, 1990). A report by Abdel-Ghaffar (1998) described the shoot and root weights of maize and soybeans in response to the application Auxins. Another study also suggested a pronounced effect of IBA application (an Auxin) on the vegetative growth of plants in the presence of VAM (Dutra *et al.* 1996).

2.2.2. Root dry weight (gms)/plant

The fact that the presence of VAM significantly increased the root dry weight is in complete agreement with several previous reports. Several investigators have reported that VAM increases the root weight and the rooting depth as compared to non-VAM plants (Busse and Ellis, 1985; Kothari *et al.* 1990; Aboul-Nasar,1998; Thanuja *et al.* 2002).

Drought did not cause any significant variation in the root dry weight probably because soybean is considered to be drought tolerant. This report is unlike most of the reports which suggest a decrease in the root growth under water stress (Ruiz-Lozano, *et al.* 1994). The hormonal application significantly increased the root dry weight of plant, especially the IAA application. The interactive effect of VAM x Hormone x Drought was responsible for a 10% increase. This can be attributed to the combined effect of auxins as suggested by Brian (2003) that mycorrhizae act as root stimulants just like auxins. Mitchell *et al.*, (1986) proposed that VAM increased the IAA levels of the plants by producing some ploy phenolic compounds, which reduced the IAA oxidase activity. Carolyn (2003) found that the presence of both VAM and auxin in a medium generally resulted in better rooting of the cuttings in horticultural studies. It is possible that it can also promote the growth of roots.

2.2.3. Length of 2nd Internode (cm)

Length of 2nd internode was chosen as one of the growth variable because visually it showed variability. The results of the present study showed a 34.5% increase in the length of 2nd internode caused by the presence of VAM. This criteria has not been reported directly but rather has been looked at in terms of shoot growth in plants and shoot dry weight. A study done by Lahlil (2001) involving two species of *Glomus* revealed that both VAM species increased the shoot growth in citrus seedlings. This can be explained through the fact that VAM infected plants are better able to absorb water (Safir *et al.* 1971; Mosse, 1978) and nutrients especially phosphorus (Sylite, 1985; Menge, 1985a). In the present study the plants of all treatments were provided with a P deficient Hoagland solution, so availability of phosphorus was very limited. Clearly

VAM plants were at an advantage as compared to non- VAM plants. Another factor contributing to the improved shoot growth could be hormones produced by the VAM and transferred to the host plants to help improve growth. The results for the Length of 2nd internode are coherent with the shoot dry weights, which suggests that greater shoot length did contribute to the biomass. The arbuscules formed by the fungus inside the host roots are considered to be the organ of nutrient transfer between the host and the fungus, although it is not clearly understood whether or not they are an organ of transportation of materials from fungus to the host (Cox *et al.*, 1975). But at least for phosphorus they seemed to be the site for transfer to the host plant as reported by Cox and Tinker (1976). Gunze and Hennessy (1980) reported that IAA application increased the development of arbuscules in plants that were subjected to either defoliation or had low level of Auxins because their apex had been removed. This could be one of the reasons for enhanced growth due to Auxin application. On the other hand in a study done by Little and Joanne (2003), the exogenous application of auxin, regardless of the method of application, did not effect the shoot growth of *Pinus* trees. Sometimes application of exogenous hormones can result in inactivation of endogenous hormones (Ribnicky *et al.*1996). Traore and Sullivan (1990), On the contrary, suggested that exogenously applied ABA could enhance plant growth, especially under drought stress. In the present study the ABA application in drought stressed VAM plants did not help to increase the foliar growth but in the absence of drought it did.

2.2.4 Yield (number of pods / plant, weight of seeds /pod, total seed weight / plant)

The results of the present study support previous similar research by showing the number of mature pods to be significantly higher in VAM plants as compared to non-VAM. One of the reason that mycorrhizal plants have higher reproductive growth (flowering, pods, grain yield, etc.) is that they do not have to invest as much in the vegetative growth like roots because fungi take over the role of roots as compared to non-mycorrhizal plants who have to allocate relatively more biomass to roots (David *et al.* 2001).

There was no significant difference in the number of pods in droughted or non-droughted plants. The same is true for weight of seed/pod. The overall weight of seeds /plant however was statistically different. The reason for that conflict is that when weight of seeds /pod was being measured, the healthiest pod from each plant was chosen.

Not all the pods on a particular plant of a treatment group had the same weight. Water stress is considered to be one of the biggest restrain for crop productivity across the world (Subramanian and Charest, 1998). The treatments involving VAM x Drought and Drought x hormones interaction also did not have any significant increase in the number of pods. The weight of seeds /pod was significantly increased by VAM x Drought interaction. The total seed weight /plant however was not significantly increased by VAM x drought (A x C) interaction. These results are different from the previous literature that suggest the VAM plants subjected to drought had higher biomass and grain yield as compared to non-VAM stressed plants (Ellis *et al.* 1985) which can be attributed to the ability of VAM plants to maneuver the soil for water and nutrients better as compared to non-VAM plants (Al-Karaki, 1999). The plants in the present study were

subjected to two-drought stress cycles once at six weeks of age and then at eight weeks whrn they were flowering and filling pods. Plants were re-watered after a week when they started to show the visible signs of wilting. Soybean is considered to be drought resistant, so number of mature pods was not affected by the drought. The fungus used in this experiment was *Glomus intraradieces*, which helps to increase the growth and productivity of other crops under different kinds of stresses including water stress (El-Tohamy *et al.* 1999).

The results of the present study demonstrate that the application of hormones also increased the yield significantly over the control in all three ways (number of pods, weight of seed per pod and total seed weight /pod). The same was true for VAM x Hormone (AxB) and VAM x Hormone x Drought interaction for number of pods/plant (Table 2.4) and weight of seeds/pod (Table2.5). The exogenous application of ABA under water stress can decrease the loss of water by reducing the transpiration rate and stomatal conductance as proposed by Ahmed *et al.* (2002). The application of auxins can affect the increase in the size of grains/seeds by promoting cell division. These effects coupled with the role that VAM performs by influencing the status of other plant hormones as well as nutrient relations and plant resource allocation probably led to a significant increase in yield of VAM plants that received exogenous application of auxins (Powel and Bagyaraj, 1984). However one should keep in mind that the interaction of plants with mycorrhizae is much more complex so there are definitely other factors that control this symbiosis.

2.2.5 Phosphorus concentration mgs/gm of plant

The results of the present study show that VAM helps the plant to absorb phosphorus from the soil. The results demonstrate that VAM has helped to ameliorate the adverse effects of drought in colonized plants. The VAM x Drought interaction had a significant increase in the uptake of P from the soil for the plant. A limited supply of nutrients and a scarcity of water can really halt plant growth, but the extraradical fungal mycelia extend the root surface area and enhance the acquisition of nutrients and water. The effects of mycorrhizae on plants have often been associated with improved water and nutrient up take, for example in a study involving soybeans and *Glomus* (Abdel-Fattah, 1997) and other host plants (Vijaya and Srivasuki, 2001). However it has also been reported, that the effect of these fungi may be independent of P uptake (Bethlenfalvay *et al.*1988).

The treatments involving the interaction of VAM with other factors (Hormonal application and Drought) continue to show statistically significant uptake of phosphorus (Table 2.7). This effect of VAM can be attributed to the findings that the Phosphatase activity is higher in the rhizosphere around AM than in non-mycorrhizal roots (Dodd *et al.* 1987). In the present study the data for total seed weight /plant correlates to the phosphorus level of the plants across some treatments except for the hormonal application. So it is safe to conclude that the phosphorus uptake by VAM plants promotes growth and productivity.

There are many reports regarding the role of VAM in the uptake of nutrients studied in stressed plants or otherwise, but there is hardly any evidenced pointing to the role of hormonal application, alone or along with VAM under water stressed conditions.

This study found that the exogenous application of ABA and Auxin had no significant effect on the uptake of phosphorus. So if the hormonal application increased the vegetative or reproductive growth of the VAM plants that was definitely independent of the involvement of hormonal application in the absorption of phosphorus. It can be attributed however to the VAM's classic roles to help withstand the stress or to the hormones ability to promote cell division and development.

Compare the results for Hormone x drought interaction for the phosphorus concentration in Table 2.7 to the number of pods in Table 2.4 and the total seed weight / plant in Table 2.6. If these plants had a significant improvement in the absorption of P then why is this not reflected in the yield? Similarly the interactions involving VAM x Hormone and VAM x Hormone x drought did not contributed to any significant improvement of the total seed weight / plant although they exhibit a statistically significant improvement in phosphorus content of the plants.

As far as the application of hormone (ABA/auxin) in drought stressed plants is concerned, the auxin was probably not able to help with drought so even though the plants had accumulated enough P in the tissue, they could not assimilate it because of water unavailability and because water stress decreases the endogenous auxin level in plants (Rubin *et al.* 2002).

The exogenously applied ABA along with the ABA possibly synthesized by the fungi affected the plants in the interaction of VAM X Hormone and VAM x Hormone x Drought probably by altering their stomatal behavior, causing a decrease in stomatal conductivity, resulting in reduction of photosynthesis and ultimately lower productivity of the plants. Although stomatal conductivity were not measured in the present study but

there are several reports suggesting that ABA acts as a non-hydraulic root signal when the plants are subjected to drought (Davies *et al.* 1994). ABA tends to be involved in controlling the stomatal conductivity of plants (Jarvis and Davies, 1998). VAM fungi can alter the host balances of ABA, furthermore VAM hyphae apparently can produce ABA (Esche, *et al.* 1994).

2.2.6 Root /shoot dry weight ratio before stress

Fig 2.8.a and 2.8.b illustrate that VAM plants exogenously supplied with ABA had the highest root/shoot ratio. Droughted VAM plants had higher root/shoot dry weight ratio as compared to droughted non-VAM plants. *Glomus* fungi have been reported to increase the root biomass as well as the shoot biomass resulting in higher root/shoot ratio in some cases as compared to non-VAM plants (Ortas *et al.* 2002). Others have reported that some species of *Glomus* decrese the root /shoot ratio under drought stress (Busse and Ellis, 1985). In this regard a lower root/shoot ratio could be considered a way of VAM to increase the drought tolerance of the soybeans, by making them have an efficient use of their root system. In the first case one can assume that improved root biomass would be positively correlated to the increased shoot dry weight, but may not be contributing towards the drought tolerance but rather drought adaptation.

2.2.7 Percent colonization of roots (hyphal, arbuscular + vesicular) before stress

Hyphal colonization was affected positively by the exogenous application of auxin and ABA as compared to the plants that did not receive any hormonal application, (Table 2.9) but the arbuscular + vesicular colonization was influenced negatively by hormonal application.

2.2.8 Percent colonization of roots (hyphal, Arbuscular + vesicular) at harvet

The results presented in Table 2.11 show that the presence or absence of drought influenced the rate of colonization significatly. The mean values of % hyphal colonization in droughted plants were higher than non droughted plants. Table 2.12 shows that the arbuscular + vesicular colonization was lower in drought stressed plants. There are conflicting reports regarding the impact of drought on the extent of VAM colonization. For example Al-kararaki and Clark (1998) found that the colonization of *Glomus intraradieces* was increased significantly in squash plants under low irrigated conditions. Similarly (Aboul-Nasr, 1998) reported that the colonization was increased significantly in response to drought stress in durum wheat. On the other hand drought stress has also been known to decrease the mycorrhizal colonization (Iqbal and Tauqir, 1982).

It is commonly understood that the effects of drought on VAM colonization will vary depending upon the host and the VAM species involved. Also the experiments in pots will yield variable results as compared to the field for the same host and fungus involved (Auge, 2001).

Hyphal colonization was increased significantly due to auxin application while arbuscular + vesicular colonization was found to be highest in plants that did not receive any hormonal application in the presence of drought. Carolyn (2003) found that the auxin application increased the root colonization by *Glomus moseae.* In the absence of drought ABA treated plants had the highest hyphal colonization and the control had the

lowest. The arbuscular and hyphal colonization however was found to be lowest in auxin treated plants suggesting an influence of auxin treatment on the growth of the VAM.

2.3. Conclusion

Mycorrhizal symbiosis between plants and fungi of different genera occur in almost all land plants in almost every environment. This symbiosis offers protection and a carbon source for the fungus and the plants benefit from increased nutrient uptake and drought resistance. The VAM can influence the carbon dynamics of the host plant leaves. Photosynthesis in VAM plants have been found to be, sink regulated at least to some degree, and stimulated by the mycorrhizal roots (Wright *et al.*1998). It has been suggested that 5-20 % of the carbon assimilated by VAM plants eventually ends up in the fungal structure (Harris et al. 1985; Wang *et al.* 1989). By acquiring so much carbon assimilates the roots act as a sink. That creates a lower concentration of carbon in the leaves. The stomata are sensitive to the concentration of carbon in the leaves so they open. The host plant stomatal conductivity increases resulting in an enhanced photosynthetic activity of the plants that eventually leads to a better growth and productivity (Jarvis and Davies, 1998). The VAM effect on leave's carbon concentration could directly influence, the stomatal behavior and the water balance of the plant (Eissenstat et al. 1999; Douds and Pfeffer, 2000). This might conflict with what I reported regarding the lower seed weight due to ABA produced by VAM, which can lower the stomatal conductivity and decrease the photosynthesis rate. Perhaps this would make more sense if we consider that the final out come is a net result of several processes working together at the same time.

It had been reported previously that the drought-stressed VAM plants had less growth of roots as compared to non-droughted VAM plants, and that increase in root growth is because of irrigation and not due to VAM inoculation (Martin and Stutz, 1994). The potential for VAM fungi to promote the host growth varies greatly due to host and VAM differences, but it is generally believed that the leguminous plants that have poorly branched roots (like soybeans) with fewer root hair can benefit more from VAM hyphae than plants like grasses and cereals with finely branched root system supplied with abundance of root hair.

Table 2.1 Three-way analysis of variance for shoot dry weight (gms)/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus intraradieces*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	2.992	2.992	7.446	0.007	0.026
VAM present	3.483						
VAM absent	3.167						
Hormone (B)		2	12.705	6.325	15.808	<0.001	0.113
Control Vs Hormone		1	5.403	5.403	13.440	<0.001	0.048
ABA Vs Auxin		1	7.302	7.302	18.169	<0.001	0.065
ABA	3.173						
Auxin	3.777						
Control	3.025						
Drought (C)		1	22.594	22.594	56.218	<0.001	0.201
Drought	2.891						
No drought	3.759						
Interactions							
AxB		2	13.977	6.988	17.389	<0.001	0.124
AxC		1	11.120	11.120	27.699	<0.001	0.099
BxC		2	3.170	1.585	3.944	0.022	0.028
AxBxC		2	2.199	1.099	2.735	0.069	0.019
Unexplained		108	43.404	0.402			0.386
Total		119	112.164				

Table 2.2 Three-way analysis of variance for root dry weight gms/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus intraradieces*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.176	0.176	8.073	0.005	0.051
VAM present	0.749						
VAM absent	0.672						
Hormone (B)		2	0.211	0.105	4.848	<0.001	0.062
Control Vs Hormone		1	0.078	0.078	3.756	<0.001	0.022
ABA Vs Auxin		1	0.133	0.133	6.199	<0.001	0.039
ABA	0.651						
Auxin	0.733						
Control	0.746						
Drought (C)		1	0.019	0.019	0.089	0.346	0.005
Drought	0.697						
No drought	0.723						
Interactions							
AxB		2	0.193	0.096	4.425	0.014	0.056
AxC		1	0.019	0.019	0.089	0.346	0.005
BxC		2	0.136	0.007	3.120	0.048	0.040
AxBxC		2	0.285	0.142	6.535	0.002	0.083
Unexplained		108	2.358	0.021			0.693
Total		119	3.400				

Table 2.3 Three-way analysis of variance for the length of 2nd internode (cm) as a response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces),* presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	665.052	665.052	2.956	<0.001	0.345
VAM present	10.450						
VAM absent	5.741						
Hormone (B)		2	21.554	10.777	1.560	0.214	0.011
Control Vs Hormone		1	7.526	7.526	1.089	0.298	0.004
ABA Vs Auxin		1	14.028	14.028	2.031	0.156	0.007
ABA	8.337						
Auxin	7.500						
Control	8.454						
Drought (C)		1	20.418	20.418	2.956	0.088	0.010
Drought	7.683						
No drought	8.508						
Interactions							
AxB		2	327.404	163.702	23.705	<0.001	0.169
AxC		1	36.852	36.852	5.336	0.022	0.011
BxC		2	26.362	13.181	1.908	0.153	0.014
AxBxC		2	83.679	41.839	6.058	0.003	0.043
Unexplained		108	745.825	0.387			0.287
Total		119	1927.148				

Table 2.4 Three-way analysis of variance for the no of pod/plant as the response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces)*, presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	180.935	180.935	50.751	<0.001	0.220
VAM present	8.639						
VAM absent	6.220						
Hormone (B)		2	112.695	56.345	15.805	<0.001	0.136
Control Vs Hormone		1	51.127	51.127	14.341	<0.001	0.061
ABA Vs Auxin		1	60.832	60.832	17.063	<0.001	0.073
ABA	8.731						
Auxin	7.025						
Control	6.530						
Drought (C)		1	21.237	21.237	5.957	0.016	0.025
Drought	7.016						
No drought	7.898						
Interactions							
AxB		2	51.853	25.926	7.272	0.001	0.061
AxC		1	10.044	10.044	2.817	0.096	0.012
BxC		2	07.654	3.827	1.073	0.345	0.010
AxBxC		2	67.252	33.626	9.432	<0.001	0.081
Unexplained		108	385.034	3.565			0.464
Total		119	829.700				

Table 2.5 Three-way analysis of variance for weight of seeds (gms)/pod as the response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus intraradieces*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.048	0.048	36.940	<0.001	0.130
VAMpresent	0.245						
VAM absent	0.204						
Hormone (B)		2	0.065	0.032	24.816	<0.001	0.177
Cntrol Vs Hormone		1	0.064	0.064	49.291	<0.001	0.175
ABA Vs Auxin		1	0.000	0.000	00.340	0.560	0.001
ABA	0.239						
Auxin	0.243						
Control	0.192						
Drought (C)		1	0.000	0.000	00.026	0.872	0.000
Drought	0.224						
No Drought	0.225						
Interactions							
AxB		2	0.066	0.033	25.316	<0.001	0.180
AxC		1	0.021	0.021	15.637	<0.001	0.057
BxC		2	0.004	0.002	01.293	0.278	0.011
AxBxC		2	0.022	0.011	08.496	<0.001	0.059
Unexplained		108	0.141	0.001			0.384
Total		119	0.367				

Table 2.6 Three way analysis of variance for total seed wt/plant as a response variable in a balanced fully-crossed factorial design with presence or absence of VAM (*Glomus intraradieces*), presence or absence of hormones and presence or absence of drought as main factors, with N=10 plants /treatments.

Source of variation	Mean(SD)	DF	SS	MS	F	P	R-Sq
VAM (A)		1	1.856	1.856	35.202	0.001	0.158
VAM present	1.290						
VAM absent	1.04						
Hormone (B)		2	2.489	1.244	23.611	<0.001	0.212
Control vs Hormone		1	1.039	1.039	19.723	<0.001	0.088
ABA vs Auxin		1	1.449	1.449	27.499	<0.001	0.123
ABA	1.366						
Auxin	1.097						
Control	1.034						
Drought (C)		1	0.218	0.128	4.136	0.044	0.018
Drought present	1.123						
Drought absent	1.210						
Interactions							
AxB		2	0.776	0.388	7.368	0.001	0.066
AxC		1	0.166	0.166	3.149	0.078	0.014
BxC		2	0.304	0.152	2.890	0.059	0.025
AxBxC		2	0.230	0.115	2.190	0.116	0.019
Un-explained		108	5.694	0.052			0.485
Total		119	11.737				

Table 2.7 Three-way analysis of variance for the phosphorus content (mgs)/gm of the plant tissues as a response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces),* presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	14.856	14.856	330.084	<0.001	0.573
VAM present	1.205						
VAM absent	0.507						
Hormone (B)		2	0.199	0.099	2.220	0.135	0.007
Control Vs Hormone		1	0.004	0.004	0.090	0.763	0.000
ABA Vs Auxin		1	0.196	0.196	4.357	0.039	0.007
ABA	0.816						
Auxin	0.914						
Control	0.852						
Drought (C)		1	2.196	2.196	48.793	<0.001	0.084
Drought	0.725						
No drought	1.205						
Interactions							
AxB		2	0.697	0.348	7.749	<0.001	0.026
AxC		1	1.811	1.811	40.245	<0.001	0.045
BxC		2	0.281	0.140	3.122	0.048	0.010
AxBxC		2	1.031	0.515	11.455	<0.001	0.039
Unexplained		108	4.860	0.045			0.187
Total		119	25.921				

Table 2.8 Three-way analysis of variance for the root dry weight/shoot dry weight ratio as a response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces)*, presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.002	0.002	0.315	0.575	0.002
VAM present	0.23						
VAM absent	0.22						
Hormone (B)		2	0.071	0.035	6.242	0.002	0.077
Control Vs Hormone		1	0.059	0.059	10.409	0.001	0.064
ABA Vs Auxin		1	0.012	0.012	2.137	0.146	0.013
ABA	0.229						
Auxin	0.203						
Control	0.261						
Drought (C)		1	0.062	0.062	10.876	0.001	0.067
Drought	0.255						
No drought	0.206						
Interactions							
AxB		2	0.059	0.029	5.224	0.006	0.064
AxC		1	0.042	0.042	7.465	0.007	0.045
BxC		2	0.056	0.028	4.952	0.008	0.061
AxBxC		2	0.005	0.002	0.445	0.6410	0.005
Unexplained		108	0.616	0.005			0.671
Total		119	0.918				

Table 2.9 Three-way analysis of variance for % Hyphal colonization of roots before stress as a response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces),* presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	9091.712	9091.712	961.691	<0.001	0.845
VAM present	17.361						
VAM absent	00.000						
Hormone (B)		2	92.068	46.034	4.869	0.009	0.008
Control Vs Hormone		1	81.925	81.925	8.665	0.003	0.007
ABA Vs Auxin		1	10.522	10.522	1.113	0.293	0.001
ABA	9.054						
Auxin	9.660						
Control	7.772						
Drought (C)							
Interactions							
AxB		2	92.068	46.034	4.869	0.009	0.008
Unexplained		108	1021.019	9.454			0.094
Total		119	10752.742				

Table 2.10 Three-way analysis of variance for % Arbuscular + vesicular colonization of roots before stress as a response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces)*, presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	6297.420	6297.420	1333.354	<0.001	0.882
VAM present	14.424						
VAM absent	00.000						
Hormone (B)		2	48.504	24.252	5.134	0.007	0.006
Control Vs Hormone		1	38.027	38.027	8.051	0.005	0.005
ABA Vs Auxin		1	10.21	10.21	2.163	0.144	0.001
ABA	6.575						
Auxin	7.204						
Control	8.258						
Interactions							
AxB		2	48.504	24.252	5.134	0.007	0.006
Unexplained		108	510.083	4.723			0.071
Total		119	7130.078				

Table 2.11 Three-way analysis of variance for % hyphal colonization of roots at harvest as a response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces),* presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	12995.735	12995.735	150.374	<0.001	0.798
VAM present	20.741						
VAM absent	00.000						
Hormone (B)		2	427.760	213.880	18.932	<0.001	0.026
Control Vs Hormone		1	191.658	191.658	16.965	<0.001	0.012
ABA Vs Auxin		1	238.886	238.886	21.146	<0.001	0.014
ABA	9.739						
Auxin	13.033						
Control	8.830						
Drought (C)		1	99.106	99.106	8.772	0.003	0.006
Drought	11.391						
No drought	9.666						
Interactions							
AxB		2	427.760	213.88	18.932	<0.001	0.026
AxC		1	99.106	99.106	8.772	0.003	0.006
BxC		2	536.926	268.463	23.764	<0.001	0.033
AxBxC		2	536.926	268.463	23.764	<0.001	0.033
Unexplained		108	1220.073	11.300			0.075
Total		119	16272.018				

Table 2.12 Three-way analysis of variance for % arbuscular + vesicular colonization at harvest as a response variable in a fully-crossed factorial design with presence or absence of VAM *(Glomus intraradieces),* presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	23565.993	23565.993	1825.452	<0.001	0.865
VAM present	27.895						
VAM absent	0.000						
Hormone (B)		2	676.968	338.484	26.219	<0.001	0.024
Control Vs Hormone		1	647.980	647.980	50.193	<0.001	0.023
ABA Vs Auxin		1	30.809	30.809	2.386	0.012	0.001
ABA	13.122						
Auxin	11.753						
Control	17.780						
Drought (C)		1	71.804	71.804	5.562	0.020	0.002
Drought	13.337						
No drought	15.050						
Interactions							
AxB		2	676.968	338.484	26.219	<0.001	0.024
AxC		1	71.804	71.804	5.562	0.020	0.002
BxC		2	544.512	272.256	21.089	<0.001	0.020
AxBxC		2	544.512	272.256	21.089	<0.001	0.020
Unexplained		108	1394.245	12.910			0.051
Total		119	27415.833				

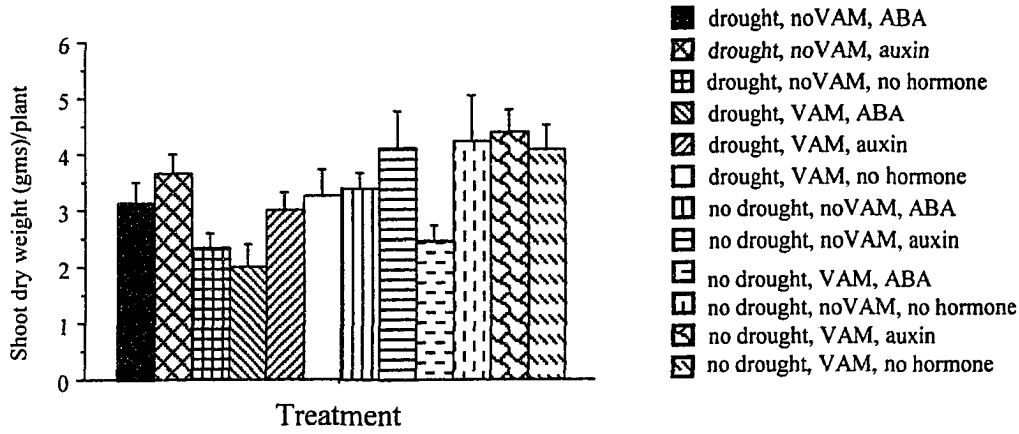

Fig 2.1.a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with shoot dry weight (gm) as the response.

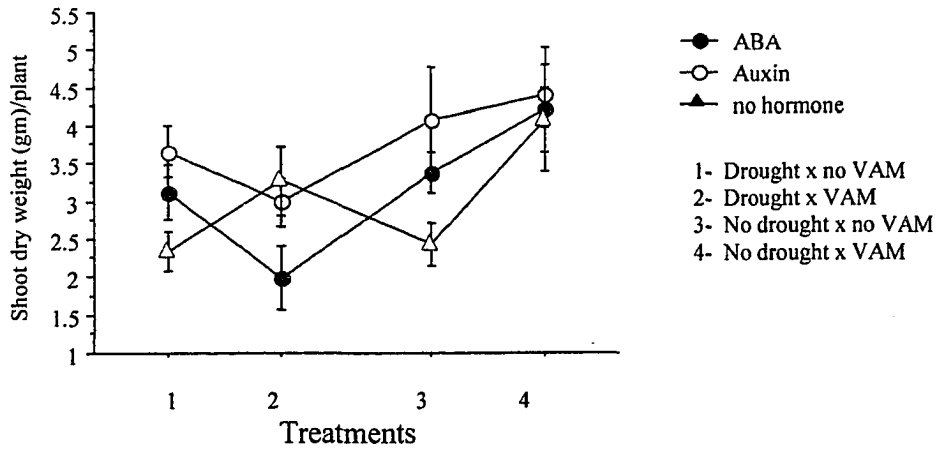

Fig 2.1.b. Mean shoot dry weight (gm) in interaction with drought, hormone and VAM

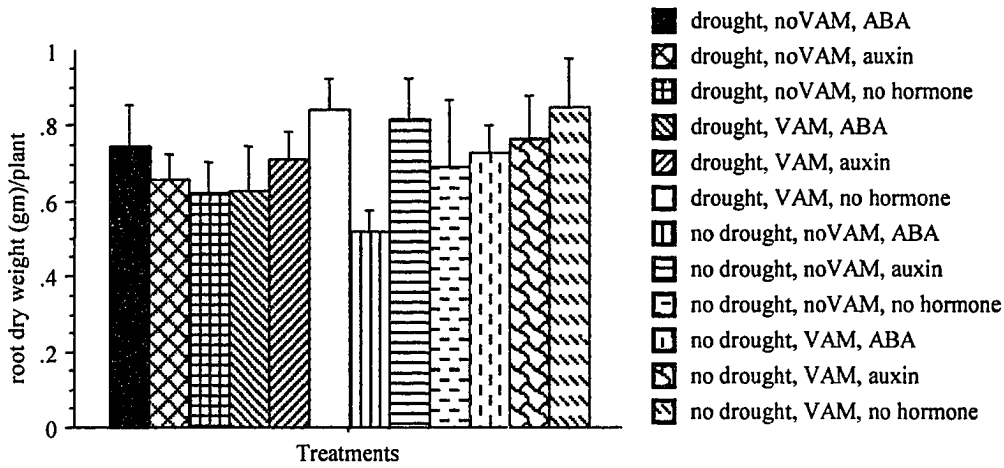

Fig 2.2.a. Means with 95 % confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with root dry weight (gm) as the response.

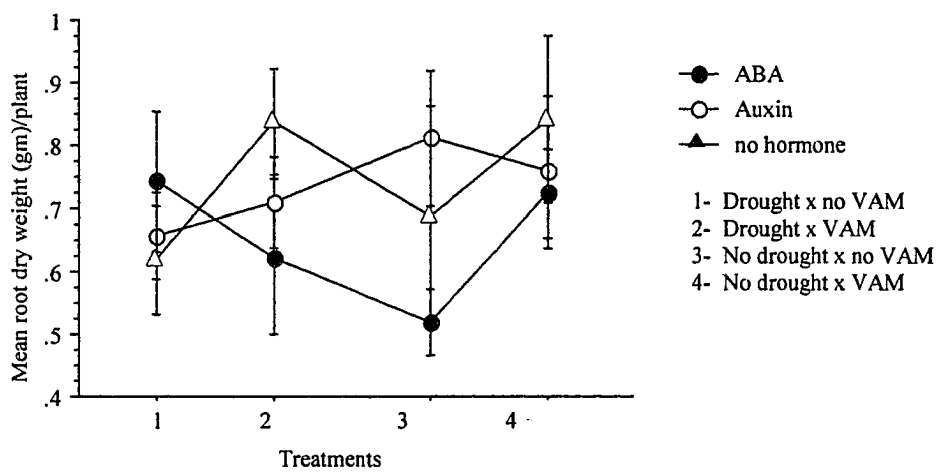

Fig 2.2.b. Mean root dry weight (gm) in interaction with drought, hormone and VAM status.

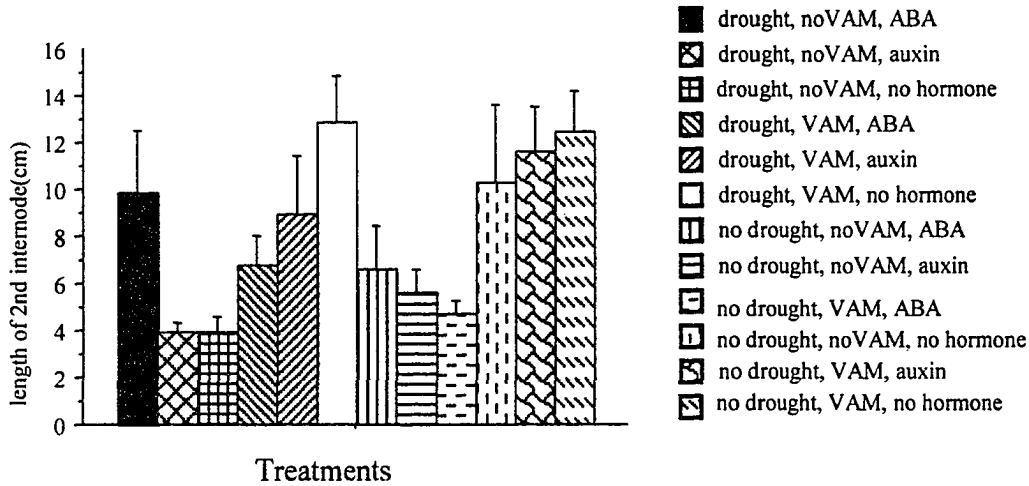

Fig: 2.3.a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with length of 2nd inter-node as the response.

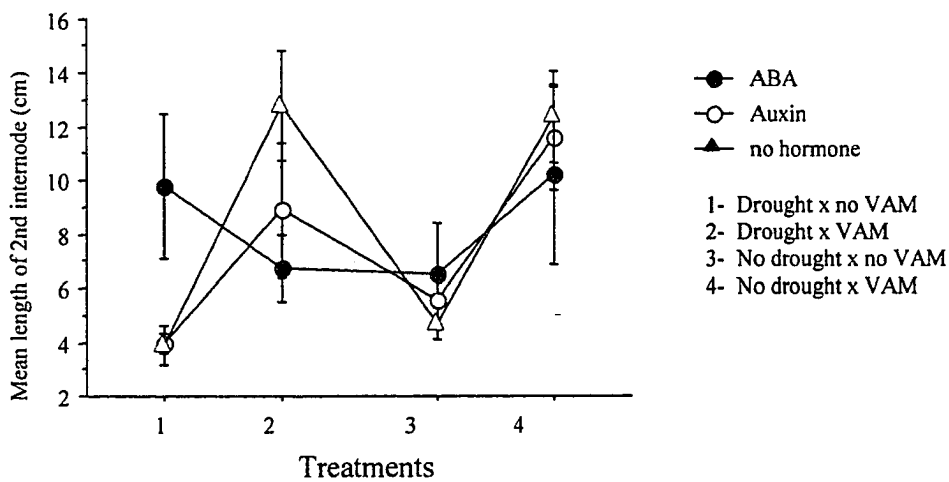

Fig 2.3 b. Mean length of 2nd internode in interaction with drought, hormone and VAM status

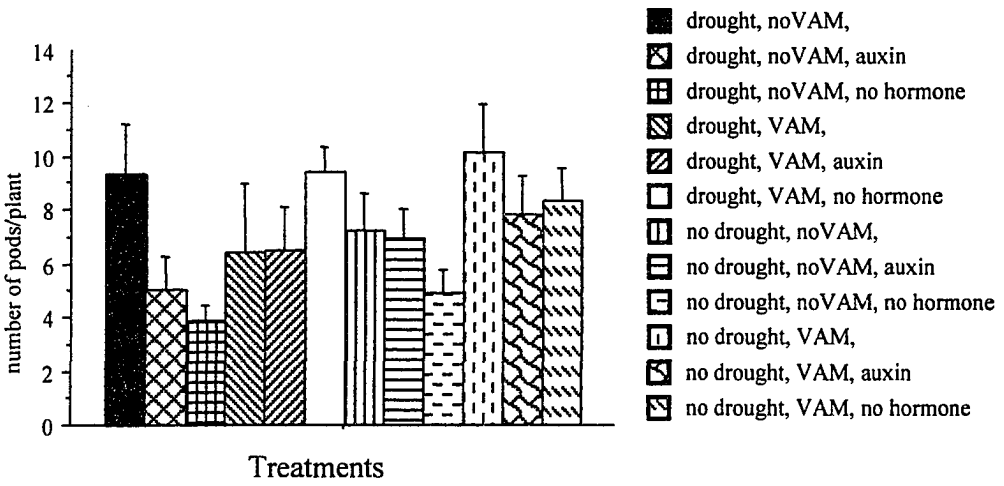

Fig: 2.4.a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with number of pods as the response.

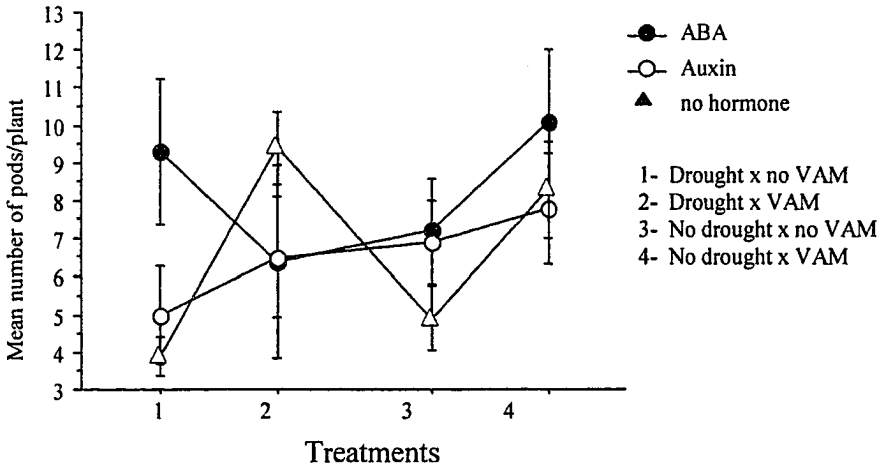

Fig 2.4.b. Mean number of pods in interaction with drought, hormone and VAM status.

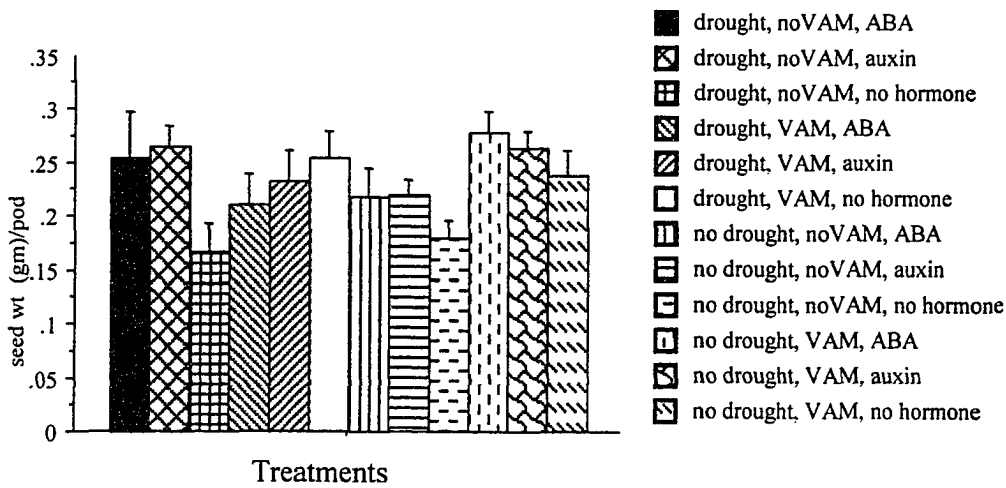

Fig 2.5.a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with seed dry weight (gm)/pod as the response.

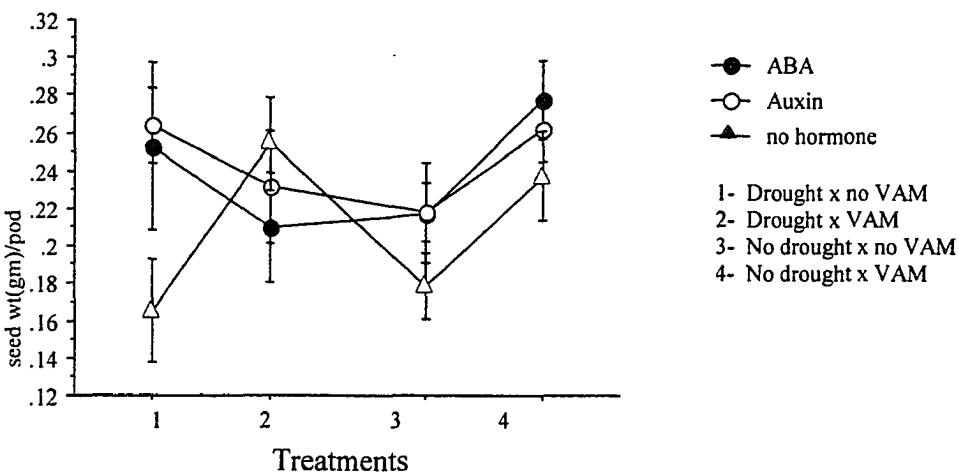

Fig 2.5b. Mean seed dry weight (gm)/pod in interaction with drought, hormone and VAM

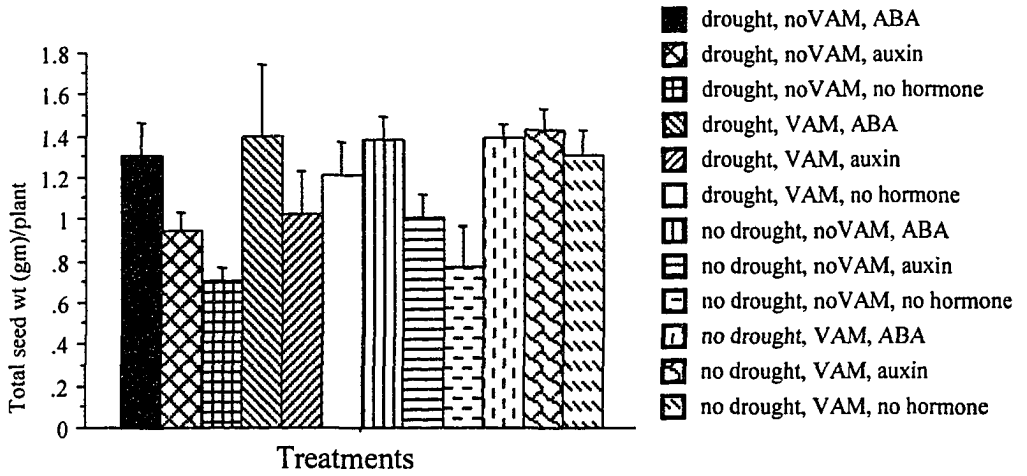

Fig 2.6.a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with total seed weight (gm)/plant as the response.

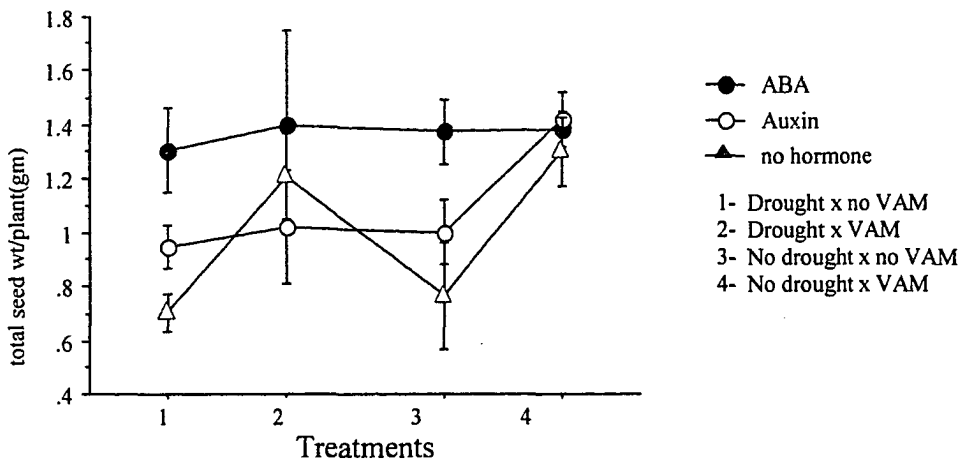

Fig 2.6.b. Mean total seed weight (gm)/plant in interaction with drought, hormone and VAM status.

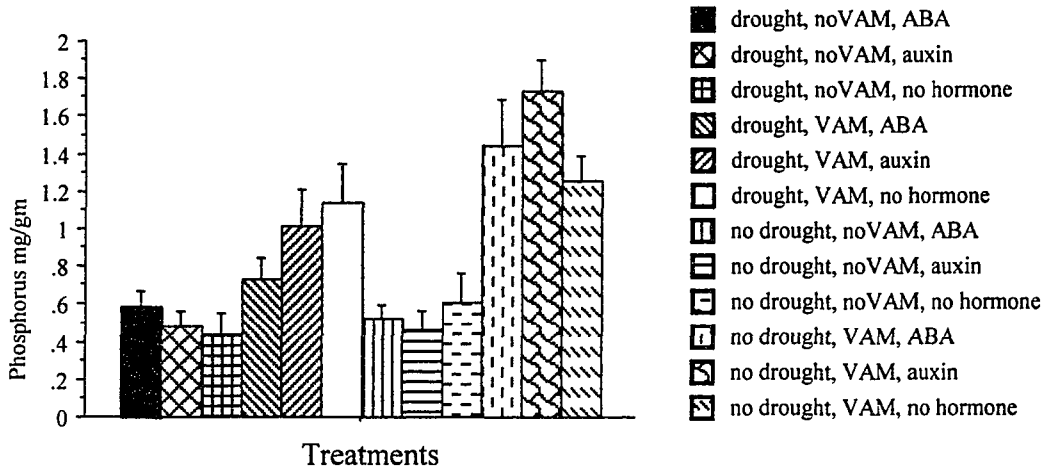

Fig 2.7.a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with Phosphorus content (mg/gm of plant) as the response.

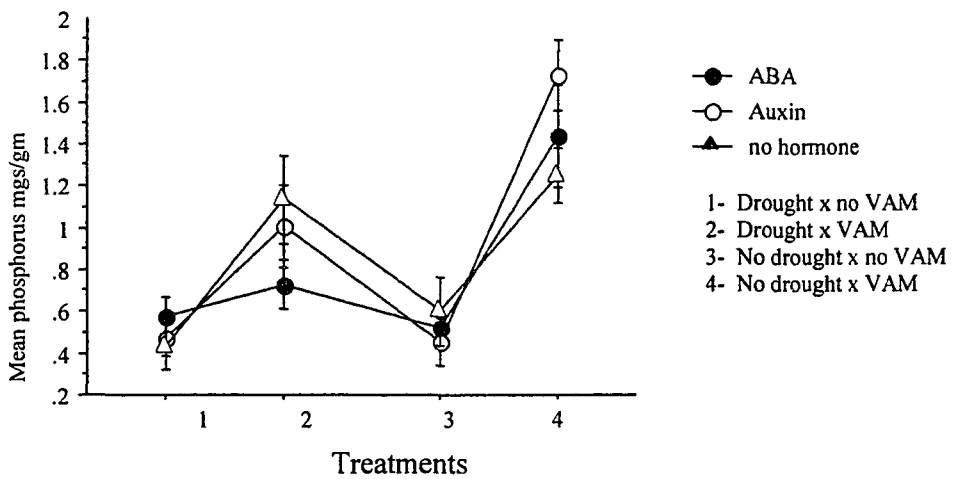

Fig 2.7.b. Mean phosphorus (content mg/gm of plant) in interaction with drought, hormone and VAM status.

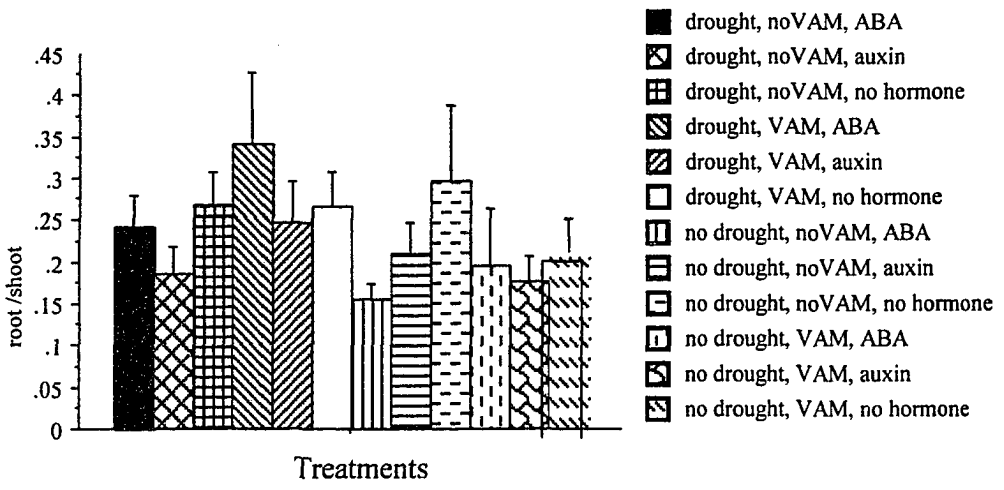

Fig 2.8a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with root/shoot dry weight ratio as the

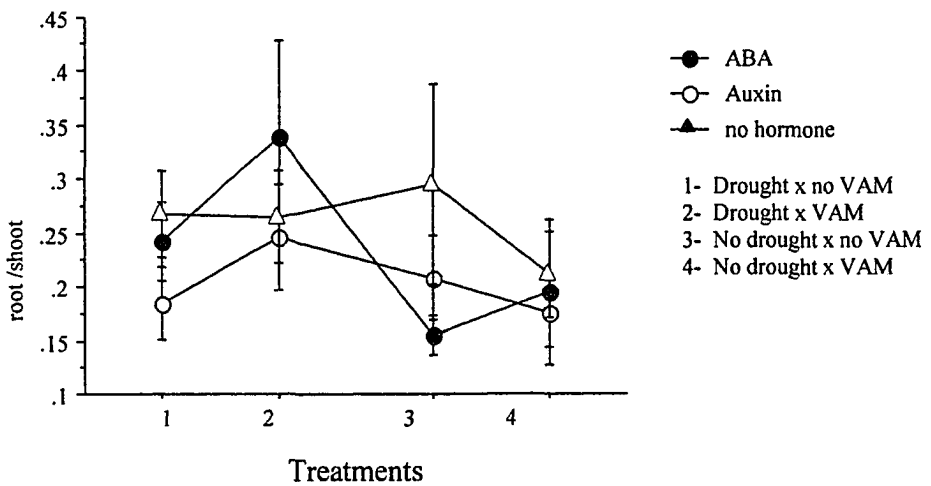

Fig 2.8.b. Mean root/shoot dry weight ratio in interaction with drought, hormone and VAM status.

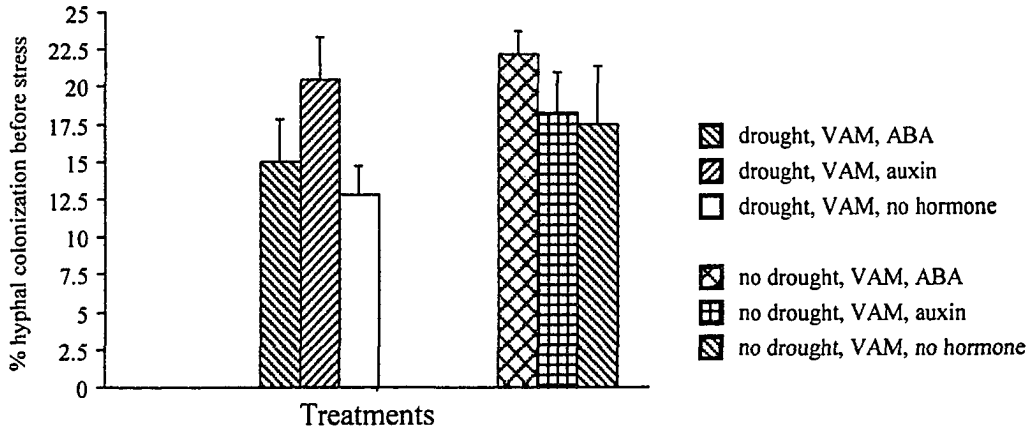

Fig 2.9.a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with % hyphal colonization before

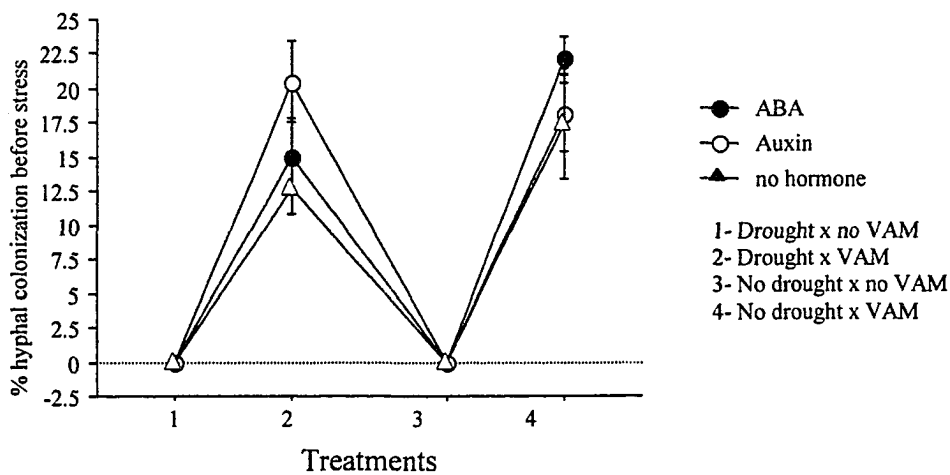

Fig 2.9.b. Mean % hyphal colonization before stress in interaction with drought, hormone and VAM status.

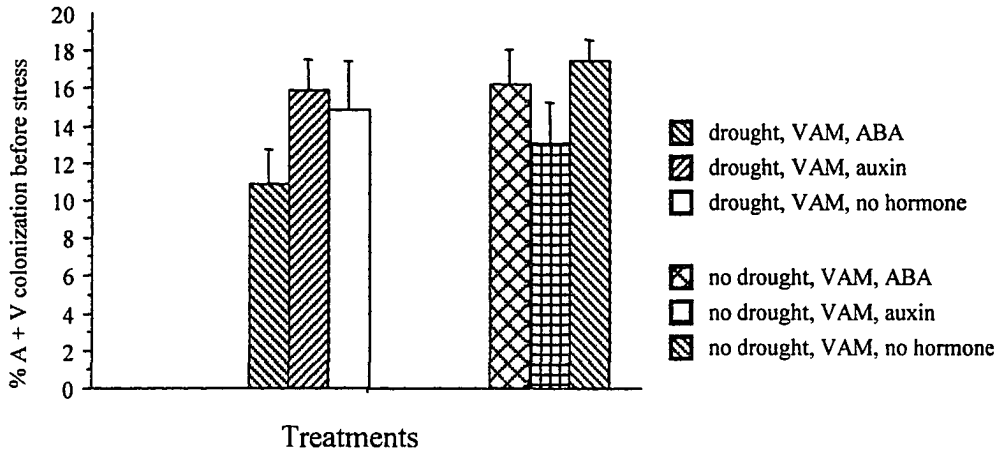

Fig 2.10. a. Means with 95% confidence intervals for all combinations of factors in a three way design of drought, VAM and hormones with % arbuscular + vesicular colonization before

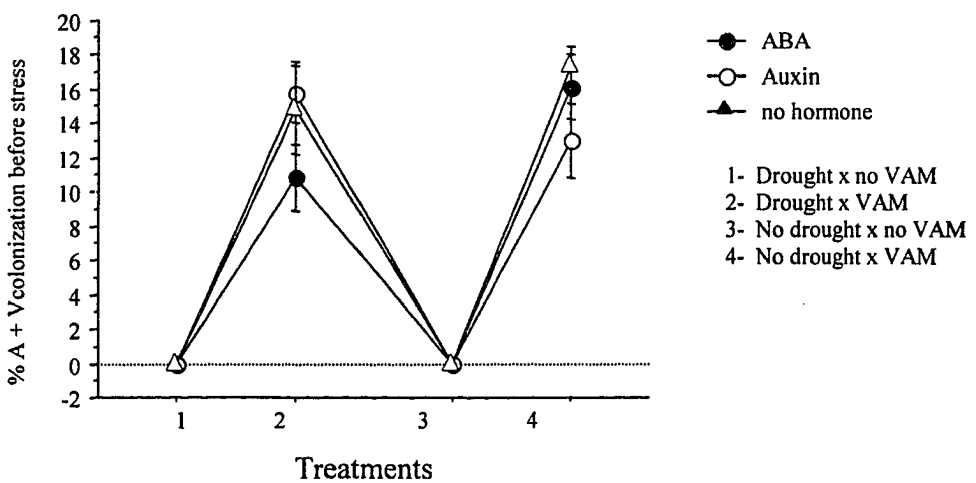

Fig 2.10.b. Mean % arbuscular + vesicular colonization in interaction with drought, hormone and VAM status.

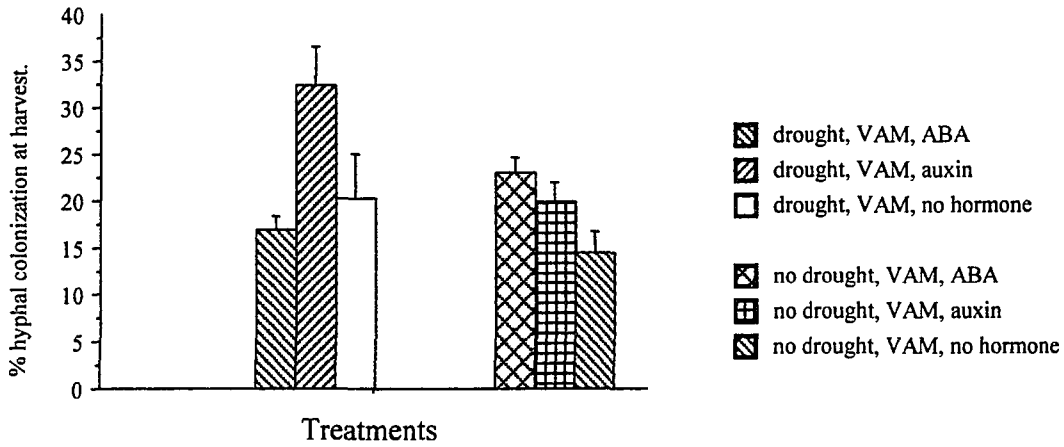

Fig 2.11.a Mean with 95% confidence interval for all combinations of factors in a three-way design of drought, VAM and hormones with % hyphal colonization at harvest as the response.

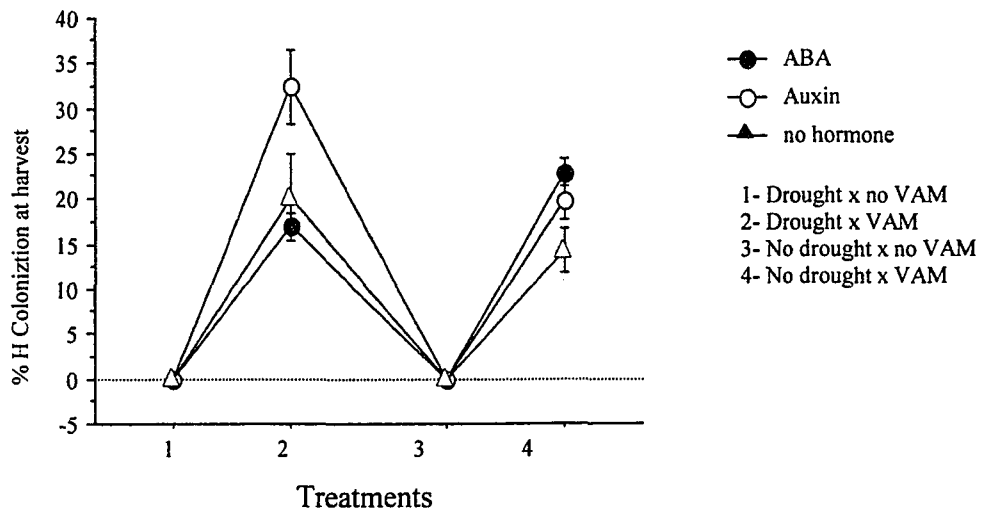

Fig 2.11.b. Mean % hyphal colonization at harvest in interaction with drought, hormone

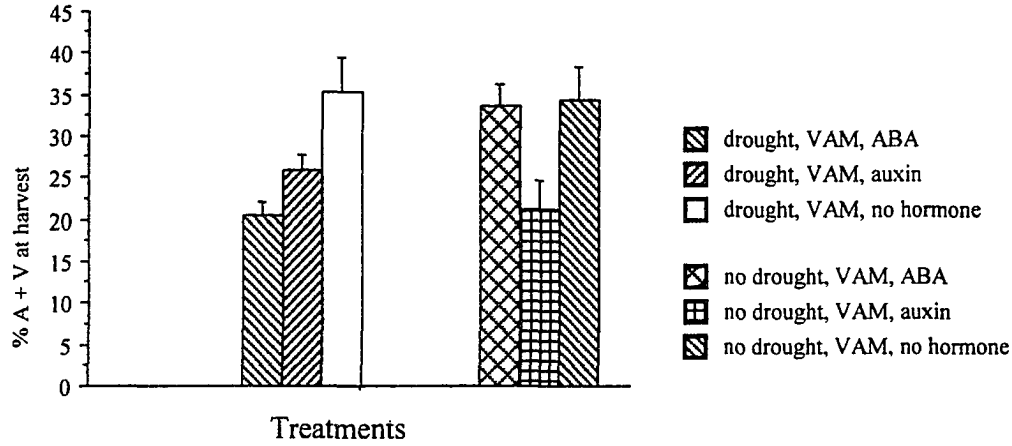

Fig 2.12.a. Mean with 95% confidence intervals for all combinations of factors in a three-way design of drought, VAM and hormones with `% arbuscular + vesicular colonization at harvest as the response.

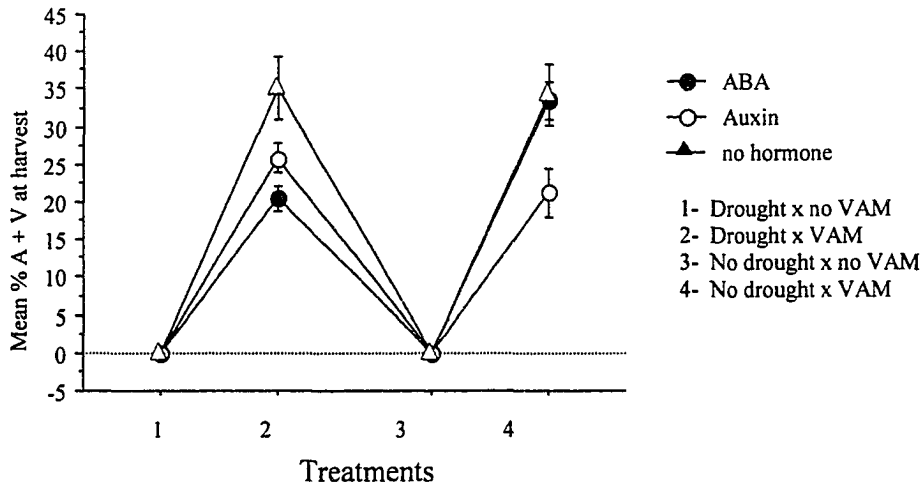

Fig 2.12.b. Mean % arbuscular + vesicular colonization in interaction with drought, hormone and VAM status.

Fig 2.13 Control Vs VAM x no drought x no hormone

Fig 2.14 Control Vs no VAM x Drought x Auxin

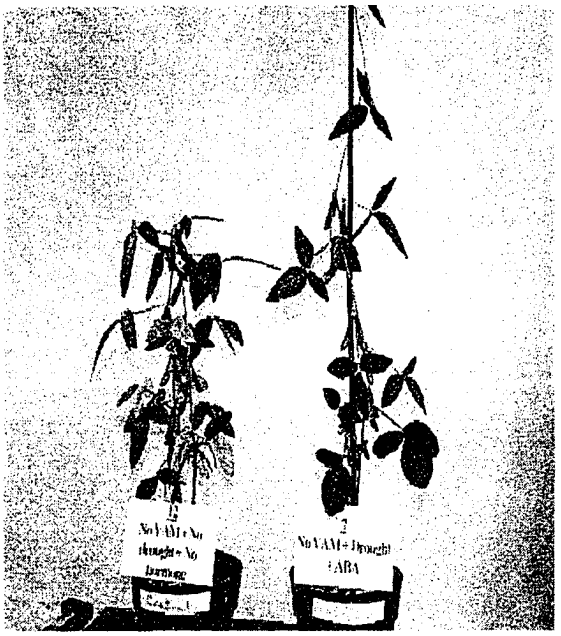

Fig 2.15 Control Vs no VAM x Drought x ABA

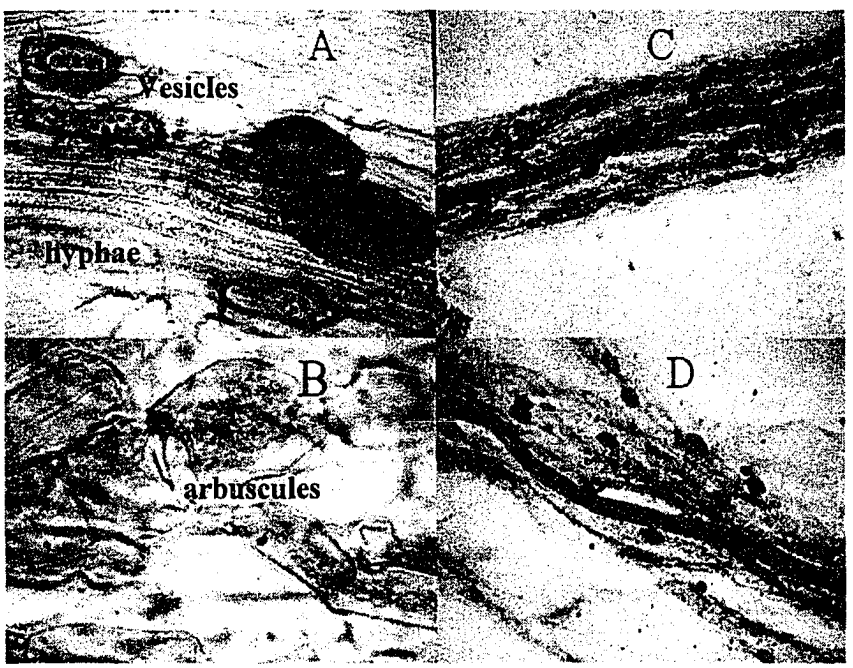

Fig 2.16 Squashed preparation of *Glomus intraradieces* infected root of of soybeans showing A; vesicles (400 x) with mature endospores B; arbuscules (400 x) C and D at 200 X showing infection by hyphae , arbuscules and vesicles.

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Chapter 3

Experiment 2

"The effect of *Glomus fasciculatum* on the vegetative growth, yield and phosphorus uptake of drought stressed soybeans along with exogenous application of ABA/Auxin".

3.1. Results

3.1.1. Vegetative growth: shoot dry weight (gms)/plant

The results of three-way ANOVA are presented in Table 3.1 and show that the VAM (*Glomus fasciculatum*) treatment influenced the shoot dry weight significantly ($P<0.001$) with an R-sq of 58.8 %. The hormonal application also caused a significant difference in shoot dry weight as compared to control, as the result of an orthogonal comparison show ($P<0.001$) with an R-sq of 5.2 %. However, the result of a second orthogonal comparison showed no significant differences in terms of the impact of ABA versus IBA on shoot dry weight. The droughted plants had significantly, lower shoot dry weight as compared to the non-droughted plants ($P<0.001$), with drought accounted for 14.2 % for the variability in shoot dry weight. The interaction of VAM x Hormone and VAM x Drought also caused significant differences in the shoot dry weight $P=0.003$ and $P<0.001$ respectively. The mean values for shoot dry weights are given in Fig 3.1.a. Figure 3.1.b illustrates the interactive effect of VAM, Drought and hormones on the shoot dry weight. Fig 3.13-3.15 illustrate growth of plants of different treatments.

3.1.2 Root dry weight (gms)/plant

The VAM plants root dry mass was significantly different from non-VAM plants ($P<0.001$) as shown in Table 3.2. VAM treatment accounted for 10.3 % variability. None of the other treatments had any significant effect on the root dry weight except for the three way interaction of VAM x Hormones and Drought ($P<0.001$) which was responsible for 12.9 % of the variability. Fig. 3.2.a and 3.2.b present the bar graph for mean values and the interactive effect of all three hormones on the root dry weight.

3.1.3. Length of 2nd internode (cm)

The three-way analysis of variance given in Table 3.3 shows that the VAM was responsible for 44.8% of the variability in the length of 2nd internodes; a statistically significant difference as compared with non-VAM plants. Hormonal application caused a significant difference over the control ($P<0.001$) with an R-sq of 4.5 %. However, the influence of both hormones was not different from one another. Droughted plants had significantly smaller 2nd internodes as compared to the non-droughted plants ($P<0.001$). Drought was responsible for 16 % variability of the length of 2nd internodes. The interaction of VAM x Hormone was accounted for 5 % variability; a statistically significant contribution ($P<0.001$). The interaction of VAM x Hormone also caused a statistically significant variation ($P<0.001$) with an R-sq of 11.4%. None of the other interaction had any significant effect on this dependent variable (Fig. 3.3.b).

3.1.4. Reproductive growth: number of pods/plant

The number of pods was significantly higher in the VAM infected plants ($P<0.001$) as compared to non-VAM, the R-sq for the treatment was 34.6 % (Table 3.4). The hormonal treatment has an R-sq of 5.3 %.

As shown by the orthogonal comparisons, there was a significant difference in the number of pods of the plants that received the hormonal application as compared to the control (P = 0.001) and also that the output of ABA and IAA was also significantly different from one another (P= 0.001). The cell bar graph in Fig 3.4.a describes the relative differences in the mean values of all the treatments.

The drought also had a significant effect (P<0.001) with an R-sq of 6.1 %. The interaction of VAM x Hormone caused a significant variability of number of pods/plant with an R-sq of 5.8 % and a P<0.001. The interaction of all three factors i.e. VAM x Hormone X drought also caused a statistically significant variation with a P= 0.01 and an R-sq of 3.2 %. Fig 3.4.b describes the result of interaction of VAM and drought along with hormonal application.

3.1.5. Reproductive growth: weight of seeds (gms) / pod

Table 3.5 describes the results of three-way-ANOVA of seed weight/pod of the plants of all the treatment groups. The VAM infected plants had a significantly higher weight of seed /pod as compared with non-VAM plants (P<0.001). The variability due to VAM was 62 %. The next biggest contributor was hormonal application, statistically significant (P<0.001) with an R-sq of 8 %. The cell bar chart in Fig 3.5.a describes the differences in the mean values. No difference was revealed in the influence of ABA versus IAA on the weight of seed /pod. Drought caused a variability of 3.4 %, a significant contribution (P<0.001). None of the interactions caused any statistically significant difference in the seed weight /pod of the plants.

3.1.6. Reproductive growth: total seed weight (gms) /plant

The VAM treated plants had a significantly different weight of seeds /plant ($P<0.001$) with, variability of 39 % as shown by Table 3.6. The hormonal application also caused a significant difference ($P<0.001$) and contributed a 6.5 % of the differences due to treatment effects. The results of orthogonal comparisons show that not only there was significant difference between the hormone versus control plants, but also between the plants that received ABA were significantly different from the plants that received IAA in this respect.

Drought caused a variability of 9.5 % in the total seed weight /plants; a statistically significant effect ($P<0.001$). All the interactions also caused significant difference in the total seed weight of the plants. VAM x Hormone was responsible for 4.4% of the variability. The VAM x drought interaction resulted in an R-sq of 11.2%. Hormone x Drought 3.6 % and VAM x Hormone x Drought responsible for 1.3 % $P=0.5$, which is still statistically significant outcome. The results of VAM, drought and ABA/IAA or control interaction are illustrated in Fig. 3.6.b

3.1.7. Phosphorus content mgs/gm of plant tissue

Table 3.7 reports the results of a, three-way-ANOVA for the phosphorus content of the plants. The phosphorus content of the VAM infected plants came out to be 20.3 % different from the non-VAM plants ($P< 0.00$). The plants that received the hormonal application also showed significantly different phosphorus content of the plants ($P=0.003$) with R-sq of 1.3 %. The results of orthogonal comparisons revealed a significant difference in the uptake of ABA treated versus Auxin treated plants ($P=0.003$). The drought caused a variability of 20.3 % in the phosphorus content of the

plants with P <0.001. The cell bar reveals the relative differences of the mean value. All the interactions caused a significant difference in the phosphorus content. The VAM x Hormone interaction caused a variability of 2.7 % and VAM x Drought 2.2 %. VAM x Drought was responsible for 5.4 % R-sq and VAM x Hormone x Drought caused a variability of 2.8%. Fig. 3.7.a. describes how drought decreased the phosphorus content of ABA/IBA or control plants

3.1.8. Root/shoot ratio

Glomus fasciculatum was found to be responsible for 35.7 % variability in the root/ shoot ratio of the soybeans (Table 3.8). As the mean values given in Table 3.8 reveal that VAM infection lowered the root/shoot dry weight ratio. The hormonal application (ABA/IBA also lowered the root/shoot ratio as shown by the mean values given in Table 3.8. The difference of root/shoot ratio of the plants that received hormonal application was significantly lower than the control (P<0.001); the treatment accounted for 13.8 % of the variability. No significant differences were found in ABA versus Auxin treated plants in this regard (P 0.57). The droughted plants had significantly higher root/shoot ratio as compared to the non- droughted ones (P<0.001). Drought treatment yielded 3.8% R-sq. The Fig. 3.8.a reveals the mean values in the form of cell bar charts. All the interaction treatments had statistically significant effect, except VAM x Drought. VAM x Hormone caused a difference of 6.1 %, Hormone x Drought 2.8 %, and VAM x Hormone x Drought 5.5 %. Fig. 3.8.a describes how the presence of VAM lowered the root shoot ratio of droughted and non droughted plants in ABA/IAA and Control plants.

3.1.9. VAM colonization of roots before water stress (% Hyphal and %arbuscular + vesicular colonization)

The results of three way ANOVA (Table 3.9) for the % hyphal colonization of roots show that 88.7 % variability of this response was caused by VAM itself and the rest was contributed by other treatments. The only other treatment that caused a significant effect at the 5 % level (P = 0.01) was the interaction of VAM x Hormone (R-sq 0.6%). The mean values show, that the plants that received the hormonal application had lower hyphal colonization than the control (Figure 3.9.a and Fig 3.9.b). In the case of % arbuscular + vesicular colonization before stress, the most variability was caused by VAM itself with an R-sq of 92 .2% (Table 3.10). The hormonal application contributed significantly (P = 0.001) with an R-sq of 0.6% only. There were no differences in terms of the application of ABA versus IAA on the root colonization of the VAM (Fig 3.10.a and 3.10.b).

3.1.10. VAM colonization of roots at harvest (% Hyphal and % arbuscular +vesicular colonization)

At the time of harvest % hyphal colonization variability was due to VAM, with an R-sq of 75 %. The hormonal application accounted for 1.7 % of R-sq a statistically significant effect (P<0.001). The result of orthogonal comparisons revealed a significant difference between hormone treated versus control, but no difference in the influence of ABA versus IAA application in this arena, as shown by the interaction plot in Fig3.11.b. Non-droughted plants had significantly higher hyphal colonization as compared to the drought stressed plants (P <0.001).

As far as the interactions of different factors are concerned, they all contributed significantly towards the variability of hyphal colonization of plants across the treatments. VAM x Hormone interaction caused a variability of 1.7 % while the VAM x drought 3.3%. The interaction of Hormone x Drought and VAM x Hormone x Drought had an R-sq of 2.1 % each. Fig 3.11.b describes the mean for all the treatments

The % arbuscular + vesicular colonization was also subjected to three-way ANOVA (Table 3.12), and it was revealed that 91 % of the variability was due to VAM. The hormonal application caused a variability of 0 .9 % with a $P<0.001$, a significant difference from control. There was however no significant difference due to ABA versus IAA treatment on the arbuscular + vesicular colonization of root in the absence of drought (Fig3.12.b). In the presence of drought however there was no significant differences in the infection of ABA/IAA or control plants (Fig. 3.12. and Fig3.12.b.). Droughted plants had a significantly higher infection (mean values given in Table 3.12) as compared with non- droughted plants R-sq of 1.9%.

The interaction of all the independent variable combinations altered the Arbuscular + vesicular colonization significantly, each had $P<0.001$. The variability caused by VAM x Hormone interaction was 0.9% Due to VAM x Drought 1.9%. Hormone x Drought and VAM x Hormone x Drought each had an R-sq of 0.4 %. Fig 3.16 describes some characteristics of *Glomus fasciculatum* infection in the roots.

3.2. Discussion

3.2.1. Vegetative growth: shoot dry weight (gms)/plant

There is mounting evidence regarding the dependence of plants on VAM. In the present study, plants infected with *Glomus faciculatum* had greater shoot dry weight than non-VAM plants. This could be due to increased photosynthetic rate in *Glomus fasciculatum* infected plants, as compared to non-VAM plants (Bildusas et al. 1986). Although in the present study the plants of all the treatments were grown at the same phosphorus level, the mycorrhizal plants had improved foliage growth in drought stressed plants as compared to non- Mycorrhizal plants even if the later are supplied with extra phosphorus (Goicoechea *et al.*, 1995). Subhan *et al.*, (1998) found that *Glomus fasciculatum* increased the shoot height number of leaves and dry weights of shoots and roots of micrpropagated *Sesbania sesban* (Fabaceae) throughout the growth period. Another reason for the increased biomass production could be enhanced uptake of phosphorus as explained later. Satpal and Kapoor (1980) reported increased grain and straw yield of mungbean followed by *G. fasciculatum* infection alone or along with other phosphorus solubilizing microorganisms.

The results of the present study showed an increased shoot dry weight of VAM inoculated drought stressed plants as compared to non-VAM droughted plants. Many other investigators (Ruiz-Lozano *et al.*, 1995; Ryan and Ash 1996) have reported similar findings. This can be due to lower uptake of phosphorus or a better absorption of water by VAM infected plants (Busse and Ellis, 1985). It was also found in the present study that drought stressed non-VAM, plants had lower phosphate content as compare to the VAM plants which were subjected to drought stress.

There are few reports regarding the study of plant responses to exogenously applied hormones. Zuzana *et al.,* (2000) found that ABA application moderately increased the dry mass production of tomato plants as compared to control. The results of the present study showed that exogenously applied hormones to VAM plants can increase shoot growth as compared to VAM alone (Fig. 3.1.b). It is possible that exogenously applied auxin can increase the endogenous level of this hormone in the plants and promote growth as was found by Abdel-Gaffar (1998) in soybeans and maize plants. In contrast, Little *et al.,* (2003) found no influence of auxin application on the foliage parts of the two evergreens trees. Further study is needed to make a concrete statement regarding the impact of exogenous application of hormones, especially in combination with mycorrhizae and drought stress.

3.2.1. Root dry weight (gms)/plant

The VAM infected plants are known to have thicker roots and less dense root hairs as compared to non-VAM plants (Xio *et al.,* 1991; Esche *et. al.,* 1994). The results of the present study agree with these reports, showing a significantly higher root mass of VAM plants. Drought stressed VAM plants that received exogenous application (VAM x Hormone x Drought) also had improved growth of roots, especially when treated with IBA, which gave the highest root dry mass. Exogenously applied IAA can increase the root dry weight of the soybeans and maize plants (Abdel-Gaffar 1998). As discussed in the previous chapter regarding the role of *Glomus intraradieces,* mycorrhizae act as root stimulants just like auxins (Bucaklew, 2003). Another point of view is that the decreased activity of IAA oxidizing enzymes in the roots can enhance the level of IAA in the roots, hence promoting the root growth (Mitchell et al., 1986).

The mode of VAM influence on the root growth is supported by another piece of evidence that the presence of VAM and Auxin simultaneously in a medium generally promotes better rooting (Carolyn, 2003). Exogenous application of IAA can also stimulate lateral rhizogenesis (Karabaghli *et al.*, 1998).

3.2.3. Length of 2nd internode

Fig. 3.3.b. describes the increased length of the 2nd internode in the VAM infected plants that received hormonal application as opposed to non-VAM plants. Another interaction that increased the length of 2nd internode was VAM x Drought. Tawaraya *et al.*, (2001) found that the shoot growth was highly dependent on the mycorrhizal colonization.

Similarly Feng *et al.* (2002) reported that mycorrhizal maize plants maintained higher root and shoot dry weights and higher Phosphorus uptake. The increased shoot growth can be attributed to higher phosphate uptake. Although it was not included in this study, there is a considerable body of evidence regarding the improved uptake of other nutrients, such as nitrogen by *Glomus* infected plants, as compared to non-VAM grown at similar nutrient levels (Azcon and Tobar 1998). *Glomus fasciculatum* has also been known to increase the vegetative growth of other crops such as rice (Secilia and Bagyaraj, 1994). Other species of *Glomus* have been known to improve growth in plants such as *Abutilon theophrasti* Medic (Lu and Koide 1994), maize and sorghum under drought stress (Osonubi, 1994). One reason for improved growth of VAM infected, drought stressed plants is that during the recovery periods the CO_2 assimilation rates have been reported to increase as compared to non-VAM plants. This suggests another aspect of VAM's influence on host plants in addition to improved phosphorus uptake.

3.2.4. Root to shoot ratio

Different types of abiotic environmental factors influence the productivity of the crops throughout the world; and drought is one of them. In disadvantageous environmental conditions like insufficient nutrients in the soil, growth promoting and yield enhancing effects of the universally present vesicular arbuscular mycorrhizae cannot go unnoticed. In the present study, VAM along with the hormonal application increased the shoot dry weight of droughted as well as non-droughted plants. VAM also helped to increase the root dry weight significantly, but increase in the shoot dry weights was much higher. Therefore, the root/shoot ratio of VAM infected plants was lower than non-VAM plants that did not receive any hormonal application. The findings of Al-Karaki (1998) support the results of present study. Ruiz-Lozano *et al.* (1995) found that the root/shoot ratios of drought stressed *G. fasciculatum* infected plants were lower than the non-stressed inoculated plants. This clearly indicated the ability of *G. fasciculatum* to help soybean plants with water stress. Most likely, by improving the uptake of the nutrients, as the phosphorus content of the drought stressed VAM plants was not significantly lower than the VAM plants, which received plentiful water.

3.2.5. Reproductive growth (number of pods, seed weight (gms)/pod and total seed weight (gms) /plant

From ecological agricultural perspectives, we are interested in characterizing the drought tolerance of both partners in a mycorrhizal symbiosis, in terms of growth yield, and survival. VAM symbiosis appears to affect these mostly through drought avoidance, often associated with improved phosphorus uptake. In about 80 –90 % of the

mycorrhizal studies, the VAM plants had higher growth and yield than their non-mycorrhizal counterparts. This supports the hypothesis that mycorrhizae can play an important role in promoting the drought tolerance of their host. The results of the present study show that VAM alone, in combination of hormonal application and along with hormonal application and drought increased the number of pods in the soybean plants, as compared to droughted and non-mycorrhizal plants. *Glomus fasciculatum* has been reported to cause a significant increase in the number of pods and grain yield in other crops (Mukherjee and Rai, 2000). The VAM x Drought interaction did not influence any aspect of the reproductive yield but the combination of VAM x Drought ABA improved the total seed weight significantly.

This may not be true at all times. For instance ABA treated plants subjected to water logging stress can have lower photosynthetic rates, due to stomatal closure caused by ABA (Ahmed *et al.*, 2002). In another study *Glomus* infected plants subjected to drought stress had higher grain yield and over all above ground biomass. It is possible because VAM also promotes root growth and rooting depth, which mean acquisition of more nutrients and water in a water stressed environment (Ellis, *et al.*, 1985).

VAM alone, in the absence of drought caused a highly significant increase in the yield. Busse and Ellis (1985) reported an increase in the total seed weight of plants infected with *Glomus fasciculatum* as compare to non-VAM plants. In case of water stress, *Glomus fasciculatum* infected plants had a higher number of pods, number of seed, and total seed weight, suggesting a potential benefit of *Glomus fasciculatum* to drought stressed soybeans. In the present study, VAM inoculated drought stressed plant had significantly higher total seed weight/plant.

The number of pods and seed weight /pod was not influenced by this combination significantly, (Fig. 3.4.a and Fig. 3.4.b for number of pods/plant and Fig. 3.5.a and Fig. 3.5.b for total seed weight /pod).

3.2.6. Phosphorus content mgs/gm of plant

Most of the phosphorus present in the soil is in unavailable forms with low solubility (Sanyal and De Datta, 1991). Microorganisms present in the rhizosphere use their enzyme systems to help solubilize this phosphorus and make it available to the plants (Kapoor *et al.*, 1989; Kucey *et al.* 1989). Mycorrhizal plants can take up more phosphorus than non- mycorrhizal plants (Barea. 1991). The Phosphorus is known to perform an important function in several biosynthetic pathways in the plants. The infection of root by *Glomus fascuculatum* can play an important role in the uptake and translocation through the host plant (Shnyreva and Kulaev, 1994).

In the present study, the mycorrhizal plants had greater root dry weights than the non- VAM plants and also had higher phosphate contents as compared to non-VAM plants. There is a positive correlation between the root size and the amount of nutrients that are absorbed through them and transfer to the host tissue across specialized interfaces which are the result of the coordinated development of the organisms (Smith *et al.*, 1994)). The enhanced efflux from the fungus can be important for symbiotic phosphorus uptake by plants via mycorrhizal fungi (Smith *et al.*, 1994). Future research to understand how a balance is sustained across those interfaces for the exchange of materials between the symbionts will help enhance our understanding of the nature of relationship between the VAM and the host.

Results of the present study reflect that VAM plants had a significantly higher phosphorus content whether they were droughted or non droughted (Fig 3.7.a and 3.7.b). The non-droughted VAM plants however had higher phosphorus content than the droughted mycorrhizal plants. Sometime, VAM plant growth also exceeds non-VAM plant growth in well-watered plants of equal phosphorus level, indicating that VAM influence occurred by relieving phosphorus stress rather than drought stress.

Nevertheless, VAM plants had higher yield in dry soils as compared to poorly nourished non-mycorrhizal plants that were well watered. (Busse and Ellis, 1985). Another aspect that can be explored in terms of soybean–*Glomus* symbiosis under drought stress is the effect of added phosphorus in the stressed and non-stressed mycorrhizal plants. Because that has been reported to increase the drought tolerance of other crops (Osonubi, 1994).

3.2.7. % VAM infection (hyphal , Arbuscular + Vesicular)

In the present study the hyphal colonization was decreased due to the water stress in the root samples collected at harvest (mean values for droughted and non-droughted in Table 3.10). The change in the intensity of hyphal colonization due to drought varies from plant to plant, from one fungus species to another and is also influenced by the age of the plant. Nelson and Safir (1982) found that drought stress did not effect the colonization of soybean roots by the fungi. Busse and Ellis (1985) found a decrease in the infection immediately after the stress. Kasetsart (2000) observed an increase in the hyphal growth of different VAM fungi with age from 6 weeks to harvest. The present study was done in pots inside the greenhouse, but chronic drought may have different impact in terms of the intensity of hyphal or arbuscular colonization (Auge, 2001).

The % arbuscular and vesicular infection after drought stress was increased significantly (mean values in Table 3.12), regardless of the exogenously applied hormones (Fig 3.11.a and Fig 3.12.b). This can be due to the fact that the plants were grown in a limited phosphorus environment. Therefore, the amount of phosphorus became even less toward the end of the life cycle of the plants. The plants suffering from phosphorus deficiency accompanied by water stress stimulated the vesicular-arbuscular hyphal growth (Bolgiano *et al* 1983; Elias and Safir, 1984).

The hyphal colonization of the droughted plants that received hormonal application was lower than the plants that did not receive hormonal application. On the contrary, the treatment of shoot tip with IAA can increase the arbuscular intensity in some plants (Gunze and Hennessy, 1980). The present study shows that the benefits of exogenous application of hormones on soybean plants are independent of the influence of hormones on the vesicular and arbuscular colonization of the VAM.

Comparison of the mean values for Arbuscular + Vesicular infection before stress and at harvest as illustrated in Fig 3.10.a-b and 3.12.a-b shows that the intensity of these structures increases, with the life of the plant. The reproductive growth of fungus occurs more rapidly when root growth ceases or slows down (Abbot and Robson, 1991). The mycorrhizal dependency of the actively growing host delays the reproductive growth of the fungus. The arbuscules are the sites of nutrient transfer and their presence is indicative of the mutualism (Koske, 1992). Therefore, arbuscule number increases as the plant size increases which shows the mycorrhizal dependency of the host.

Development of arbuscules in plants is controlled by host nutrient demand (Koide and Li, 1990). High nutrients demand in plant is likely to be associated with active growth when respiratory and photosynthesis rates are higher and when mycorrhizae may be involved in the transport of nutrients (Sanders and Fitter, 1992).

Table 3.1 Three-way analysis of variance for shoot dry weight (gms) /plant as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	136.859	136.859	506.524	<0.001	0.588
VAM present	4.017						
VAM absent	1.881						
Hormone (B)		2	12.917	6.012	23.905	<0.001	0.052
Control Vs Hormone		1	12.269	12.269	45.411	<0.001	0.052
ABA Vs Auxin		1	0.648	0.648	2.398	0.124	0.002
ABA	3.085						
Auxin	3.265						
Control	2.497						
Drought (C)		1	32.920	32.920	121.841	<0.001	0.142
Drought	2.425						
No drought	3.473						
Interactions							
AxB		2	3.258	1.629	6.029	0.003	0.014
AxC		1	14.943	14.939	55.308	<0.001	0.064
BxC		2	1.051	0.525	1.946	0.147	0.004
AxBxC		2	1.493	0.746	2.763	0.067	0.006
Unexplained		108	29.180	0.270			0.125
Total		119	232.627				

Table 3.2 Three-way analysis of variance for root dry mass/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM(*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.375	0.375	18.025	<0.001	0.103
VAM present	0.777						
VAM absent	0.665						
Hormone (B)		2	0.095	0.047	2.298	0.105	0.026
Control Vs Hormone		1	0.013	0.013	0.651	0.421	0.003
ABA Vs Auxin		1	0.082	0.082	3.945	0.049	0.023
ABA	0.681						
Auxin	0.746						
Control	0.736						
Drought (C)		1	0.111	0.111	5.311	0.023	0.030
Drought	0.691						
No drought	0.751						
Interactions							
AxB		2	0.168	0.084	4.046	0.020	0.046
AxC		1	0.066	0.066	3.190	0.079	0.018
BxC		2	0.083	0.042	1.993	0.141	0.022
AxBxC		2	0.469	0.234	11.246	<0.001	0.129
Unexplained		108	2.251				0.622
Total		119	3.620				

Table 3.3 Three-way analysis of variance for length of 2nd internode as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	721.525	721.525	283.646	<0.001	0.448
VAM present	9.685						
VAM absent	4.780						
Hormone (B)		2	72.562	36.281	14.262	<0.001	0.045
Control Vs Hormone		1	72.490	72.490	28.497	<0.001	0.045
ABA Vs Auxin		1	0.072	0.072	0.028	0.866	0.000
ABA	7.812						
Auxin	7.752						
Control	6.133						
Drought (C)		1	258.280	258.280	101.535	<0.001	0.160
Drought	5.765						
No drought	8.700						
Interactions							
AxB		2	81.456	40.728	16.011	<0.001	0.050
AxC		1	183.892	183.892	72.291	<0.001	0.114
BxC		2	6.506	3.253	1.278	0.282	0.004
AxBxC		2	10.974	5.487	2.157	0.120	0.006
Unexplained		108	274.725	2.453			0.171
Total		119	1609.922				

Table 3.4 Three-way analysis of variance for number of pods/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM(*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	343.408	342.408	88.876	<0.001	0.346
VAM present	9.566						
VAM absent	6.183						
Hormone (B)		2	53.150	26.575	6.877	0.001	0.053
Control Vs Hormone		1	43.350	43.350	11.219	0.001	0.042
ABA Vs Auxin		1	9.800	9.800	2.536	0.001	0.010
ABA	8.650						
Auxin	7.950						
Control	7.025						
Drought (C)		1	60.208	60.208	15.582	<0.001	0.061
Drought	7.166						
No drought	8.583						
Interactions							
AxB		2	58.316	29.158	7.546	<0.001	0.058
AxC		1	6.075	6.075	1.572	0.212	0.006
BxC		2	21.116	10.558	2.732	0.069	0.021
AxBxC		2	31.550	15.775	4.082	0.019	0.032
Unexplained		108	417.300	3.863			0.421
Total		119	991.125				

Table 3.5 Three-way analysis of variance for weight of seeds(gms)/pod as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.458	0.458	283.608	<0.001	0.623
VAM present	0.315						
VAM absent	0.192						
Hormone (B)		2	0.059	0.029	18.461	<0.001	0.080
Control Vs Hormone		1	0.058	0.058	36.346	<0.001	0.080
ABA Vs Auxin		1	0.001	0.001	0.576	0.449	0.001
ABA	0.272						
Auxin	0.266						
Control	0.222						
Drought (C)		1	0.025	0.02	15.751	<0.001	0.034
Drought	0.239						
No drought	0.268						
Interactions							
AxB		2	0.001	0.000	0.607	0.546	0.001
AxC		1	0.004	0.004	2.579	0.111	0.005
BxC		2	0.005	0.002	1.674	0.192	0.006
AxBxC		2	0.006	0.003	1.877	0.157	0.007
Unexplained		108	0.174	0.002			0.236
Total		119	0.735				

Table 3.6: Three-wayanalysis of variance for the total seed weight/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	15.747	15.747	173.591	<0.001	0.390
VAM present	1.694						
VAM absent	0.970						
Hormone (B)		2	2.615	1.307	14.413	<0.001	0.065
Control Vs Hormone		1	0.624	0.624	6.881	0.009	0.015
ABA Vs Auxin		1	1.990	1.990	21.946	<0.001	0.049
ABA	1.541						
Auxin	1.225						
Control	1.230						
Drought (C)		1	3.862	3.862	42.583	<0.001	0.095
Drought	1.152						
No drought	1.511						
Interactions							
AxB		2	1.682	0.841	9.273	<0.001	0.042
AxC		1	4.551	4.551	50.172	<0.001	0.112
BxC		2	1.477	0.738	8.143	<0.001	0.036
AxBxC		2	0.554	0.227	3.054	0.051	0.013
Unexplained		108	9.797	0.090			0.243
Total		119	40.287				

Table 3.7 Three-way analysis of variance for Phosphorus content (mg)/gm of plant tissue as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	13.490	13.490	175.763	<0.001	0.203
VAM present	1.836						
VAM absent	0.757						
Hormone (B)		2	0.913	0.456	5.949	0.003	0.013
Control Vs Hormone		1	0.243	0.243	3.174	0.077	0.003
ABA Vs Auxin		1	0.669	0.669	8.724	0.003	0.010
ABA	1.237						
Auxin	1.410						
Control	1.232						
Drought (C)		1	13.490	13.490	175.763	<0.001	0.203
Drought	0.961						
No drought	1.631						
Interactions							
AxB		2	1.837	0.918	11.968	<0.001	0.027
AxC		1	1.418	1.418	18.481	<0.001	0.022
BxC		2	3.591	1.795	23.395	<0.001	0.054
AxBxC		2	1.854	0.927	12.084	<0.001	0.028
Unexplained		108	8.289	0.076			0.125
Total		119	66.325				

Table 3.8 Three-way analysis of variance for root/shoot (dry weight) ratio as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	1.163	1.163	120.468	<0.001	0.357
VAM present	0.204						
VAM absent	0.401						
Hormone (B)		2	0.450	0.225	23.312	<0.001	0.138
Control Vs Hormone		1	0.447	0.447	46.304	<0.001	0.137
ABA Vs Auxin		1	0.003	0.003	0.320	0.572	0.001
ABA	0.265						
Auxin	0.253						
Control	0.389						
Drought (C)		1	0.125	0.125	12.962	<0.001	0.038
Drought	0.335						
No drought	0.270						
Interactions							
AxB		2	0.199	0.099	10.334	<0.001	0.061
AxC		1	0.001	0.001	0.187	0.666	0.000
BxC		2	0.093	0.046	4.825	0.009	0.028
AxBxC		2	0.179	0.089	9.291	<0.001	0.055
Unexplained		108	1.043	0.009			0.320
Total		119	3.256				

Table 3.9 Three-way analysis of variance for % hyphal colonization of roots before stress as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	4305.779	4305.779	1201.364	<0.001	0.887
VAM present	11.980						
VAM absent	00.000						
Hormone (B)		2	30.872	15.436	4.306	0.015	0.006
Control Vs Hormone		1	28.790	28.790	8.032	0.005	0.006
ABA Vs Auxin		1	2.081	2.081	0.580	0.447	0.000
ABA	5.482						
Auxin	5.805						
Control	6.682						
Interactions							
AxB		2	30.872	15.436	4.306	0.015	0.006
Unexplained		108	387.080	3.584			0.080
Total		119	4852.604				

Table 3.10 Three-way analysis of variance for % vesicular + arbuscular colonization of roots before stress as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

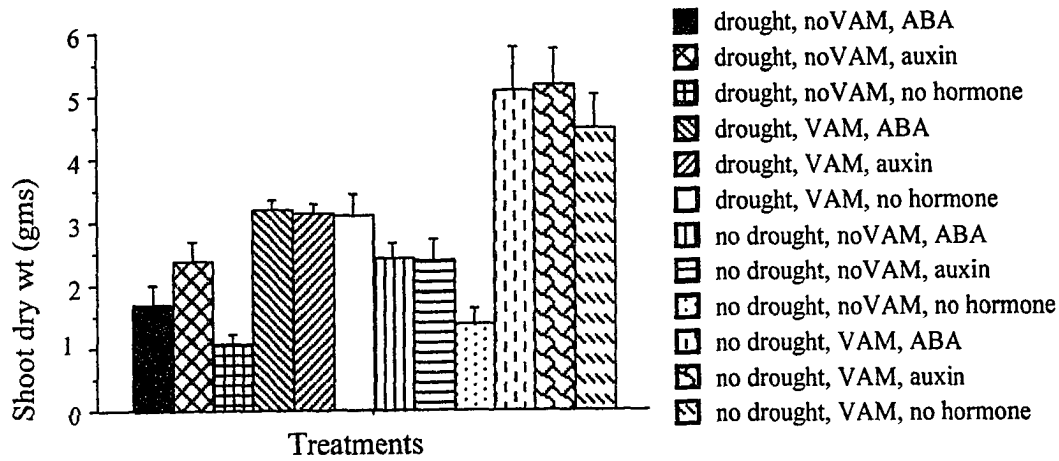
Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	13430.800	13430.800	2181.690	<0.001	0.922
VAM present	21.158						
VAM absent	00.000						
Hormone (B)		2	86.085	43.042	6.991	0.001	0.006
Control Vs Hormone		1	72.833	72.833	11.831	<0.001	0.005
ABA Vs Auxin		1	13.251	13.251	2.152	0.145	0.001
ABA	9.621						
Auxin	11.392						
Control	11.681						
Interactions							
AxB		2	86.085	43.042	6.991	0.001	0.006
Unexplained		108	664.863	6.160			0.046
Total		119	14568.522				

Table 3.11 Three-way analysis of variance for the % hyphal colonization at harvest as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	9123.788	9123.788	34.836	<0.001	0.751
VAM present	17.439						
VAM absent	00.000						
Hormone (B)		2	214.694	107.347	9.162	<0.001	0.017
Control Vs Hormone		1	201.938	201.938	17.235	<0.001	0.016
ABA Vs Auxin		1	12.756	12.756	1.088	0.299	0.001
ABA	8.201						
Auxin	7.403						
Control	10.554						
Drought (C)		1	408.162	408.162	34.836	<0.001	0.033
Drought	6.875						
No drought	10.563						
Interactions							
AxB		2	214.694	107.347	9.163	<0.001	0.017
AxC		1	408.162	408.162	34.836	<0.001	0.033
BxC		2	250.933	125.466	10.708	<0.001	0.021
AxBxC		2	250.933	125.466	10.708	<0.001	0.021
Unexplained		108	1265.399				0.104
Total		119	12136.786				

Table 3.12 Three-way analysis of variance for the % Arbuscular + vesicular colonization of roots at harvest as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), presence or absence of hormones and the presence or absence of Drought as main factors, with N=10 plants/treatment. The sum of squares due to Hormonal treatment was decomposed by two orthogonal comparisons.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	27545.000	7545.000	4458.794	<0.001	0.911
VAM present	30.301						
VAM absent	00.000						
Hormone (B)		2	299.606	149.803	24.249	<0.001	0.009
Control Vs Hormone		1	270.584	270.584	43.800	<0.001	0.008
ABA Vs Auxin		1	29.022	29.022	4.697	0.032	0.001
ABA	14.691						
Auxin	13.486						
Control	17.274						
Drought (C)		1	583.911	583.911	94.519	<0.001	0.019
Drought	17.356						
No drought	12.944						
Interactions							
AxB		2	299.606	149.803	24.249	<0.001	0.009
AxC		1	583.911	583.911	94.519	<0.001	0.019
BxC		2	125.794	62.897	10.183	<0.001	0.004
AxBxC		2	125.794	62.897	10.183	<0.001	0.004
Unexplained		108	667.189				0.022
Total		119	30230.813				



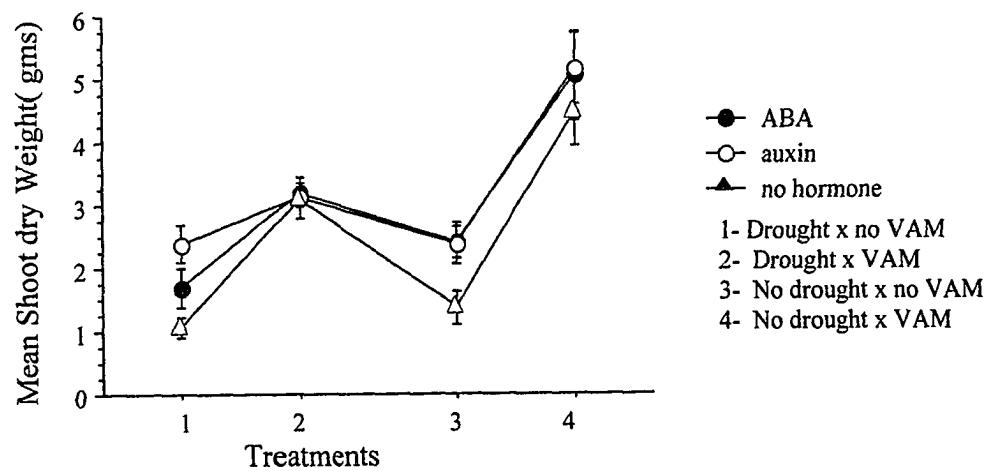


Fig 3.1.b. Mean shoot dry weight (gms) in interaction with drought hormone and VAM status

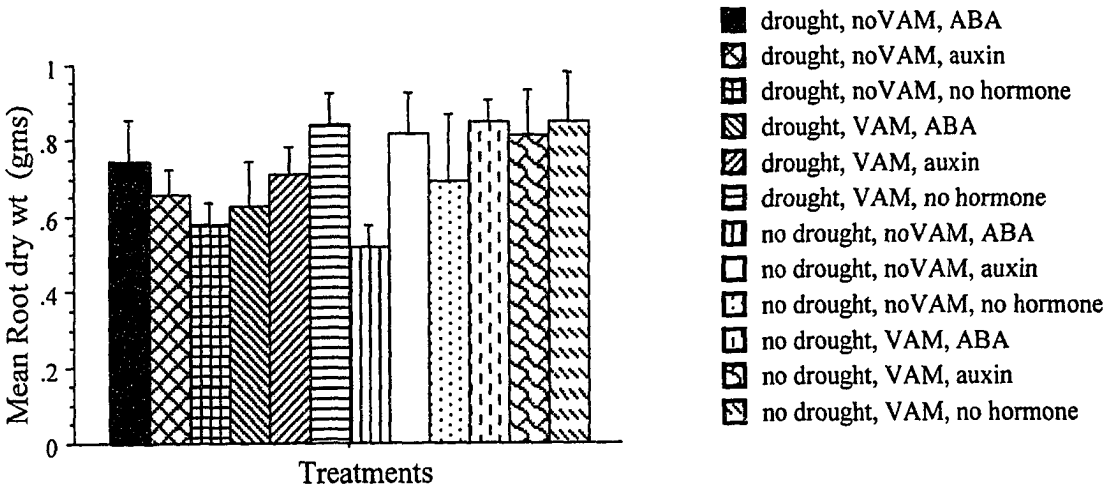


Fig 3.2.a. Mean with 95 % confidence intervals for all combination of factors in three-way design of drought, VAM and hormone with root dry weight (gm) as the response.

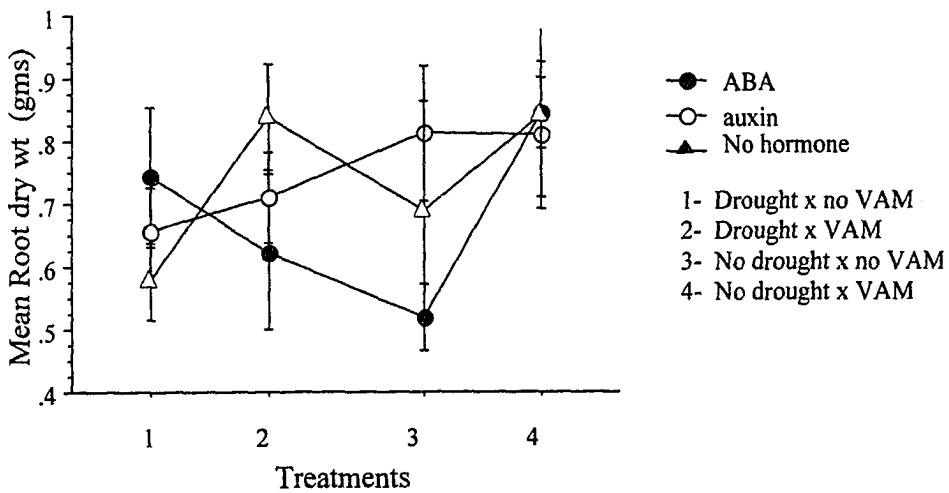


Fig 3.2.b. Mean root dry weight (gm) in interaction with drought hormone and VAM status

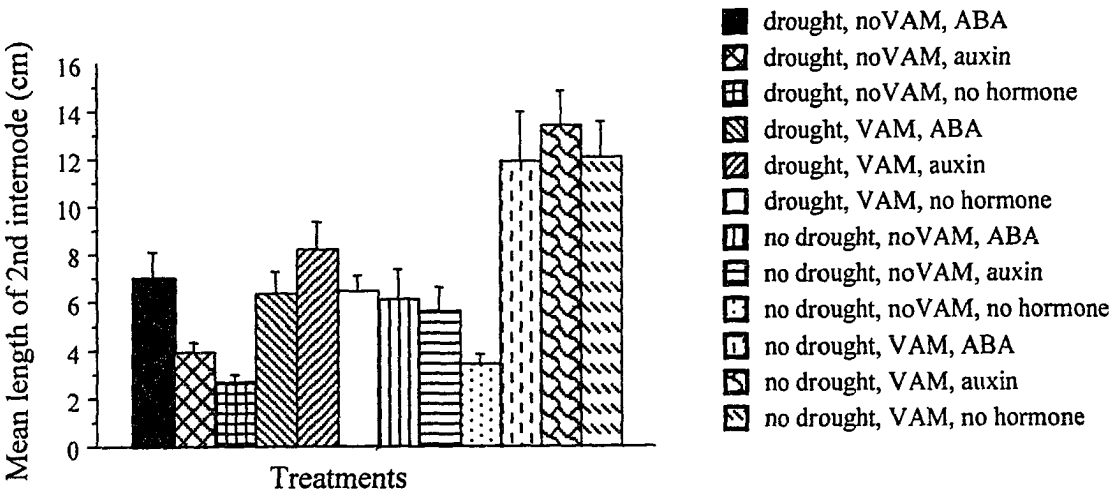


Fig 3.3.a. Mean with 95 % confidence intervals for all combination of factors in three-way design of drought, VAM and hormone with length of 2nd internode (cm) as the response.

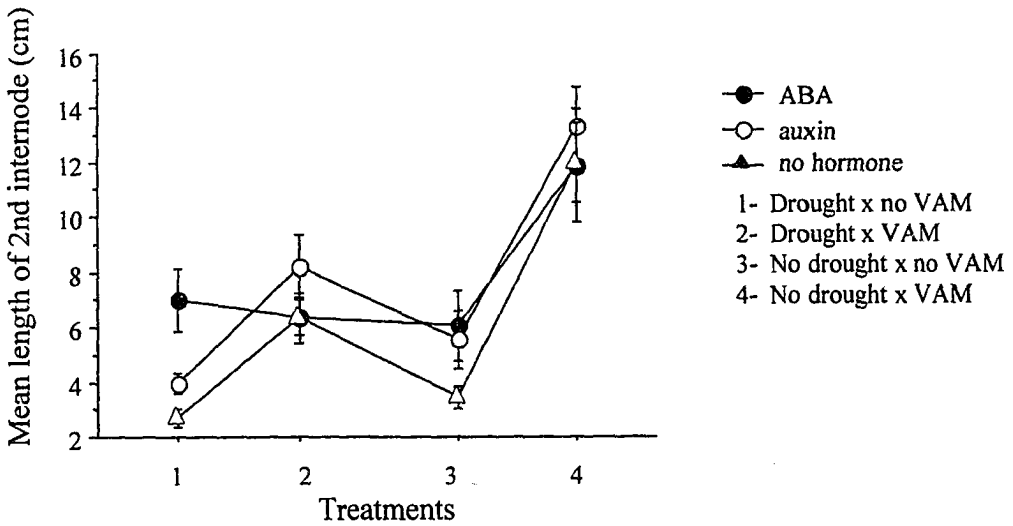


Fig 3.3.b. Mean length of 2nd internode in interaction with drought hormone and VAM status

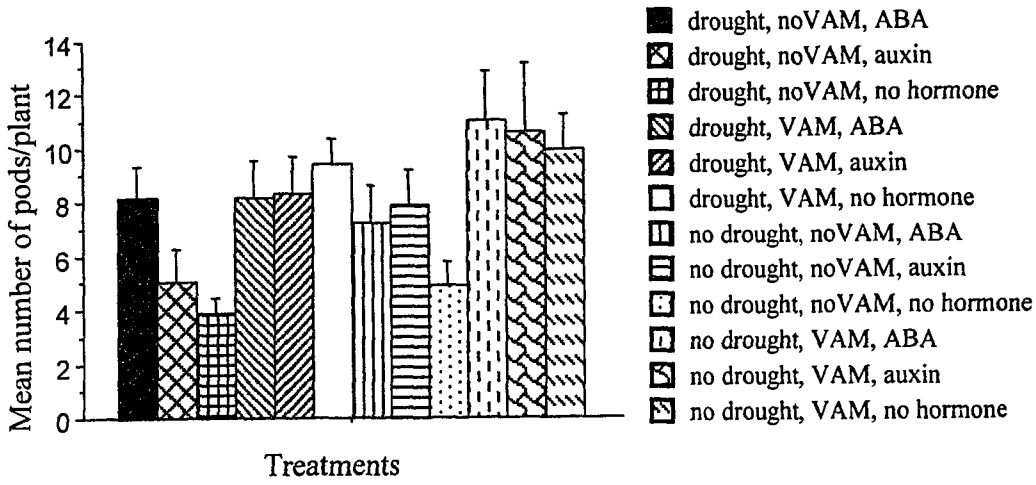


Fig 3.4.a. Mean with 95 % confidence intervals for all combination of factors in three-way design of drought, VAM and hormone with number of pods/plant as the response.

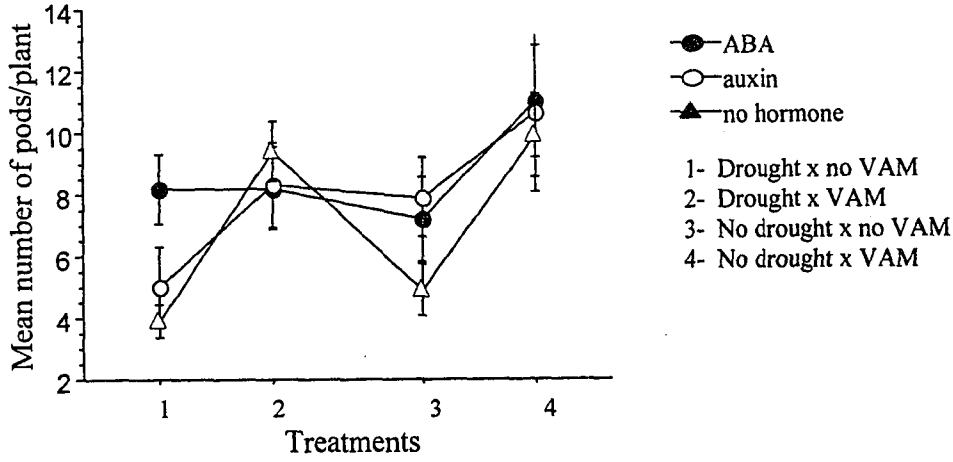


Fig 3.4.b. Mean number of pods/plant in interaction with drought hormone and VAM status.

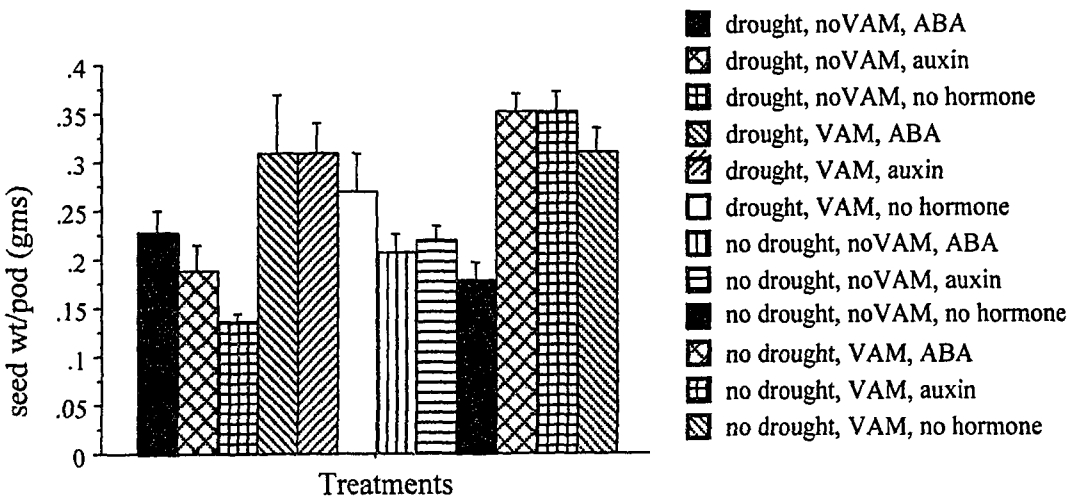


Fig 3.5.a. Mean with 95 % confidence intervals for all combination of factors in a three-way design of drought, VAM and hormone with seed weight/pod (gms) at harvest as the response.

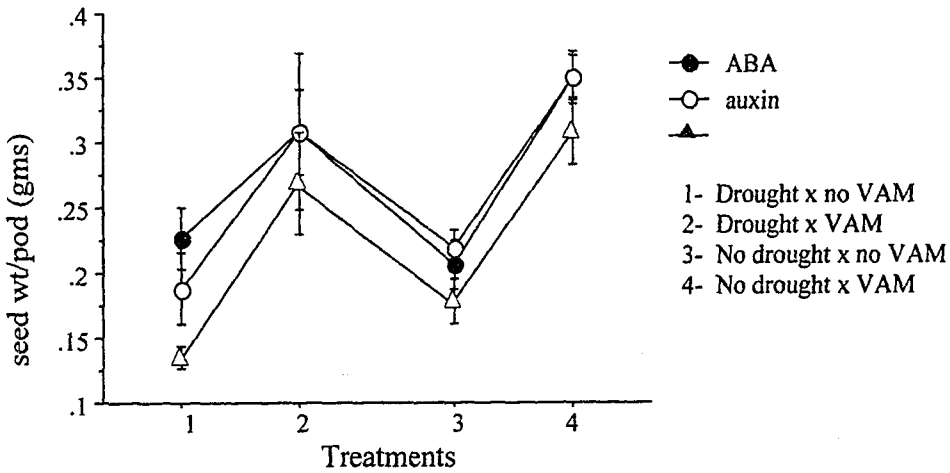


Fig 3.5.b. Mean seed weight/pod (gms) in interaction with drought hormone and VAM status.

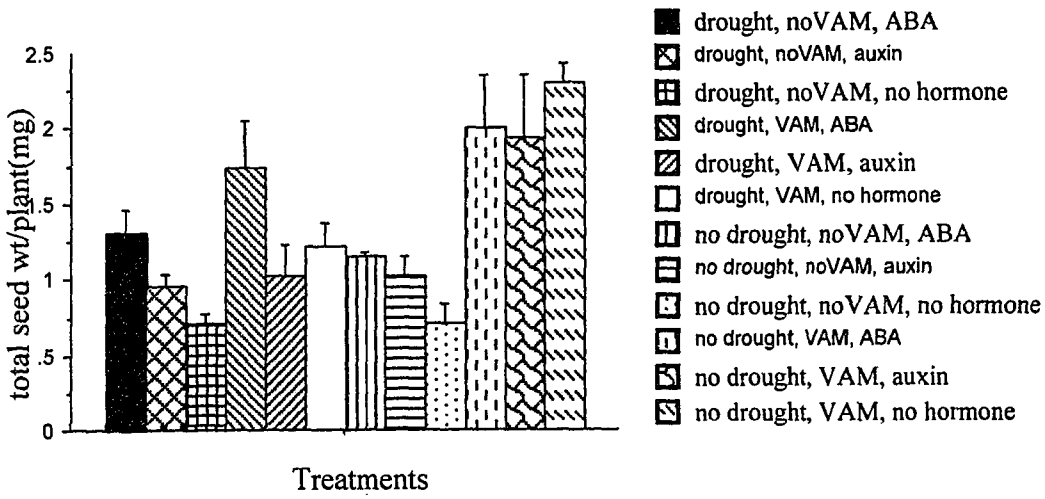


Fig 3.6.a. Mean with 95 % confidence intervals for all combination of factors in three-way design of drought, VAM and hormone with total seed weight/plant as the response.

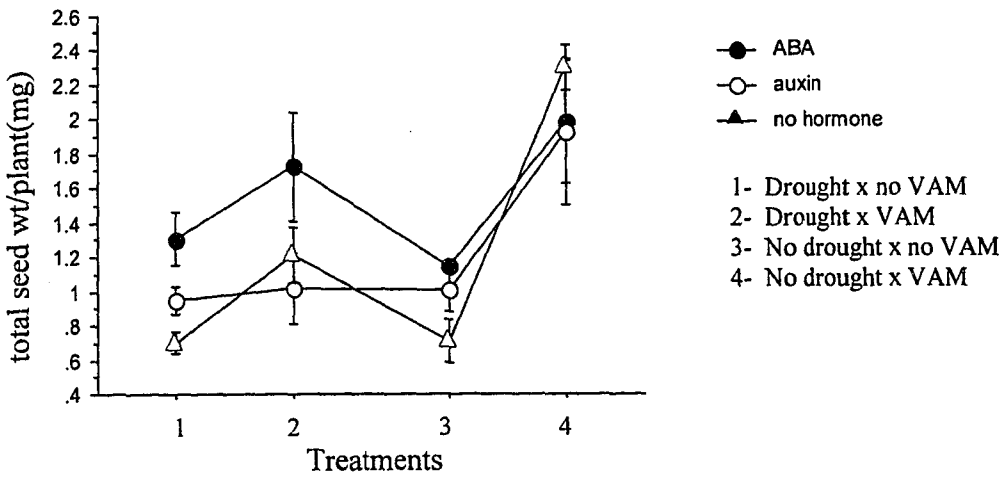


Fig 3.6.b. Mean total seed weight/plan in interaction with drought hormone and VAM status

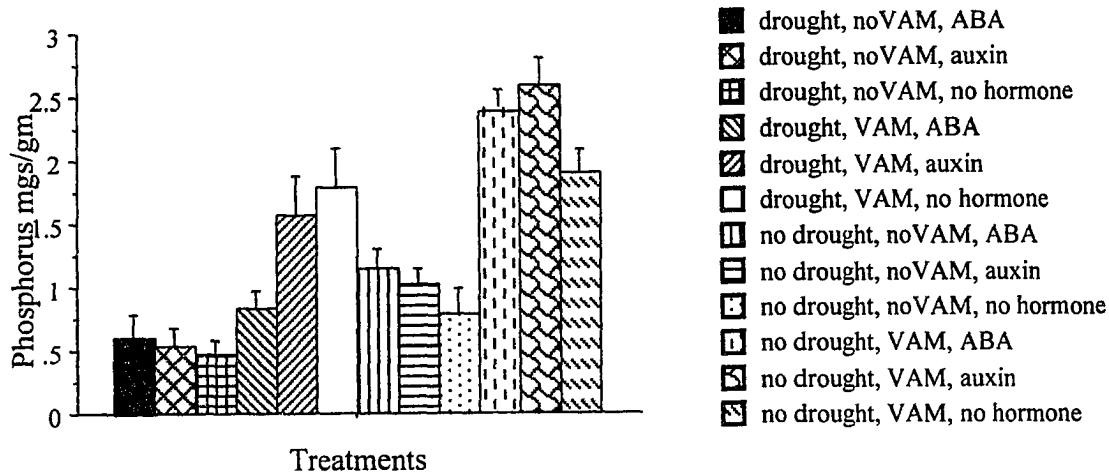


Fig 3.7.a. Mean with 95 % confidence intervals for all combination of factors in three-way design of drought, VAM and hormone with phosphorus mg/gm as the response.

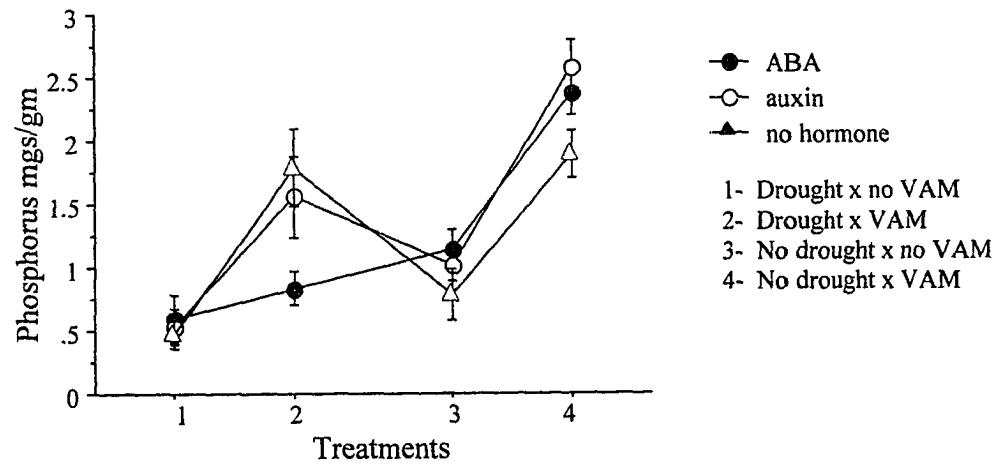


Fig 3.7.b. Mean phosphorus mg/gm in interaction with drought hormone and VAM status

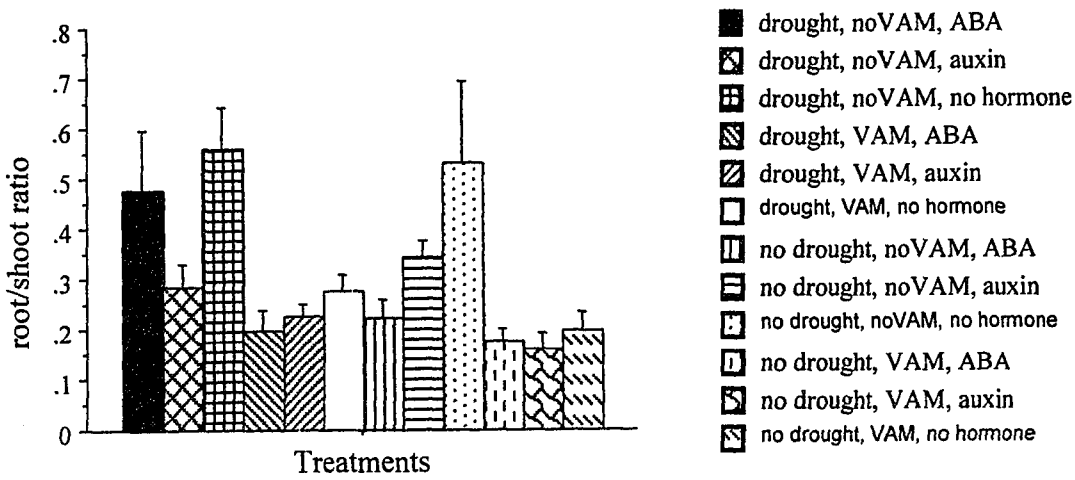


Fig 3.8.a. Mean with 95 % confidence intervals for all combination of factors in three-way design of drought, VAM and hormone with root/shoot ratio as the response.

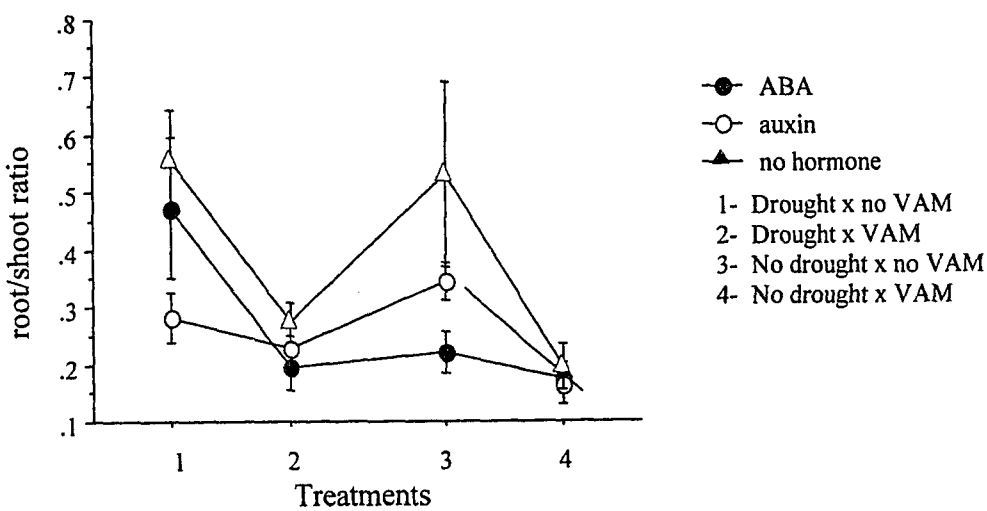


Fig 3.8.b. Mean root/shoot ratio in interaction with drought hormone and VAM status

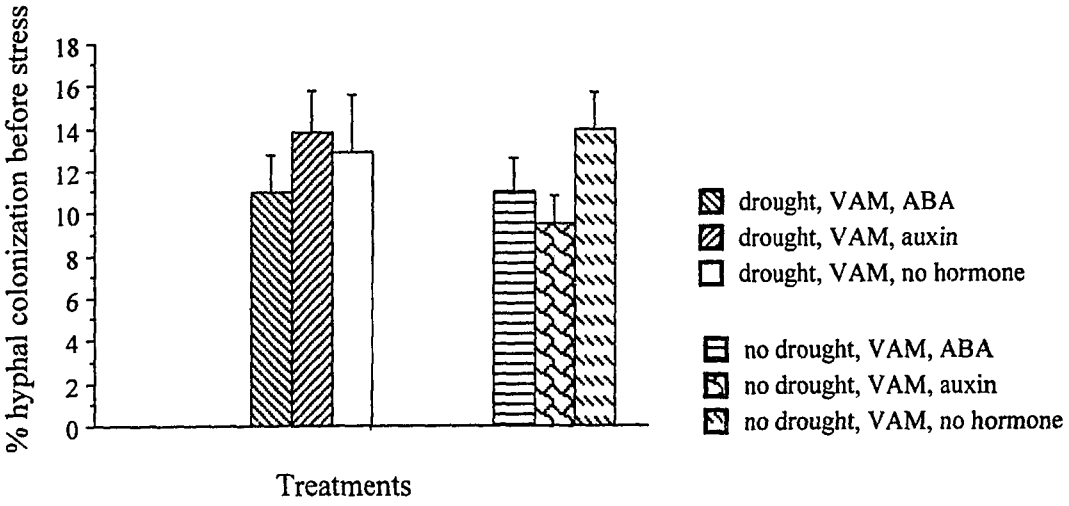


Fig 3.9.a. Mean with 95 % confidence intervals for all combination of factors in three-way design of drought, VAM and hormone with % hyphal colonization before stress as the response.

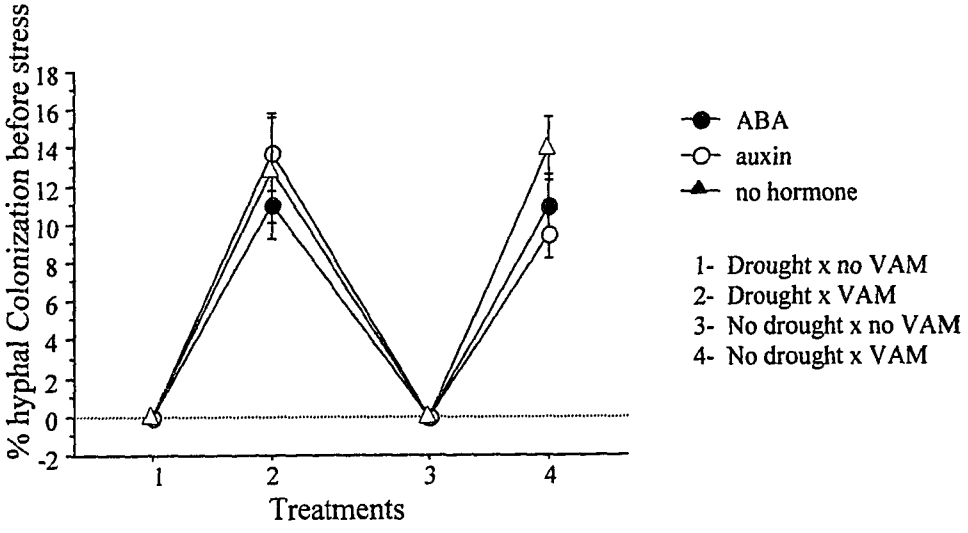


Fig 3.9.b. Mean % hyphal colonization before stress in interaction with drought hormone and VAM status

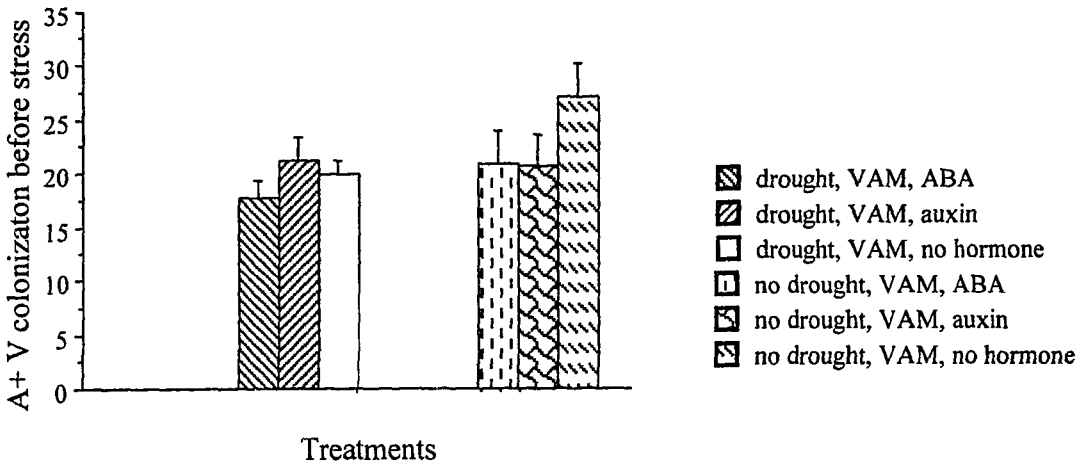


Fig 3.10.a. Mean with 95 % confidence intervals for all combination of factors in a three-way design of drought, VAM and hormone with A+V colonization before stress as the response.

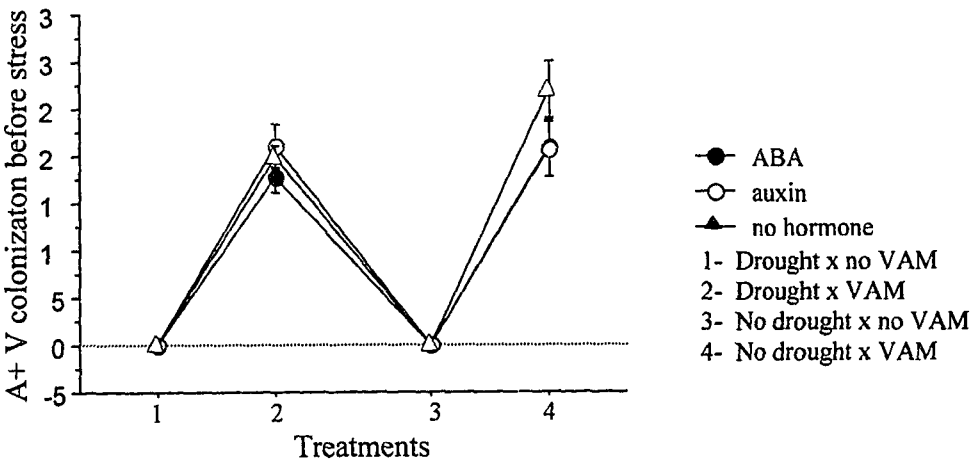


Fig:3.10.b. Mean A+V colonization before stress in interaction with drought hormone and VAM status

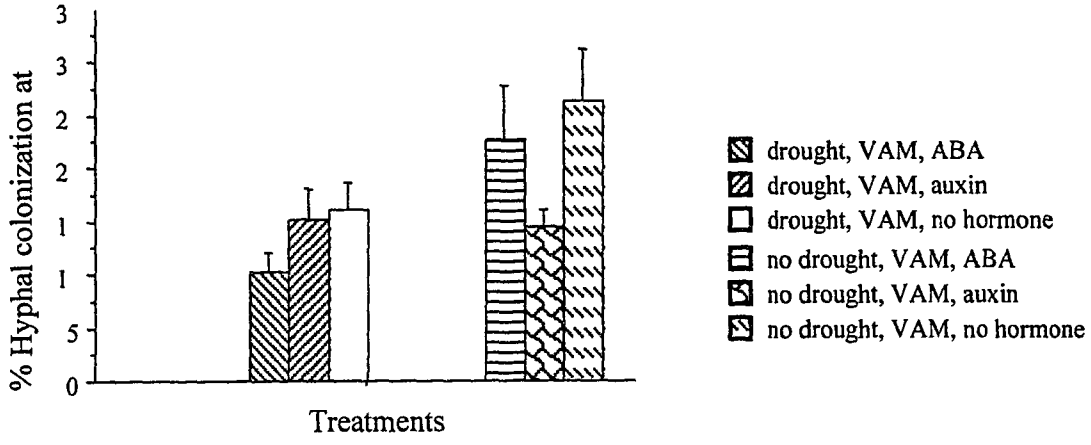


Fig 3.11.a. Mean with 95 % confidence intervals for all combination of factors in a three-way design of drought, VAM and hormone with % hyphal colonization at harvest as the response.

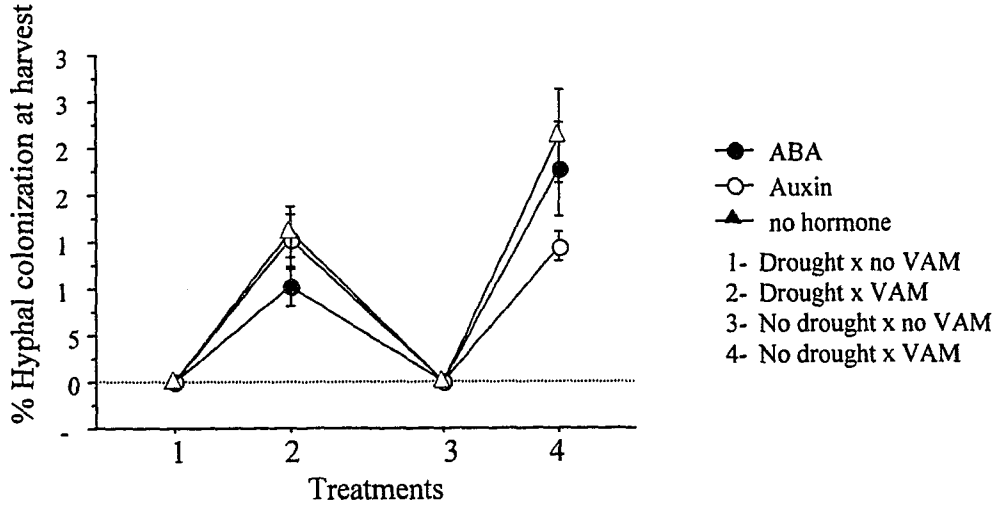


Fig 3.11.b. Mean % hyphal colonization at harvest in interaction with drought hormone and VAM status

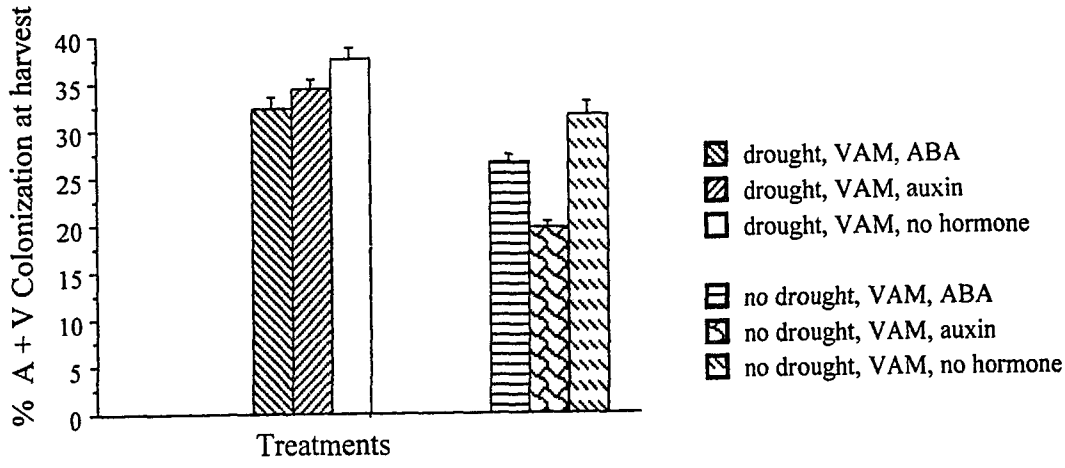


Fig 3.12.a. Mean with 95 % confidence intervals for all combination of factors in a three-way design of drought, VAM and hormone with % A + V Colonization at harvest as the response.

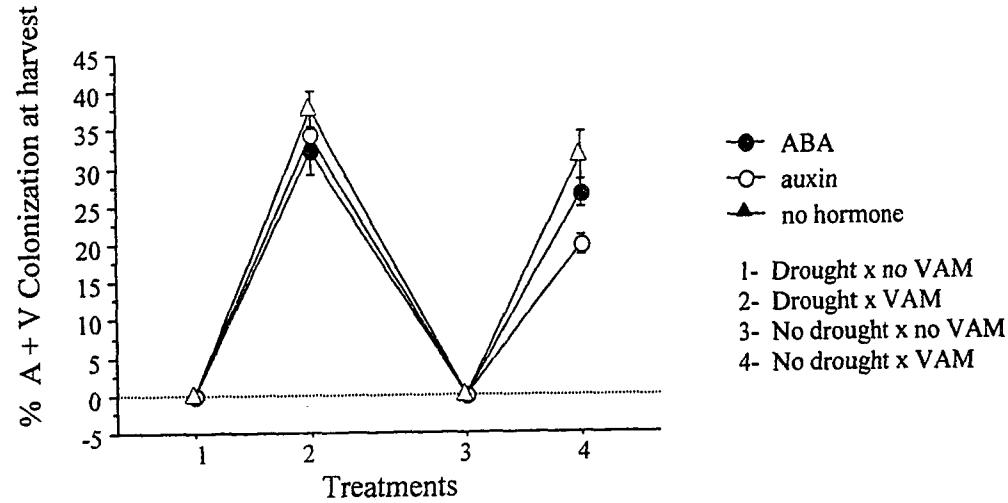


Fig 3.12.b. Mean % A + V Colonization at harvest in interaction with drought hormone and VAM status

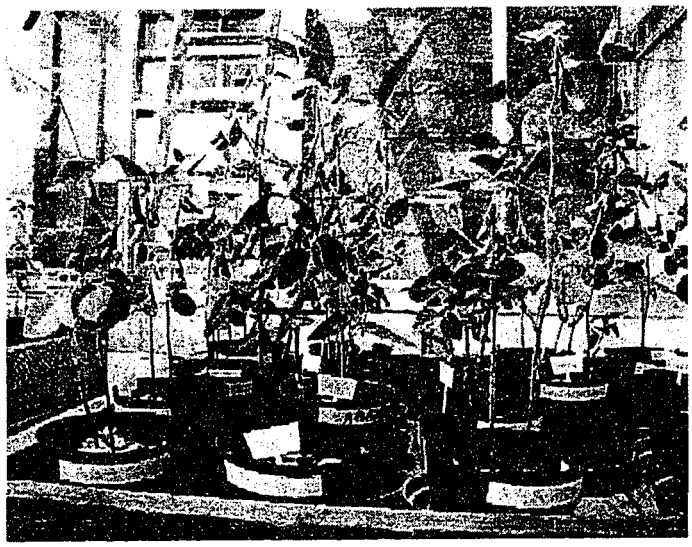


Fig 3.13 Ten weeks old no VAM x no Drought x ABA treated plants

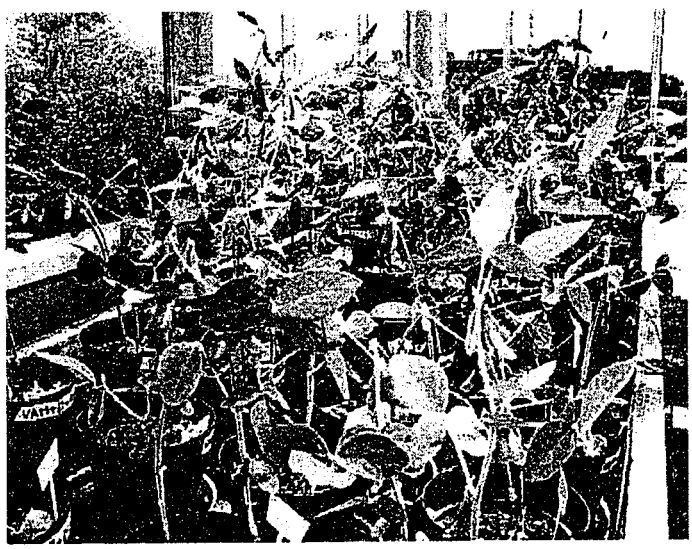


Fig 3.14 Ten weeks old VAM x Drought x no hormone treated plants

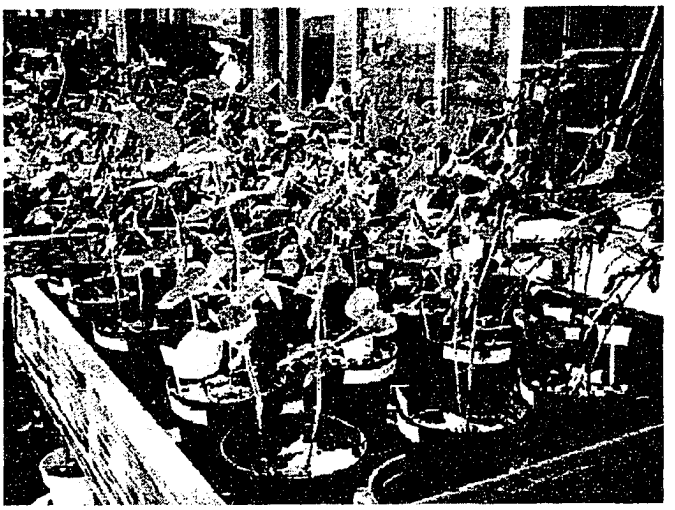


Fig 3.15 Ten week old VAM x Drought x Auxin treated plants

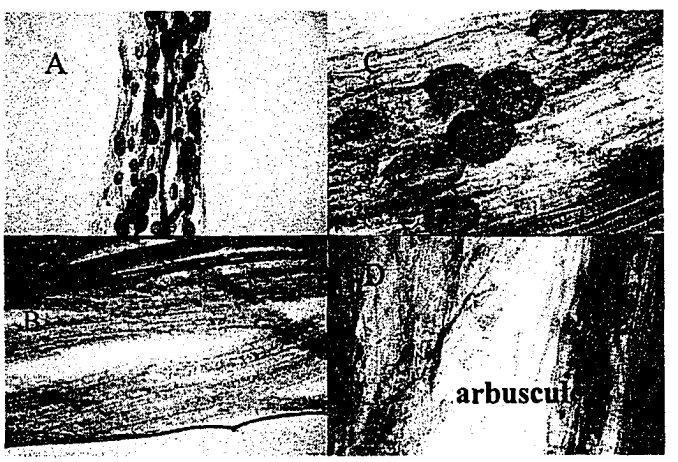


Fig 3.16 Squashed preparation of *Glomus fasciculatum* infected root of soybeans showing: A; vesicles, hyphae and arbuscules (200 x) and B; non mycorrhizal root C; vesicles with mature endospores D; arbuscules (400 x)

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Chapter 4

"Glomus intraradieces versus *Glomus fasciculatum* for drought stressed soybeans."

4.1. Results

The results of three-way ANOVA done separately on the host growth and yield data for two different species of VAM, that were used in these experiments seemed sufficiently different to justify further data analysis. The experiments with *G. intraradieces* were done in 1999 and 2000 (April- July). The experiments with *G. fasciculatum* were done in 2001 and (April-July) and 2002. The environmental conditions inside the greenhouse were the same during all the experiments, with a light period of 14-16 hour and relative humidity of 30-40 %. In order to compare the influence of both species of VAM fungus on the host the data for all the variables for both species were analyzed by one-way ANOVA, with VAM species being the only factor in this comparison. The data were also analyzed by MANOVA.

4.1.1. Shoot dry weight/plant (gms)/plant

The results of one-way ANOVA given in Table 4.1.a show that the mean shoot dry weight of the soybeans due to two VAM species was significantly different ($P<0.001$). The results of all pairwise comparisons, Tukey-Kramer HSD test (Table 4.1 b) show that the influence of both species and the presence or absence of VAM was significantly different from one another. The order of the mean shoot dry weight from highest to lowest was *G. fasciculatum* > *G intraradieces* > No VAM. Mean shoot dry weights are illustrated in Fig. 4.1.

4.1.2. Root dry weight (gms)/plant

The results of one-way ANOVA (Table 4.2.a) showed that overall the VAM species caused a significant increase in the root dry weight over the non-VAM plants. The results of all pairwise comparisons using Tukey's test shows that difference in mass of the root was not significantly different due to two VAM species used in the experiments but it did differ significantly from non VAM plants. Fig 4.2 is the visual presentation of the means.

4.1.3. Length of 2nd internode (cm)

The results of one-way ANOVA are described in Table 4.3.a. which shows that there was a significant difference in length of 2nd internode due to VAM species ($P<0.0001$). Table 4.3.b shows a significant difference in the role that was performed by VAM as compared with the non-VAM plants although the means due to two species are not significantly different from one another.

4.1.4. Yield (no of pods/plant, weight of seeds /pod, total seed weight (gms) /plant)

The results of one-way ANOVA for the number of pods, seed weight per pod and total seed weight/pod are given in Table 4.4a-4.6a. As far as the number of pods is concerned it was not increased tremendously by VAM species so that is obvious in Tukey's test as shown in Table 4.4.b.

As shown by the one-way-ANOVA in Table 4.4.a. the number of flowers that grew to the pod was not significantly different, but the data presented in Table 4.5.a and 4.6.a show a significant difference in the weight of the seeds / pod and total weight of seeds/plant due to *G. fasciculatum* as compared to *G. intraradieces*. Fig 4.5 and 4.6 show that *G. fasciculatum* increased the yield significantly over the *G. intraradieces*.

The results of Tukey's all pairwise comparisons test and the diamond plot in Fig. 4.5 clearly show that there was a significant difference in the weight of seeds/ pod, and *G. fasciculatum* increased the weight of seeds/pod as compared to the *G. intraradieces*. The results of one-way ANOVA for total seed weight/plant are presented in Table 4.6.a. The outcomes of the measurements of total seed weight/plant were found to be significantly different for the two species used ($P<0.0001$). The results of all pair wise comparisons show that the total seed wt/plant were significantly different due to both species and each species compared to No VAM. The treatment means plotted in Fig 4.6 show that *G. fasciculatum* did significantly better in terms of increasing the yield of soybeans as compare to the *G. intraradieces.*

4.1.5. Phosphorus mgs/gm of plant

The results for analysis of phosphorus content of the plant are presented in Table 4.7.a. These results show a significant difference in the phosphorus content of the plants, infected by VAM versus No VAM and also due to different species of the VAM ($P<0.0001$). The result of all pair wise comparison Tukys test (Table 4.7.b) reveal that the phosphorus contents of the plants across al the pairs are significantly different from one another. The diamond plot and Tukey's test plotted in Fig. 4.7.b how that the mean values of phosphorus content due to different VAM species and No VAM at all are significantly different from one another.

4.1.6. %VAM colonization (hyphal ,arbuscular +vesicular) of roots before stress

The % hyphal colonization before water stress also came out to be significantly different in both species (Table 4.8.a.). The results of all pairwise comparisons. Tukey's test confirms the differences in hyphal colonization by both species of VAM. Means of %

hyphal colonization before stress, and at harvest (Fig. 4.10) were higher in *G. intraradieces*.

The results for % Arbuscular + Vesicular infection before the water stress (Table 4.9.a) and at harvest (Table 4.11.a.) describe that the roots infection by the two *Glomus* species differed significantly at both stages of the plant's life. The results of all pairwise comparisons Tuky's test (Table 4.9.b and 4,10.b 4.11.b) show that the % hyphal as well as arbuscular + vesicular colonization before the water stress was significantly different between the two VAM species used. The treatment means and the Tukey's test as illustrated in Fig. 4.9 and Fig 4.11 show that the arbuscular + vesicular colonization of *G. fasciculatum* was higher than *G. intraradieces* at both stages of life.

4.1.7. % VAM colonization of roots at harvest

Table 4.10.a presents one-way ANOVA of % hyphal colonization at the time of harvest and shows a significant difference due to the VAM species ($P<0.0001$).
All pairwise comparisons given in Table 4.10.b for the % hyphal colonization at harvest reveal that all pairs differed significantly in term of % hyphal colonization of roots at harvest. *G. intrardieces* had higher hyphal colonization than *G. fasciculatum*. The treatment means and the Tukey's test illustrated in Fig 4.10 show that *G. fasciculatum* had higher % root infection as compared to the *G. intraradieces*. The vesicular + arbuscular colonization of both species was also significantly different, at harvest (Table 4.11.a and 4.11.b). Fig. 4.11 shows that *G. fasciculatum* had the higher arbuscular + vesicular colonization at the time of harvest.

4.1.8. Root dry weight/shoot dry weight ratio

The root dry weight/shoot dry weight ratio was significantly higher in non-VAM plants followed by *G. intraradieces* and then *G. fasciculatum* (Table 4.12.a). Fig.4.12 describes the mean for root / shoot dry weight ratio. The VAM infected plants had significantly different root/shoot dry weight ratios as compared to non-VAM but there was no significant difference in the root/shoot dry weight ratio of two species.

4.1.9. Multi-way analysis of variance

The results of MANOVA done on dependent variable combination, confirm the results of all the individual ANOVA done for each of the dependent variables. The MANOVA for shoot dry weight, root dry weight and total seed weight, with VAM species being the only factors, shows a positive correlation between the three variables. The influence of both species on these variables was found to be significantly different (Wilk's Lambda and F-test $P<0.001$). Fig 4.13 gives graphic presentation of the results, showing that total seed weight was increased by *G. fasciculatum* more than the *G. intraradieces*, followed by the shoot dry weight, the root dry weight was least affected by the difference of species.

The % arbuscular + vesicular colonization at harvest showed a positive correlation with the root/shoot ratio for plants of both species. The effect was significant as shown by Wilk's Lambda ($P <0.001$). Fig 4.14 confirms the findings of Tukey-Kramers pairwise comparisons that the root/shoot ratio, of plants colonized by different *Glomus* species were not significantly different. And that this was independent of the influence of two species on the extent of arbuscular and vesicular colonization.

Total seed weight was positively correlated to the plant phosphorus content. The Wilk's Lambda test confirmed the significantly different role of the two species in this regard ($P<0.001$). The results plotted in Fig 4.14 show that phosphorus content of the plant was most significantly higher due to *G. fasciculatum* as compared to *G. intraradieces* and was followed by the total seed weight in exactly same way.

4.2. Discussion

4.2.1. Vegetative growth

As described in chapters 2 and 3, the VAM is undoubtedly useful for the host but the results of comparison done here clearly shows that the maximum benefits that can be expected from the VAM are dependent on the level of compatibility of the VAM species and the host. The finding of this analysis about the vegetative growth (shoot dry mass/plant, root dry mass /plant and length of 2nd internode) can be supported by the literature. For example, in a study done by Ruiz et al. (1995) the effect of several VAM species on water stressed lettuce plants was investigated, among them the *G. fasciculatum* proceeded the *G. intraradieces* in terms of increasing the biomass and the drought tolerance of the host plants. In another unpublished study done by Abdelgadir and Abdelaziz (1998), it was found that the rate of infectivity and the effect of different VAM species were different on soybean plants. The authors suggested the importance of evaluation of compatible fungal species and the host plants. Similarly Amerian and Stewart (2001) reported that *Glomus moseae* was more useful for CO_2 assimilation and growth of the maize plants than *Gomus intraradiecec.*

Usually when plants are subjected to an environment of limited nutrients and water that can retard the photosynthesis, the amount of resources that the plants will have

to use to produce absorbing organs can be crucial for the plants survival. In the present study the VAM infected fungi did have more root mass as compared to the non-VAM plants, but they still did well as a whole with VAM under stress as compared to the non VAM plants (chapter 2 & 3).

The results of the present study show that the role that the VAM fungi perform for the plants are independent of their influence on root growth. This is an aspect that needs further exploration using more VAM species on different varieties of the soybeans.
It has been reported often that different fungal species alter other hosts's responses to different degrees under different kind of growth conditions. Allen and Boosalis (1983) for example reported that drought stressed wheat *G. mosseae* and *G. fasciculatum* responded differently, the later was found to be more helpful in withstanding the drought by providing better osmotic adjustments. As discussed in previous chapters VAM fungi most probably send a non-hydraulic signal to the leaves most probably in the form of ABA and control the stomatal conductivity of the leaves. This acts as one of the tools that VAM fungi use to help the host under situation of stress.

A study showed the comparative roles performed by different VAM fungi to help drought stressed wheat plants by influencing the root growth. Ellis *et al.* (1985) found that G*lomus fasciculatum* increased the root dry weight and rooting of wheat plant more than *G. mosseae*. In different studies people have used different species, but in most cases *G. fasciculatum* seems to be the winner, although in present study we did not see significant difference in root dry weight due to VAM species.

Fig 4.3.c illustrates the treatment means and Tukey-Kramer pair-wise test results. One can infer from these results that even though *G. fasciculatum* increased the over all

dry mass of the shoot, it did not make much of a difference in terms of the growth of a specific part of the shoot, i.e. the second internode. In fact *G. intraradieces* did slightly better in this respect as shown by Fig 4.3 although the different was not significant. This difference can be attributed to the difference in terms of redistribution of a particular, growth hormones by VAM fungi (Thanuja *et al.* 2002). Similarly Alkaraki *et al.* (1998) also reported a difference in the shoot growth of wheat plants in response to two different species of *Glomus*.

The root dry weight/shoot dry weight ratio of the non-VAM plants, were found to be the highest, and the *G. gasciculatumiculatum* were the lowest. The shoot dry weights of the *G. fasciculatum* were significantly higher than the *G. intraradieces*, but the lower root/shoot ratio suggest that the *G. fasciculatum* did more for the above ground parts of the plants than the other species. The findingd of Ruiz *et al.* (1995) also support the results of the present study. It is possible that effects of fungi are far reaching into the physiological processes of plant growth and development rather than just absorption of water and nutrients.

4.2.2. Yield (number of pods, weight of seeds /pod and total seed weight / plant)

As mentioned elsewhere in this chapter the influence of different species of *Glomus* on various plants has always been a matter of interest for investigators. Ruiz and Lozano (2001) for example reported that *Glomus mosseae* was a better symbiont for soybeans as compared to *G. intraradieces*, because they found that it can prevent the nodule senescence in soybean plants increasing their ability to absorb nitrogen and hence increase the yield. In another study done on cassava involved the study of the influence of several VAM species including *Glomus* species. The results of that study showed that

other species of *Glomus* (*G. manohotis* and *G. occultum*) improved the growth of the host as compared to the *Glomus fasciculatum*. It is a matter of host and VAM compatibility, which is driven by several factors such as the level of moisture available for host and the fungus and some exudates produced by the roots that can promote the establishment of fungus within the host (Elias and Safir, 1987).

The differences in terms of the improved yield by one species over the other is congruent with the percent arbuscular + vesicular colonization of roots and the phosphorus content of the *G. fasciculatum* infected soybeans as compared to the *G. intraradieces*. The results of one-way ANOVA of plant phosphorus content, % colonization of roots before the water stress, and % colonization at the harvest (Table 4.7.a, 4.8.a, and 4.11.a) show a significant difference in measurements of these variables due to species and due to VAM/no-VAM soybean plants. The ANOVA means and the Tukey- Kramer tests plotted in Fig.4.7, 4.8 and 4.9 show that *G. fasciculatum* seems to be the more useful VAM fungus for soybeans, at least in the greenhouse experiments. Further experiments with mixed culture inoculum and then comparing them with the results obtained from the studies of the individual inocula may produce conclusive results. The present study shows that *G. fasciculatum* should be the inoculum of choice for soybean plants if VAM is used as a biofertilizer. There is a need, to further explore the comparative roles of different species of *Glomus* on some physiological processes of soybean like photosynthesis.

4.2.3. Phosphorus content (mgs)/gm of plant

The present study showed significantly improved uptake of P by the plants infected with *G, fasciculatum* as compared to *G. intraradieces.* The improved nutrition uptake also reflected in the total seed weight by plant which was found to be significantly higher *in G. fasciculatum* infected plants. Ruiz *et al.* (1995) reported that different species of VAM had different level of efficiencies in improving the growth and nutrient uptake of drought stressed lettuce plants. The *G. fasciculatum* preceded the *G. intraradieces* among other species. The authors also found the efficiency to be independent of the colonizing ability of the plants. The findings of the present study were that the hyphal colonization of *Glomus intraradieces* were significantly higher than *G. fasciculatum* at both stages of life for which colonization was estimated, although the arbuscular + vesicular colonization was higher in the later than the former at both stages of life. In another report published by Alkaraki *et al.* (1998), two different species of *Glomus* were found to have a different phosphorus uptake by the host plants used in that study. So it is possible for the two VAM species to behave significantly differently in the same set of conditions. Maybe in future studies other aspects of host responses and the uptake of other nutrients should be considered in order to get a complete picture. It might very well be that one would be better in some other aspects, and may be that the combination of both can lead to a more beneficial interaction. These could be issues for future experiments on soybeans and VAM fungi.

4.2.4. % root colonization (Hyphal, Arbuscular + Vesicular)

In the present study the Arbuscular + Vesicular colonization is actually higher for *G. fasciculatum* than *G. intraradieces* while the hyphal colonization is the opposite.

As described earlier sometimes the advantages offered by the VAM fungi to the plants can be independent of their extent of colonization (Ruiz *et al.*1995), but most of the times that is not the case. In present study the ranking of colonization is *G. fasciculatum* > *G. intraradiece,* which, agrees with the shoot dry weight and the total yield ranking for the two species. Alkaraki et al (1998) working on *G mosseae* and *G. monosporum* found that the former had higher colonization and caused more nutrient uptake and growth than the later. The same type of differences in plant response to *G. fasciculatum* and *G. mosseae* were reported by Allen and Boosalis (1983). This could be due to a source-sink response. VAM fungi depend on the host for their glucose. The host, which is a source, will be able to produce more glucose if it is supplied with more water and nutrients. So if a VAM fungus (sink) can do that for the host, in return it will get more of the glucose and increase its colonization (Acosta *et al.* 1996). Al-Agely and Reeves (1995) found a strong positive correlation between the mycorrizal formation and the amount of above ground plant cover, but it is hard to tell that which one is the cause and which one is the effect. However, there are reports supporting the production of phytohormones that can also contribute to the growth responses of the host (Danneberg *et al.* 1992). The exudates produced by the growing roots of the plants that are experiencing phosphorus deficiency, play a role in stimulate the growth of the VAM hyphae (Elias and Safir, 1987). The plants in the present study were also grown pots, so there was a limited amount of phosphorus avaialable to them and they were given a phophorus defeicient Hoaglands nutrient solution, so it is possible that the *G. fasciculatum* was able to flourish better than the *G. intraradieces*

4.3. Conclusion

There is a little doubt that as the plant growth changes so does the fungal colonization and vice versa. It is true that mycorrhizae can cause changes in nutrient uptake, and even alter the plant water relations. The results of the present study prove that VAM increases the uptake of phosphorus by the plant. In fact the improved plant resistance or tolerance to the drought may actually be due to the improved plant nutrients. The VA mycorrhizae can improve the plant growth and water use efficiency. The soil type, the amount of available phosphorus in the soil and the fact that mycorrhizae are totally dependent on the host for their nutrition all play a role in determining the mycorrhizal response (Sieverding, 1981). The results of this study can help determine that the role performed by different VAM fungi, in helping the colonized plant can be diverse. One VAM fungus can be more effective than the other. The VAM fungal efficiency can be evaluated in terms of the vegetative and reproductive growth of the host plants. The selection of appropriate VAM fungi to be used agriculturally in stressed environment to address specific problems is a promising, but usually neglected strategy. Suitably adapted mycorrhizal fungal isolates should be studied for their importance for maintaining and restoring the plant soil equilibrium in sustainable agricultural situations.

Table 4.1.a. One-way ANOVA for shoot dry weight (gms)/plant. Main factor was VAM species. N=240

Source of variation	f	SS	MS	F	P
VAM species	2	98.729	49.364	45.964	<0.001
Error	237	254.530	1.074		
Total	239	353.259			

Table 4.1.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between shoot dry weight means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. Intraradieces*	*G. fasciculatum*
G. fasciculatum	1.10655	0.08807	
G. Intraradieces	0.57222		
noVAM			

Table 4.2.a. One-way ANOVA for root dry weight (gms)/plant. The main factor was the VAM species. N=240

Source of variation	Df	SS	MS	F	P
VAM species	2	0.557	0.278	10.212	<0.001
Error	237	6.471	0.027		
Total	239	7.028			

Table 4.2.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between root dry means means, minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. Intraradieces*	*G. fasciculatum*
G. fasciculatum	0.046877	-0.04274	
G. Intraradieces	0.018461		
noVAM			

Table 4.3.a. One-way ANOVA for length of 2nd internode, Main factor was VAM species. N=240

Source of variation	Df	SS	MS	F	P
VAM species	2	1403.55	701.78	76.35	0.001
Error	237	2178.18	9.19		
Total	239	3581.74			

Table 4.3.b Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference) for length of 2nd internode as the response variable. Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. fasciculatum*	*G. Intraradieces*
G. Intraradieces	4.05818	-0.54047	
G. fasciculatum	3.29318		
noVAM			

Table 4.4.a One-way ANOVA for the number of pods/plant. The main factor was the VAM species. N=240

Source of variation	Df	SS	MS	F	P
VAM species	2	541.73	270.86	49.76	<0.001
Error	237	1289.92	5.44		
Total	239	1831.66			

Table 4.4.b Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. Intraradieces*	*G. fasciculatum*
G. fasciculatum	2.50498	-0.13795	
G. Intraradieces	1.63831		
noVAM			

Table 4.5.a One-way ANOVA for weight of seed (gms)/pod. The main factor was the VAM species/no VAM

Source of variation	Df	SS	MS	F	P
VAM species	2	0.55	0.27	108.64	0.001
Error	237	0.60	0.00		
Total	239	1.15			

Table 4.5.b Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level

	G. fasciculatum	*G. Intraradieces*	noVAM
G. fasciculatum		0.048797	0.098319
G. Intraradieces			0.027836
noVAM			

Table 4.6.a One-way ANOVA for total seed weight/plant. The main factor was the VAM species/no VAM

Source of variation	Df	SS	MS	F	P
VAM species	2	20.88	10.44	58.66	<0.001
Error	237	42.11	0.17		
Total	239	63.00			

Table 4.6.b Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. Intraradieces*	*G. fasciculatum*
G. fasciculatum	0.564524	0.266137	
G. Intraradieces	0.116858		
noVAM			

Table 4.7.a. One-way ANOVA for phosphorus (mgs)/ gm of plant. The main factor was the VAM species

Source of variation	Df	SS	MS	F	P
VAM species	2	59.24	29.62	158.43	<0.001
Error	237	44.31			
Total	239	103.55			

Table 4.7.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level

	noVAM	*G.intraradieces*	*G. fasciculatum*
G. fasciculatum	1.04085	0.43610	
G.intraradieces	0.41855		
noVAM			

Table 4.8.a One-way ANOVA for hyphal colonization before stress

Source of variation	Df	SS	MS	F	P
VAM species	2	14134.65	7067.33	857.62	<0.001
Error	237	1953.02			
Total	239	16087.67			

Table 4.8.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. fasciculatum*	*G. intraradieces*
G. intraradieces	16.5803	4.4344	
G. fasciculatum	10.9097		
noVAM			

Table 4.9.a. One-way ANOVA for % arbuscular + vesicular root colonization before stress. Main factor was the VAM species

Source of variation	Df	SS	MS	F	P
VAM species	2	20514.93	10257.50	1331.85	0.001
Error	237	1825.28	7.70		
Total	239	22340.21			

Table 4.9.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. intraradieces*	*G. fasciculatum*
G. fasciculatum	20.1238	5.2991	
G. intraradieces	13.6297		
noVAM			

Table 4.10.a One-way ANOVA for % hyphal colonization at harvest. Main factor was the VAM species. N=240

Source of variation	Df	SS	MS	F	P
VAM species	2	22663.91	11332.0	451.79	<0.001
Error	237	5944.51	25.1		
Total	239	28608.436			

Table 4.10.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. fasciculatum*	*G. intraradieces*
G. intraradieces	19.2198	1.4916	
G. fasciculatum	15.5715		
noVAM			

Table 4.11.a. One-way ANOVA for % Arbuscular + Vesicular colonization of roots at harvest. N=240

Source of variation	Df	SS	MS	F	P
VAM species	2	52233.60	26116.80	1014.49	0.001
Error	237	6101.21	25.70		
Total	239	58334.82			

Table 4.11.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5% level.

	noVAM	*G. intraradieces*	*G. fasciculatum*
G. fasciculatum	28.6758	0.0232	
G. intraradieces	26.4677		
noVAM			

Table 4.12.a. One-way ANOVA for root /shoot dry weight ratio. N=240

Source of variation	Df	SS	MS	F	P
VAM species	2	0.54	0.27	16.13	<0.001
Error	237	3.94	0.01		
Total	239	4.47			

Table 4.12.b. Comparisons for all pairs using Tukey-Kramer HSD. Tabled values are absolute differences between means minus LSD (Least Significant Difference). Positive values indicate pairs of means significantly different at 5 % level.

	G. fasciculatum	*G. intraradieces*	noVAM
noVAM	0.060414	0.026264	
G. intraradieces	-0.02139		
G. fasciculatum			

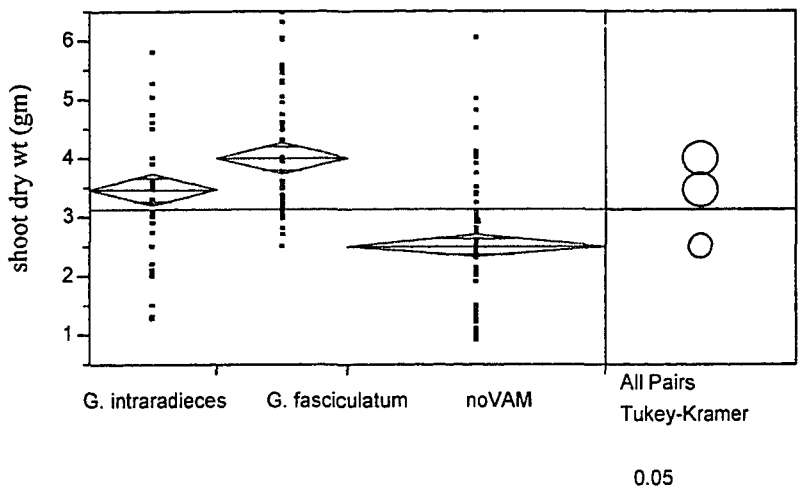

Fig 4.1. Shoot dry weight (gm)/plant by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

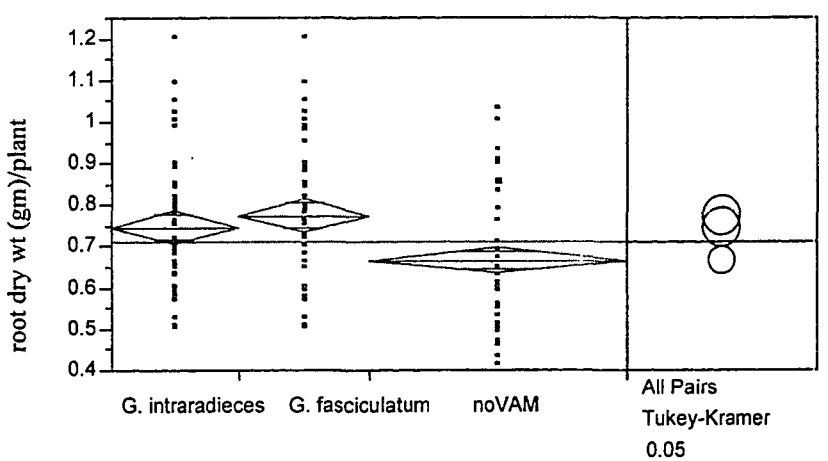

Fig 4.2. Root dry weight (gm)/plant by VAM treatments. Mean with 95%confidence intervals and visualization of Tukey-Kramer test

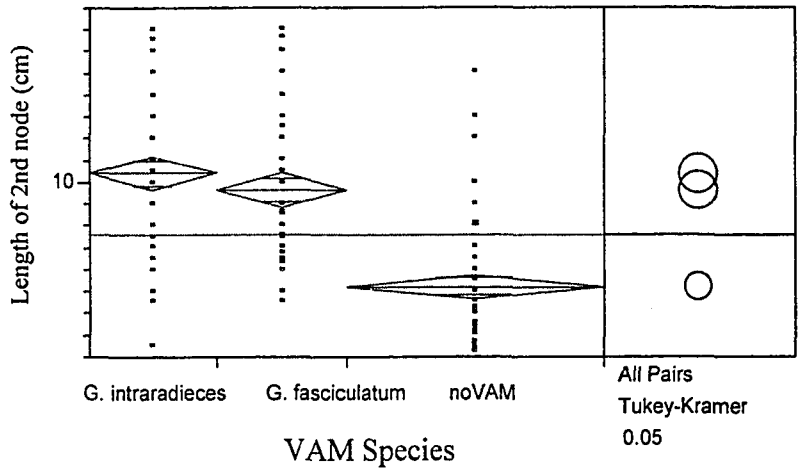

Fig 4.3. Length of 2nd internode (cm) by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

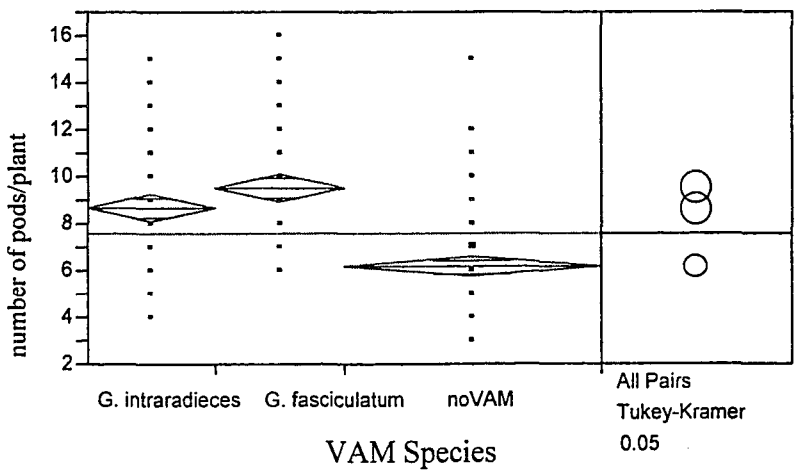

Fig. 4.4. Number of pods/plant by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

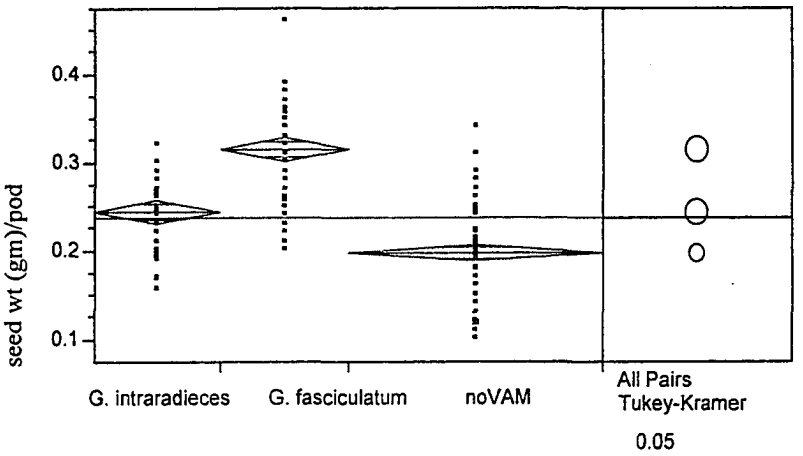

Fig. 4.5. Seed dry weight (gm)/plant by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

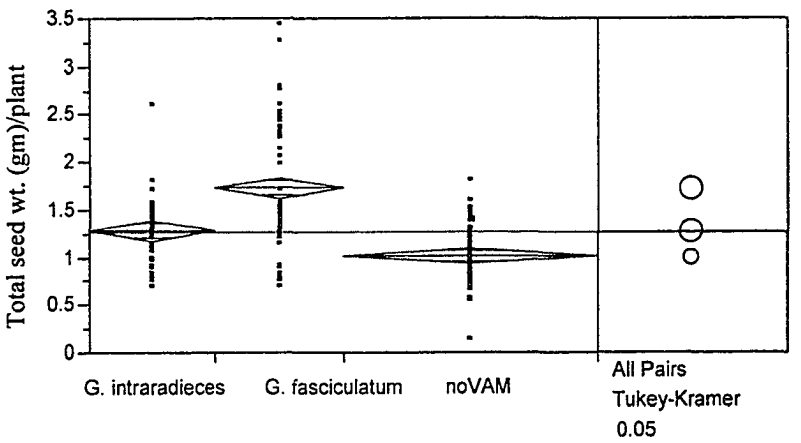

Fig 4.6. Total seed weight (gm)/plant by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

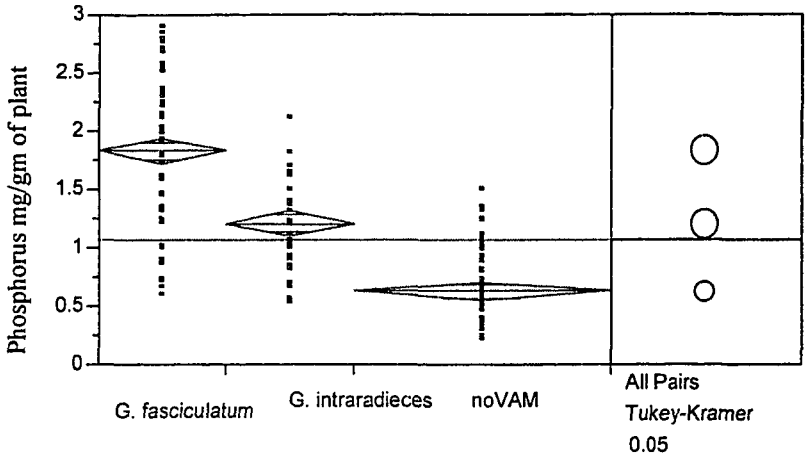

Fig. 4.7. Phosphorus mg/gm of plant (mg)/ gm of plant by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

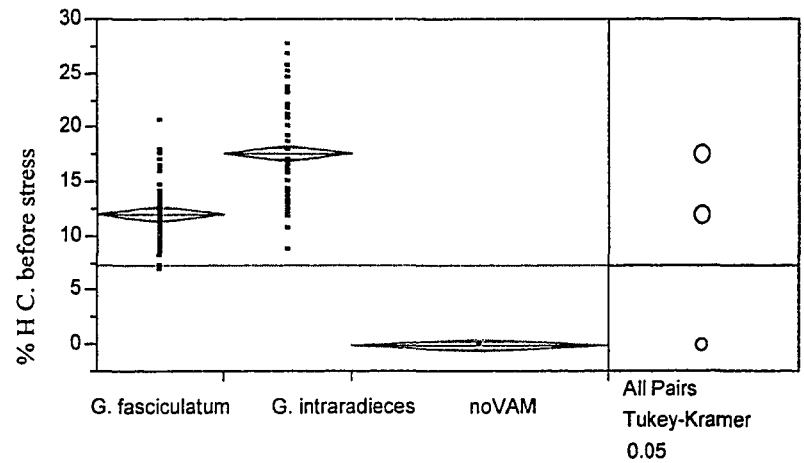

Fig 4.8. % Hyphal colonization before stress by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

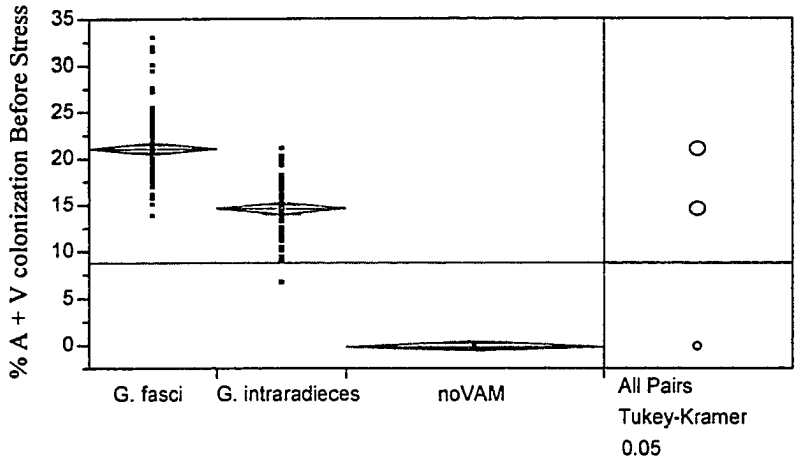

Fig 4.9. % Arbuscular + vesicular colonization before stress by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test.

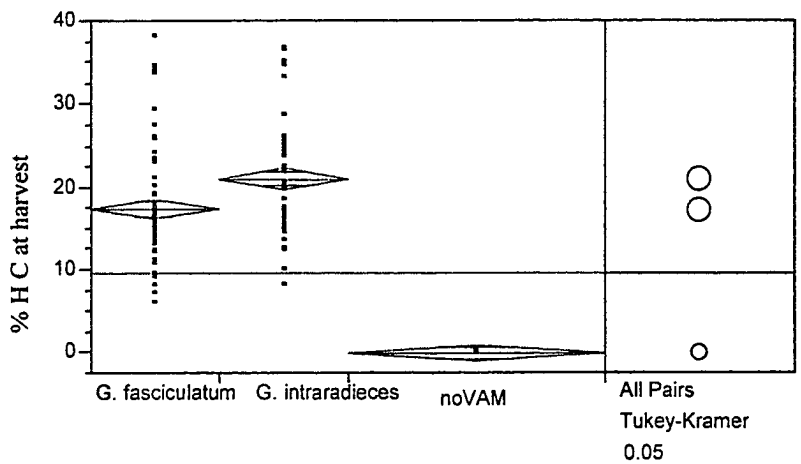

Fig 4.10. % Hyphal colonization at harvest by VAM treatments. Mean with 95% confidence intervals and visualization of Tukey-Kramer test

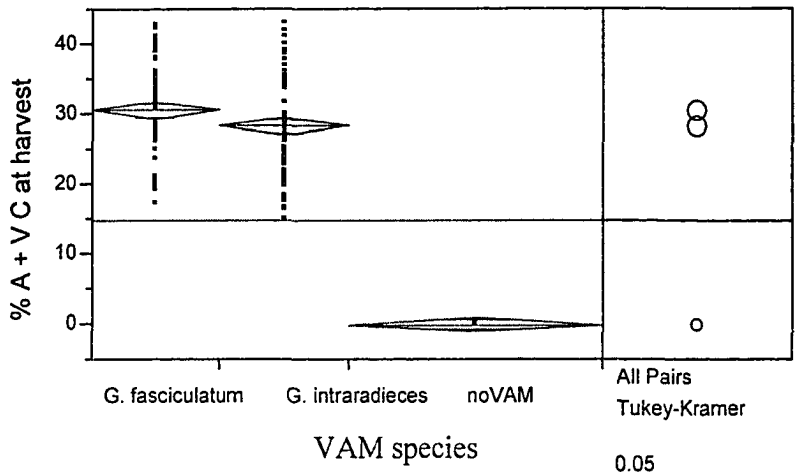

Fig 4.11. % Arbuscular + Vesicular colonization at harvest by VAM species. Mean with 95 % confidence intervals and visualization of the Tukey-Kramer test

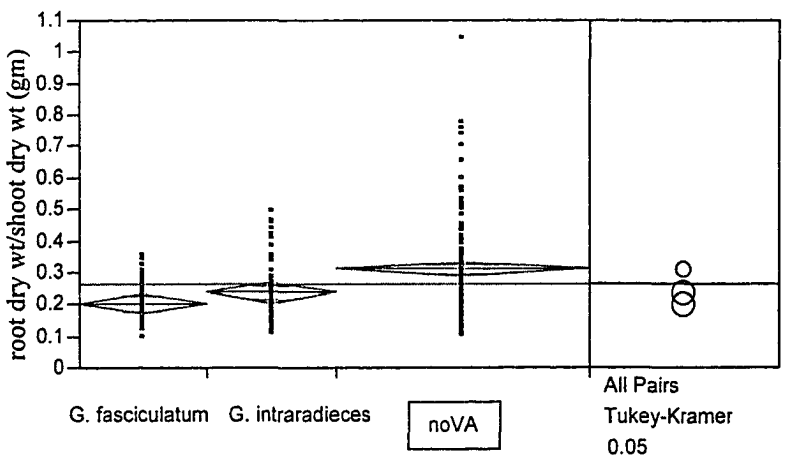

Fig. 4.12. Root dry weight/shoot dry weight by VAM treatments. Means with 95% confidence intervals and visualization of Tukey-Kramer test by VAM species

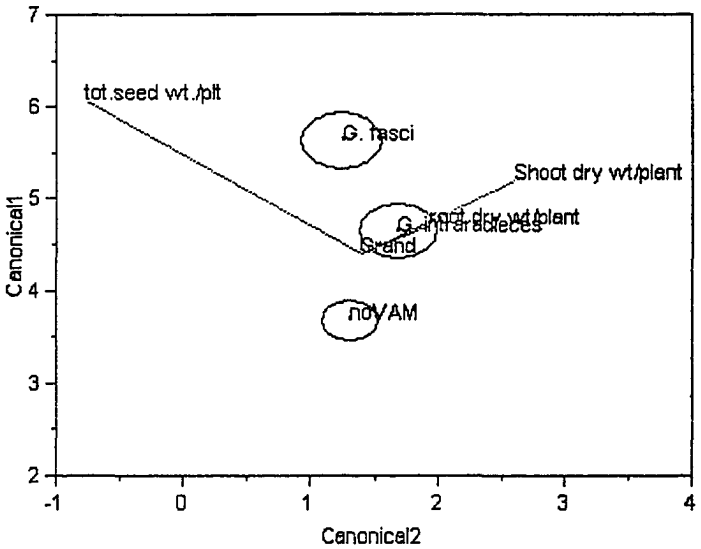

Fig.4.13. Multivariate means with the 95 % confidence intervals for shoot dry weight, root dry weight and total seed weight per plant at three levels of VAM treatments.

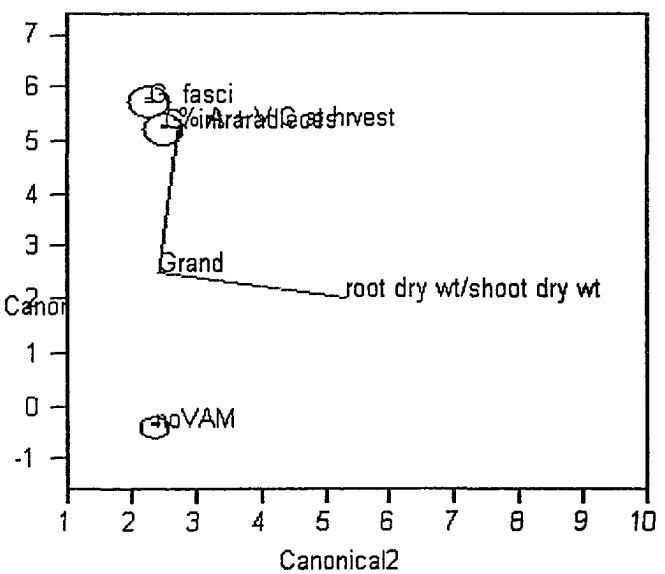

Fig.4.14. Multivariate means with 95 % confidence intervals for Arbuscular + vesicular colonization at harvest and root/shoot ratio at three levels of VAM treatments.

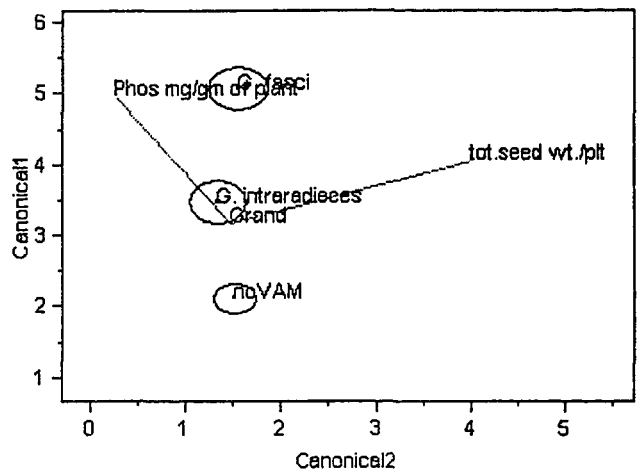

Fig.4.15. Multivariate means with 95 % confidence interval for total seed weight and phosphorus content at three levels of VAM treatments.

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Chapter 5

Experiment 3

"Endogenous levels of ABA and the plant phosphorus conten in drought stressed soybeans, inoculated with *Glomus fasciculatum.*"

5.1. Experimental design and growth conditions

This experiment was performed in a fully crossed 2x2 factorial design, with presence or absence of VAM and presence or absence of drought as main factors. The experiment was repeated twice. Plants were grown in pasteurized garden soil. The plants were given two drought stress cycles, at 5th and 8th week. The *Glomus fasciculatum* was used as VAM fungus in this experiment. Two plants were grown in each pot and shoot of one plant from each pot, was harvested after the completion of the drought stress cycle. The harvested shoots were wrapped in aluminum foil and stored in –20 °C immediately. These shoots were analyzed for endogenous levels of ABA in the plants of all treatment groups. The second plant in each group was harvested at the end of the growth season and was assessed for biomass production, phosphorus content and the % VAM colonization of roots in terms of hyphae, arbuscules and vesicles. The data were analyzed by two-way ANOVA and further tested by MANOVA.

5.2. Results

5.2.1. Effect of drought and VAM on the shoot dry weight (gms)/plant

The results of two-way ANOVA (Table 5.1) show a significant influence of VAM on the shoot dry weight (R-sq = 61.6%) with a P = <0.001. The mean values are presented in Fig 5.1.a. The drought treatment made a difference of 27 % in the shoot dry weight outcome. The VAM x drought interaction was responsible for 2 % variation (P

=0.008). The results of MANOVA showed a significant negative correlation between ABA and shoot dry weight by VAM as well as by drought (F-test yielded P<0.001). The multivariate means and the linear correlation across responses are illustrated in Fig 5.9 and 5.10.

5.2.2. The effect of drought and VAM on root dry weight (gms)/plant

The root dry weight was also altered significantly due to VAM with an R-sq of 13.7 % and P = 0.001. The Drought caused 14.4 % of the variation. The VAM x Drought interaction did not cause any significant difference to the root dry weight.

5.2.3. Root/shoot dry weight ratio

VAM plants had much lower root /shoot dry weight ratio as compared to non VAM plants as shown by the mean cell bar charts in Fig 5. 3.a. The influence of VAM was statistically significant with an R-sq of 51.7 % (Table 5.3). The drought treatment and the interaction of VAM x drought also had a significant influence on the root/shoot ratio. The drought had an R-sq 20 % and P < 0.001. The VAM x Drought had an R-sq of 4.5 % and 0.001 probability.

5.2.4. Total seed weight (gms) /plant

The results of two-way ANOVA for total seed weight (gms)/plant are presented in Table 5.4. The presence of VAM caused a variation of 58.3 % in the seed dry weight of soybean plants (P <0.001). Total seed weight/plant had an R-sq of 23.9 % in the was due to drought (P <0.001). The VAM x Drought interaction also caused 11.9 % variation of total seed weight (gms) /plant with P <0.001. The results of MANOVA illustrated in Fig 5.11 and 5.12 show multivariate means across ABA and total seed weight (gms)/plant, by VAM and drought respectively. There was a negative correlation between

the seed dry weight (gms)/plant and the ABA content (P <0.001 as given by overall F-test).

5.2.5. Plant phosphorus content (mg)/gm fresh weight of plant

The results of two-way ANOVA in Table 5.5 represent the phosphorus content of the plants, as influenced by VAM and drought. The VAM infected plants had 81.5 % variation of plant phosphorus content due to VAM (P<0.001). The drought was responsible for 10.4 % of the variation of plant phosphorus content. The VAM x Drought interaction caused 7.9 % variation in the plant phosphorus content, P value slightly higher than the rest of the treatments but still statistically significant (P = 0.02). The results of MANOVA showed a positive linear correlation between the shoot dry weight and the phosphorus content. The F-test confirmed the significance of the linear correlation (P<0.001). The multivariate means are given in Fig 5.13. ABA levels in the plant and phosphorus content (mg)/gm of the plant were found to be negatively correlated by VAM as well as by drought. Multivariate means across the responses are given in Fig. 5.14 and 5.15.

5.2.6. Percent colonization of roots (hyphal, arbuscular + vesicular) before drought stress

VAM was responsible for 92.4 % variation in hyphal colonization before stress was (Table 5.6) with P < 0.001. Arbuscular + vesicular colonization due to VAM treatment, had an R-sq of 90 % (Table 5.7) P <0.001.

5.2.7. Percent colonization of roots (hyphal, arbuscular + vesicular) at harvest

The hyphal colonization at the harvest was influenced 83 % by VAM (P<0.001) and 1.2 % by drought and the same by VAM x Drought, interaction and had P value 0.03

(Table 5.8). The arbuscular + vesicular colonization varied 87.4 % due to VAM ($P<0.001$) as shown in Table 5.9. The ABA levels were found to be negatively correlated to % hyphal colonization of roots at harvest. F-test gave $P<0.001$. The multivariate means are shown in Fig. 5.16. However ABA was positively correlated to arbuscular + vesicular colonization of roots at harvest, significant at $P<0.001$. The multivariate means across the responses are illustrated in Fig 5.16.

5.2.8. Endogenous level of ABA (picomoles/gm of fresh weight, of plant shoot

As reported in Table 5.10 ABA level of the plant shoots, was influenced significantly be all the treatments. The VAM was responsible for 9.4 % of the variation ($P = 0.006$). The endogenous level of ABA were found to be negatively correlated to the % hyphal colonization of roots by VAM at harvest but it was found to have a positive correlation with arbuscular + vesicular colonization. The F-test for the ABA level and the hyphal as well as arbuscular colonization gave a $P <0.001$. The drought caused 16 % variation and $P <0.001$. The VAM x Drought interaction brought 7.6 % variation in the plant ABA content, a statistically significant contribution ($P = 0.01$).

The results of MANOVA for ABA and phosphorus content show a significantly negative linear correlation between the two (F-test gave $P <0.001$). Multivariate means are illustrated in Fig 5. 14 and Fig. 5.15.

The MANOVA for ABA and total seed weight/plant by VAM and drought done separately also revealed a significantly negative correlation between ABA endogenous ABA level and total seed weight/plant (F-test $P <0.001$. The multivariate means are depicted in Fig 5.11 and 5.12. The results of MANOVA for endogenous levels of ABA, and shoot dry weight /plant by VAM as well as by Drought in separate tests show a

significantly negative correlation between the endogenous levels of ABA and the shoot dry weight/plant. The over all F-test yielded $P<0.001$. The multivariate means are given in Fig 5.9 and Fig 5.10.

5.3. Discussion

The results of experiment# 1 with *Glomus intraradieces* and Experiment # 2 with *Glomus fasciculatum* as described in chapter 2 and 3 helped the drought stressed soybeans to different degrees in different response variables. The current experiment (#3) took soybean –*Glomus fasciculatum* interaction study to another level. The amount of ABA present in plants of all treatment combinations were estimated through ELISA.

A number of studies have shown that the mycorrhizal plants can increase their photosynthetic rate to compensate for the needs of microbial partners, this can lead to increased surface area in young plants and an increase in the amount of CO_2 fixed per unit weight of the leaf. Studies of the ability of different plant associations to compensate for the needs of mycorrhizal partners continues to prove a useful area of research. In the present study the results have repeatedly shown an increased growth and productivity of VAM colonized plants especially in case of drought stress, as compared no non-VAM drought stressed plants. These results are supported by several other reports (Ruiz *et al.* 1995; Subhan *et al.* 1998). According to Ruiz *et al* (1995) the VAM helps to increase the drought tolerance of the mycorrhizal plants. When it comes to the dealing with drought plants use two mechanisms. One is avoidance and the other is tolerance. The avoidance of plant water deficits include adjustments such as shorter growth cycles, and different morphological and anatomical adjustments to help conserve the water or to maximize the water uptake by developing a good root system. Some VAM fungi have been reported to

enhance the growth of host plant roots. They either produce some auxins or just maintain the high levels of these growth hormones in the roots. The carbon sources of the plants tend to accumulate wherever there is a high concentration of auxins. This way the presence of auxins eventually leads to improved growth of roots (Barea and Azcon, 1982).

The other mechanism is to develop tolerance for drought by maintaining turgor through stoamatal conductance and maximum use of available water and nutrients and VAM fungi help the plants to acquire these things. The effecient organisms try to alter their physiological and biochemical processes to help them cope with the environmental stresses (Smith and Gianinazzi, 1988). Drought stress usually causes a suppression of transpiration that leads to lower photosynthetic rates in soybeans (Egli *et al.* 1983). The VAM endophytes can help the host plants to alter their physiological parameters and help them adapt to drought (Ruiz *et al.* 1995; Auge *et al.* 1993). The results of present study agree with this concept. In the present study the shoot dry weight of the droughted and non- droughted VAM plants were found to be higher than that of the non-VAM droughted as well as non- VAM non- droughted plants (Fig 5.1.a and 5.1.b.).

A negative correlation was found between the endogenous levels of ABA and the shoot dry weight of the plants. There was a positive correlation between the ABA content and the % arbuscular and vesicular colonization of roots at harvest. Keeping in mind that the ABA estimations were carried in the plants harvested immediately after the stress, the increased level of ABA in drought stressed VAM inoculated plants might have triggered an increase of the arbuscular as well as vesicular formation by the VAM endophyte. Arbuscular growth might have helped increase the nutrient transfer and the vesicular

growth for storage of polyphosphte molecules and possibly to ensure the survival of the fungus itself because under drought stressed conditions the vesicles are transformed into sporangia that house chlamydospores of the *Glomus fasciculatum*. The end result was that the reduction in biomass of soybeans that results from drought stress can be partially offset by the inoculation of VAM fungi such as *Glomus fasciculatum*.

It is generally assumed that a very branched root system has a greater absorbing power than an elongate one (Glinski, and Lipiec, 1990). The VAM colonized roots in the present study had higher biomass as compared to the non- VAM plants. A negative correlation was observed between the ABA levels of the plants and the root dry weight (gms)/plant. This is in agreement with the poor growth of drought stressed roots and higher level of ABA found in drought stresses non-VAM plants in the present study (Fig. 5.2.a and 5.2.b). The VAM inoculated plants had a lower level of ABA and higher root dry weight (gms)/plant. The root / shoot ratio of the VAM colonized droughted soybeans were lower than the non-VAM droughted plants suggesting enhanced tolerance of host due to *Glomus fasciculatum*. This finding is supported by Busse and Ellis (1984). The MANOVA revealed a significant negative correlation between ABA levels and the root/shoot ratio of the soybean plants. Which means if the ABA increased the root weight decreased resulting in lower root/shoot ratio.

Total seed weight (gms)/plant of VAM colonized, non- droughted plants were highest, followed by VAM colonized droughted plants. The non-VAM droughted plants had the lowest total seeds weight (gms)/plant (Fig. 5.4.a and 5.4.b). The MANOVA revealed a negative correlation between the ABA levels and total seed weight (gms)/plant . The results of the other experiments in the present study suggest an improved total seed

weight (gms)/plant in VAM colonized, droughted as well as non-droughted palnts. It was discussed in the previous chapters that a nonhydraulic signal is send to the leaves of droughted plants, which alters the stomatal conductance of the leaves. The nature of this nonhydraulic signal was proposed to be ABA. This nonhydraulic signal can be eliminated by VAM colonization (Auge *et al* 1994). In the present VAM colonized plants have been found to have lower levels of ABA as compared to non-VAM soybean plants, which suggests that the VAM plants when subjected to drought never experience the decrease in the stomatal conductance and eventually lower photosynthetic rates as the non-VAM drought stressed plants do (Robert *et al.* 1996). That is one of the reasons for the increase in the total seed weight /plant of VAM colonized plants as compared to non-VAM plants. A positive correlation between the ABA levels and the % arbuscular + vesicular colonization but a negative correlation between the % hyphal colonization and ABA (Fig.5.16 and 5.17) needs further exploration. An estimation of ABA at different intervals during the life cycle of the soybean plants probably can shed more light on the interaction of ABA levels in the plants and its role in vegetative and reproductive growth of the plants. This can be one avenue for future exploration of VAM and endogenous ABA interaction on drought stressed soybeans. There are some reports of increased ABA levels in VAM plants as opposed to non-VAM plants when estimated over a coarse of time for example Danneberg *et al.* (1992).

So far there has not been much success with the estimation of ABA in VAM fungi because they cannot be grown in pure cultures Thus the relative contributions of VAM and fungus to the level of ABA found in the plants are not known. The ABA is known to be a typical stress hormone. The level of ABA in plants increases in response to

conditions such as drought (Lachno and Baker, 1986) and other stresses such as flooding and salinity (Zhang and Davies, 1987; Kefu *et al.* 1991). The increases were usually observed within hours after the onset of the stress. The long term effects of ABA, however, can be involved in the regulation of host mycorrhizal relationship.

The results of the present study showed a positive correlation between the phosphorus (mgs)/gm of plant material and the shoot dry weight (gms)/plant. These results can be supported by several repots (Bethlenfalvay *et al.* 1987; Rajapakse *et al.* 1989; Mukharjee and Rai, 2000). Like many other reports (Nelsen and Safir, 1982; Fitter, 1988) the results of present study also suggests that mycorrhizae influence the plants responses especially to drought by enhanced phosphorus uptake.

In the present study the plant ABA levels of drought stressed non-VAM plants were found to be much higher than VAM and droughted plants. The same was reported by Duan *et al.* (1996) and Goicoechea *et al.* (1997a), these authors suggest that the reason for lower ABA levels was that plants were never stressed. The present study revealed a negative correlation between ABA level and the phosphorus content of plants. Which makes sense if the positive correlation between phosphorus content (mgs)/gm of plant and shoot dry weight (gms)/plant and total seed weight (gms)/plant is considered. Moreover a negative correlation between ABA levels (picomoles/gm of plant fresh weight) and shoot dry weight (gms)/plant and total seed weight (gms)/plant is found in the present study.

5.4. Conclusion.

The present study has contributed towards the classic understanding that VAM improves plant growth by enhancing phosphorus uptake especially in drought stressed plants. This is true under the growth conditions used for this study. Comparisons between ABA levels and other responses suggest the mycorrhizal and ABA interaction influences host physiology. Studies of other aspects of ABA and drought stress in soybeans along with different mycorrhizal fungi can add more to the understanding of the complex dynamics of the ABA and VAM interaction.

The increased interest in mycorrhizae in general and their interaction with plant water relation is exciting. There is however a need to improve the availability of instrumentation, for controlled environment work to produe more conclusive data. Also the mycorrhizal studies should also be extended to the field experiments more than it has been done in the past. With the addition of more replicated and repeated experiments more can be learned about the mycorrhizal plant water relations since we have only stretched the surface in this intriguing field of research.

VAM mycorrhizae can improve the plant growth and water use efficiency. The soil type, the amount of available phosphorus in the soil and the fact that mycorrhizae are totally dependent on the host for their nutrition all play a role in determining the mycorrhizal response. The results of this study can help determine that the role performed by different VAM fungi, in helping the colonized plant can be diverse.

One VAM fungus can be more effective than the other. The VAM fungal efficiency can be evaluated in terms of the vegetative and reproductive growth of the host plants. The selection of appropriate VAM fungi to be used agriculturally in stressed

environment to address specific problems is a promising, but usually neglected strategy. Suitably adapted mycorrhizal fungal isolates should be studied for their importance for maintaining and restoring the plant soil equilibrium in sustainable agricultural situations.

Table 5.1 Two-way analysis of variance for the shoot dry weight (gm)/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	89.971	89.971	381.234	<0.001	0.616
VAM present	4.176						
VAM absent	1.727						
Drought (B)		1	39.744	39.744	168.408	<0.001	0.272
Drought	2.137						
No drought	3.765						
Interaction							
VAM X drought		1	2.969	2.969	12.580	0.008	0.020
Unexplained		56	13.215	0.236			0.090
Total		59	145.900				

Table 5.2 Two-way analysis of variance for the root dry weight (gms/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.148	0.148	11.060	0.001	0.137
VAM present	0.785						
VAM absent	0.686						
Drought (B)		1	0.155	0.155	11.647	0.001	0.144
Drought	0.685						
No drought	0.786						
Interaction							
VAM X drought		1	0.029	0.029	2.165	0.146	0.026
Unexplained		56	0.749	0.013			0.732
Total		59	1.082				

Table 5.3 Two-way analysis of variance for the root/shoot dry weight ratio as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.903	0.903	122.932	<0.001	0.517
VAM present	0.201						
VAM absent	0.447						
Drought (B)		1	0.352	0.352	47.960	<0.001	0.201
Drought	0.401						
No drought	0.280						
Interaction							
VAM X drought		1	0.078	0.078	10.687	0.0018	0.045
Unexplained		56	0.411	0.007			0.235
Total		59	1.746				

Table 5.4 Two-way analysis of variance for the total seed dry weight (gm)/plant as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	14.582	14.582	571.837	<0.001	0.538
VAM present	1.850						
VAM absent	0.864						
Drought (B)		1	2.992	2.992	234.937	<0.001	0.239
Drought	1.041						
No drought	1.673						
Interaction							
VAM X drought		1	2.992	2.992	117.350	<0.001	0.119
Unexplained		56	1.428	0.025			0.057
Total		59	24.995				

Table 5.5 Two-way analysis of variance for the phosphorus content (mgs)/gm of plant tissue) as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	41.153	41.153	647.998	<0.001	0.815
VAM present	2.443						
VAM absent	0.787						
Drought (B)		1	5.244	5.244	82.580	<0.001	0.104
Drought	1.319						
No drought	1.911						
Interaction							
VAM X drought		1	0.316	0.316	4.977	0.029	0.079
Unexplained		56	3.556	0.063			0.071
Total		59	50.270				

Table 5.6 : Two-way analysis of variance for the % hyphal colonization before stress as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	2694.351	2694.351	723.395	<0.001	0.924
VAM present	13.402						
VAM absent	0.000						
Unexplained		56	208.577	3.725			0.072
Total		57					

Table 5.7 Two-way analysis of variance for the % arbuscular + vesicular colonization before stress as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	6639.592	6639.592	665.447	<0.001	0.900
VAM present	21.039						
VAM absent	00.000						
Unexplained		56	558.747	9.98			0.075
Total		57	7362.093				

Table 5.8 Two-way analysis of variance for the % hyphal colonization at harvest as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	4870.373	4870.373	329.320	<0.001	0.830
VAM present	18.019						
VAM absent	0.000						
Drought (B)		1	71.451	71.451	4.831	0.032	0.012
Drought	7.918						
No drought	10.100						
Interaction							
VAM X drought		1	71.451	71.451	4.831	0.032	0.012
Unexplained		56	828.193	14.790			0.140
Total		59	5841.471				

Table 5.9 Two-way analysis of variance for the % arbuscular + vesicular colonization at harvest as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	12782.447	12782.447	1833.039	<0.001	0.874
VAM present	29.191						
VAM absent	0.000						
Drought (B)		1	743.195	743.195	105.285	<0.001	0.050
Drought	18.094						
No drought	11.097						
Interaction							
VAM X drought		1	743.195	743.195	105.285	<0.001	0.050
Unexplained		56	390.508	6.970			0.026
Total		59	14641.346				

Table 5.10: Two-way analysis of variance for the ABA concentration Picomoles/gm of fresh weight of shoot as a response variable in a fully-crossed factorial design with presence or absence of VAM (*Glomus fasciculatum*), and the presence or absence of drought as main factors, with N=15 plants/treatment.

Source of variation	Mean	Df	SS	MS	F	P	R-Sq
VAM (A)		1	0.114	0.114	8.005	0.006	0.094
VAM present	0.342						
VAM absent	0.429						
Drought (B)		1	0.205	0.025	14.395	<0.001	0.160
Drought	0.444						
No drought	0.327						
Interaction							
VAM X drought		1	0.091	0.091	6.438	0.014	0.076
Unexplained		56	0.797	0.014			0.659
Total		59	1.208				

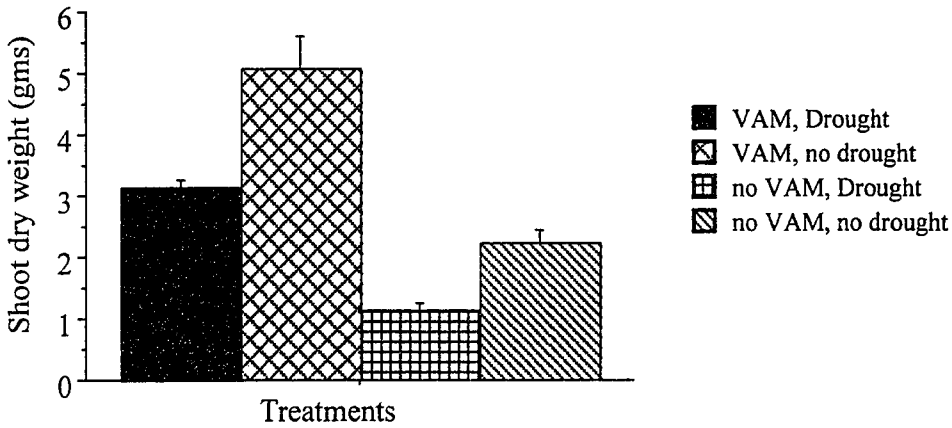

Fig 5.1.a. Means with 95 % confidence interval for all combinations of factors in a two-way design of VAM and drought, with shoot dry weight as the response.

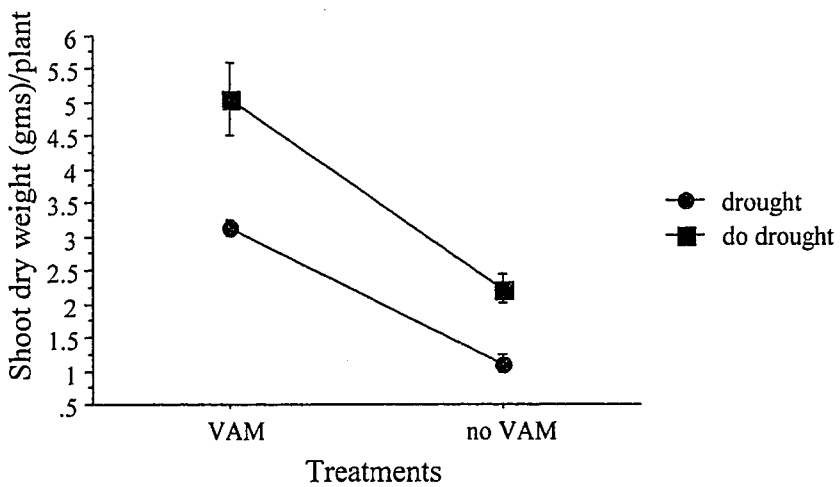

Fig 5.1.b. Mean shoot dry weight (gms)/plant in interaction with VAM and and drought. Error bars represent 95% confidence interval.

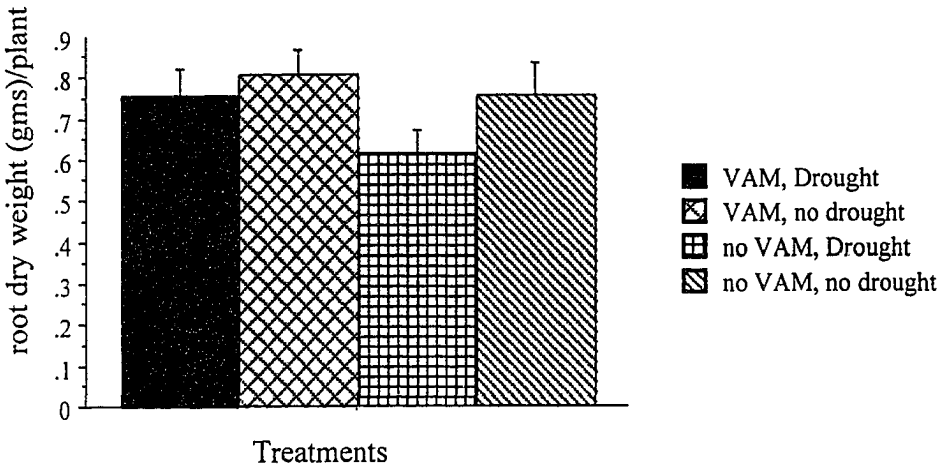

Fig:5.2.a. Means with 95 % confidence interval for all combinations of factors in a two-way design of VAM and drought with root dry weight (gms)/plant as the response.

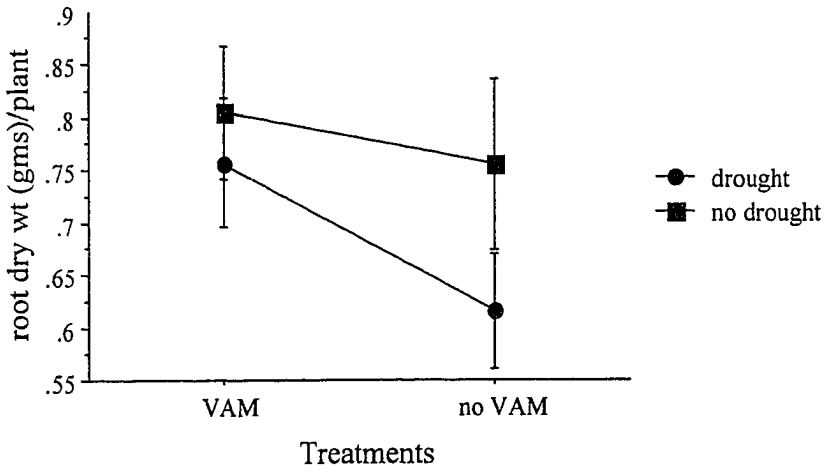

Fig 5.2.b. Mean root dry weight (gms)/plant in interaction with VAM and drought. Error bars represent 95% confidence interval.

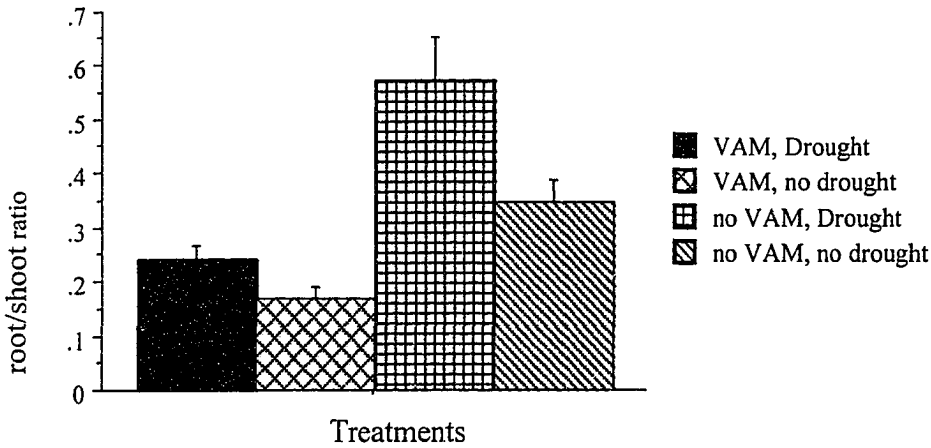

Fig 5.3.a. Means with 95 % confidence interval.s for all combinations of factors in a two way design of VAM and drought with root/shoot ratio as the response.

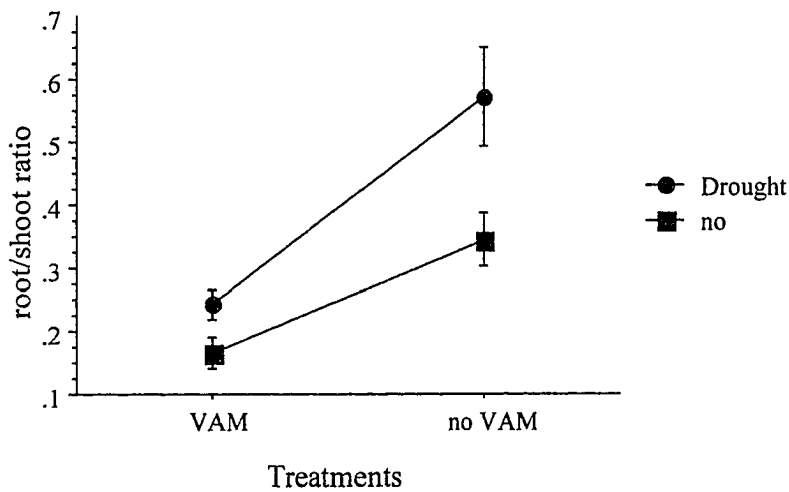

Fig: 5.3.b. Mean root/shoot ratio in interaction with VAM and drought. Error Bars represent 95% confidence interval.

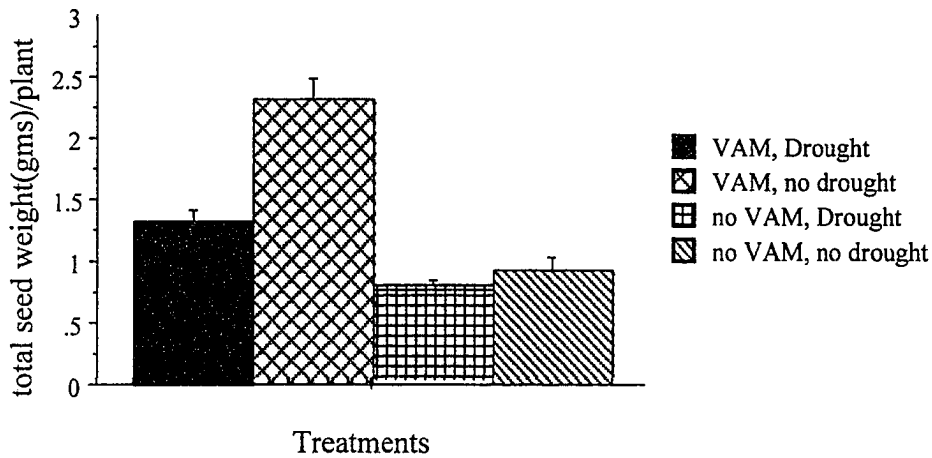

Fig 5.4.a. Means with 95 % confidence interval for all combinations of factors in a two-way design of VAM and drought with total seed weight (gms)/plant as the response.

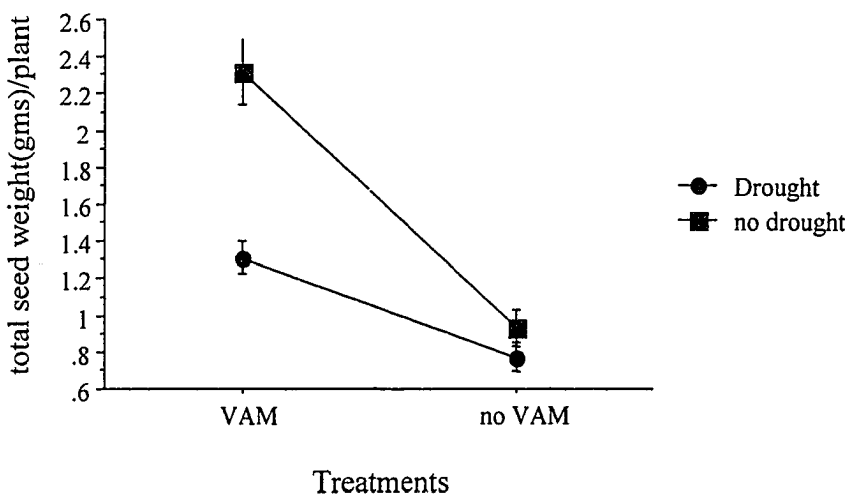

Fig 5.4.b. Mean total seed weight (gms)/plant in interaction with VAM and drought. Error bars represent 95% confidence interval.

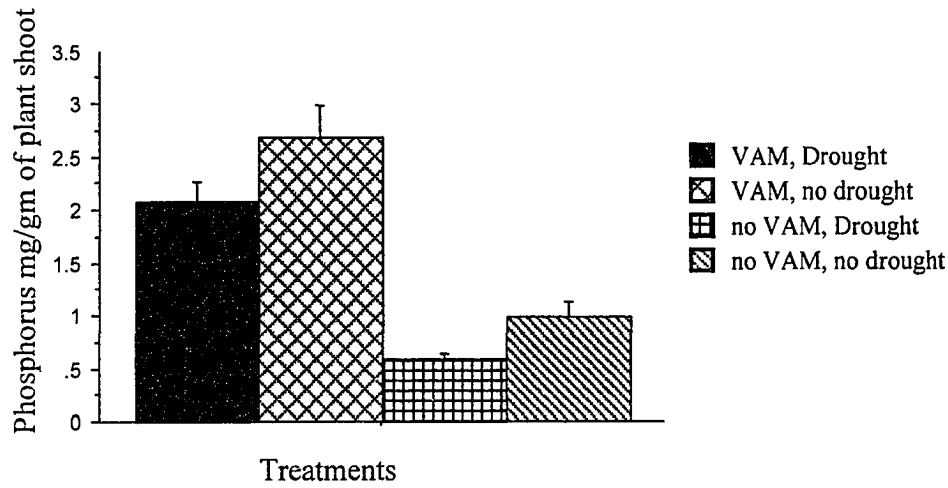

Fig 5.5.a. Means with 95 % confidence interval for all combinations of factors in a two-way design of VAM and drought with Phosphorus content (mg)/ (gm) of plant as the response.

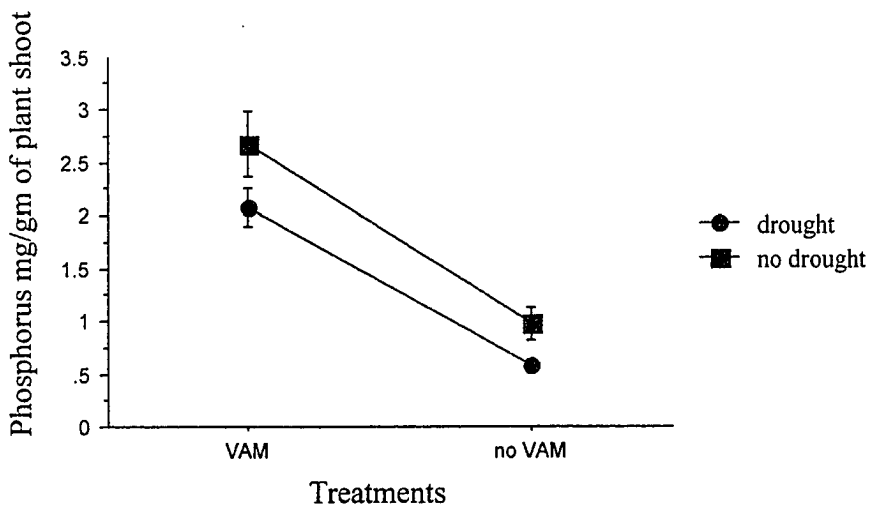

Fig 5.5.b. Mean Phosphorus content(mg)/ (gm) plant in interaction with VAM and drought. Error Bars represent 95% confidence interval.

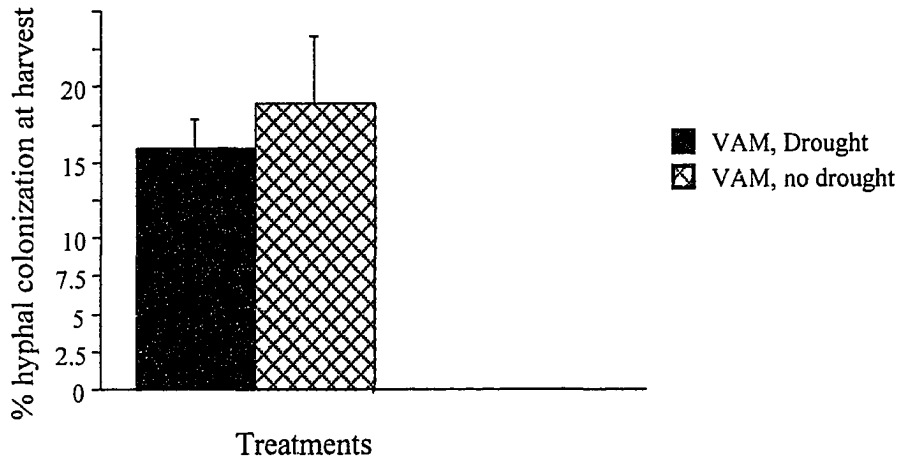

Fig 5.6.a. Means with 95 % confidence interval.s for all combinations of factors in a two way design of VAM and drought with % hyphal colonization at harvest as the response.

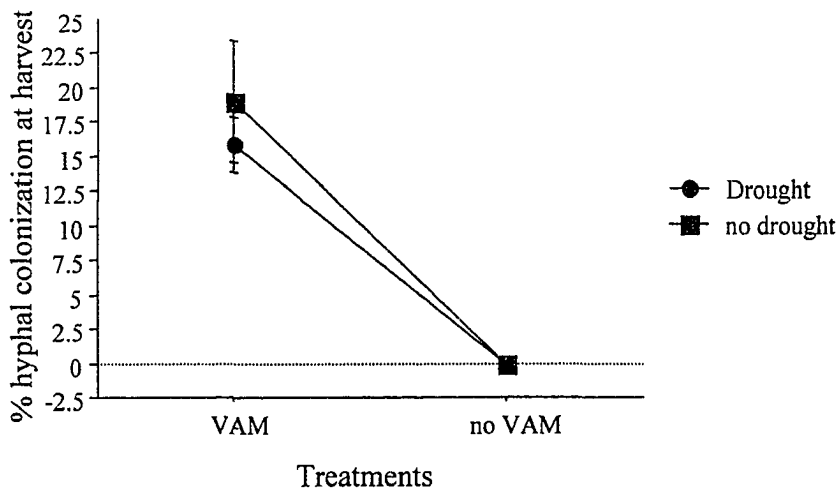

Fig 5.6.b. Mean % hyphal colonization at harvest in interaction with VAM and drought. Error Bars represent 95% confidence interval.

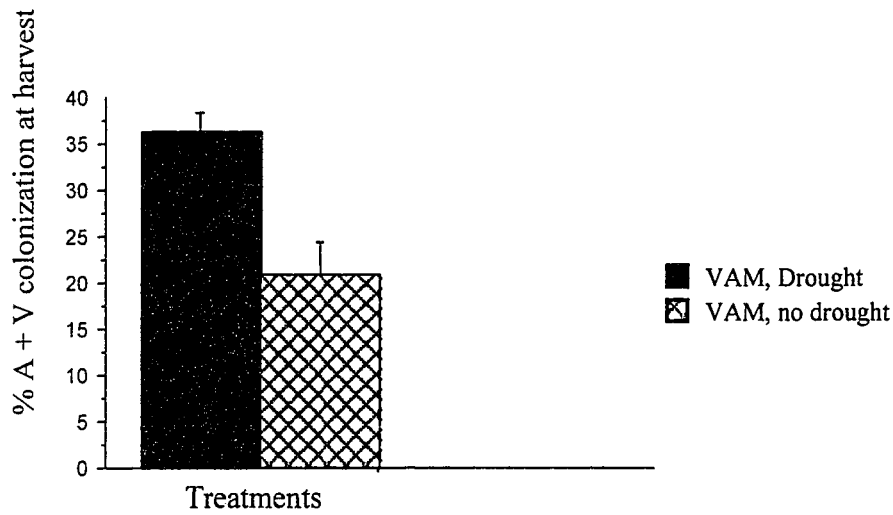

Fig 5.7.a. Means with 95 % confidence interval for all combinations of factors in a two-way design of VAM and drought with % arbuscular + vesicular colonization as the response.

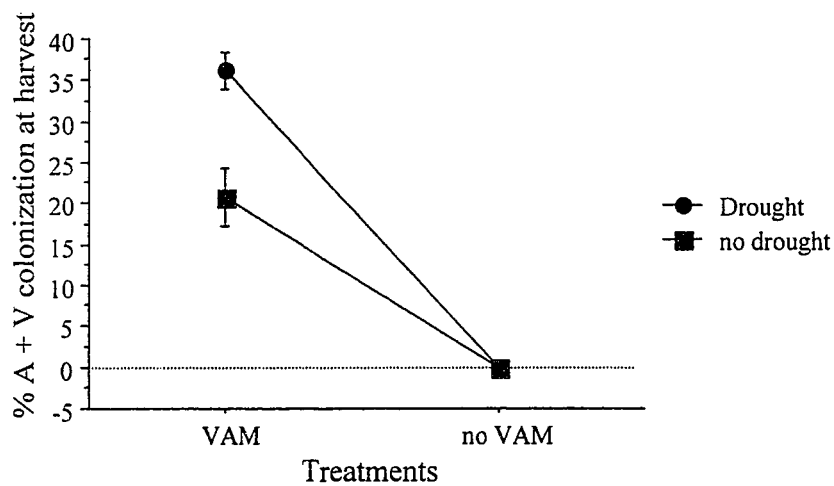

Fig 5.7.b. Mean total seed weight (gms)/plant in interaction with VAM and drought. Error Bars represent 95% confidence interval.

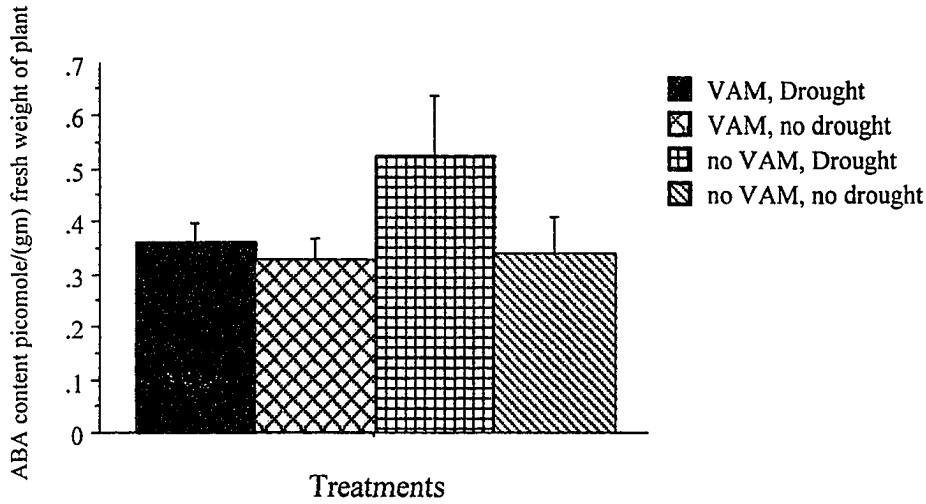

Fig 5.8.a. Means with 95 % confidence interval for all combinations of factors in a two-way design of VAM and drought with ABA level (picomoles)/gm of fresh plant weight as the response.

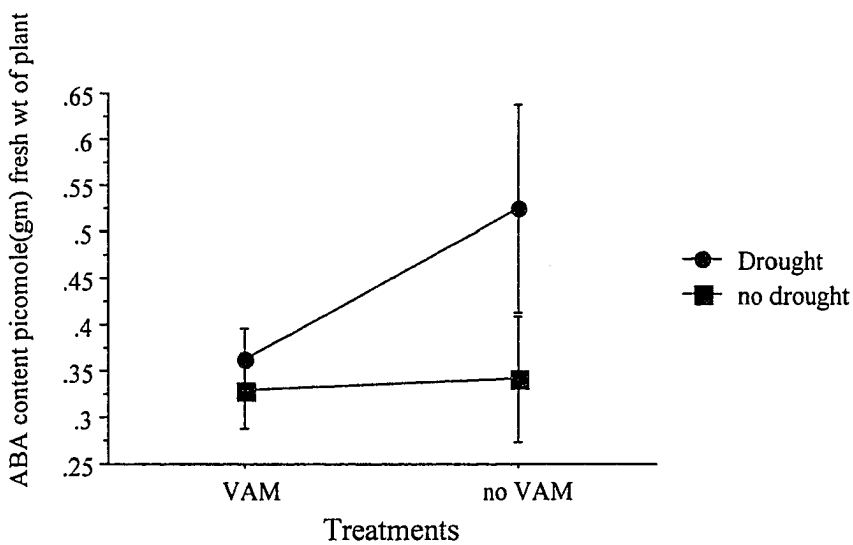

Fig 5.8.b. Mean ABA levels (picomole) /(gm) fresh weight of plant in interaction with VAM and drought. Error Bars represent 95% confidence interval.

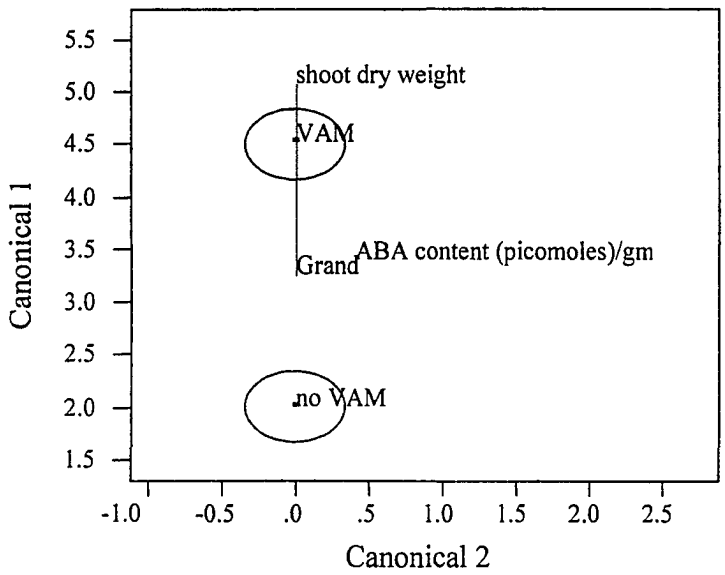

Fig. 5.9. Multivariate means with 95 % confidence interval. for shoot dry weight (gms) /plant and ABA at two levels of VAM treatments.

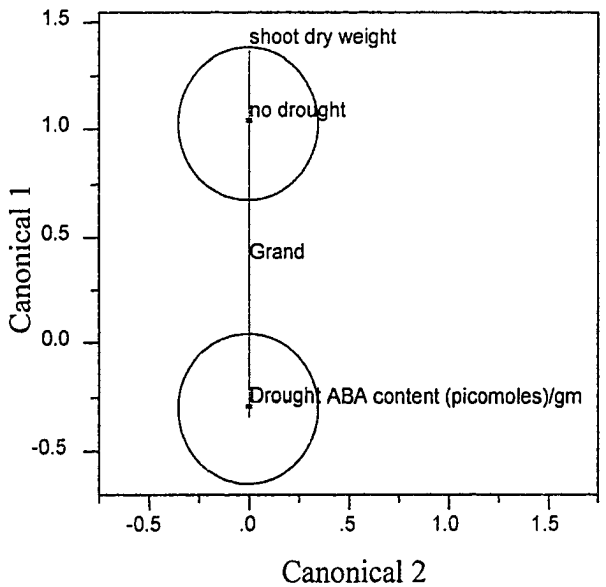

Fig. 5.10. Multivariate means with 95 % confidence interval. for shoot dry weight (gms) /plant and ABA at two levels of drought treatments.

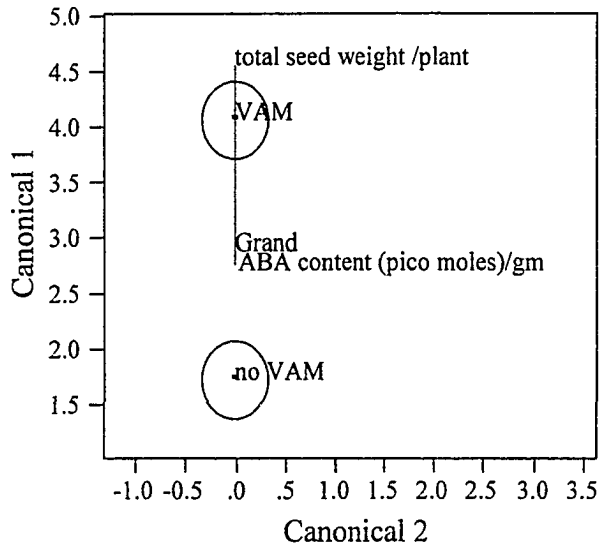

Fig. 5.11. Multivariate means with 95 % confidence interval. for total seed weight (gms) /plant and ABA, at two levels of VAM treatments.

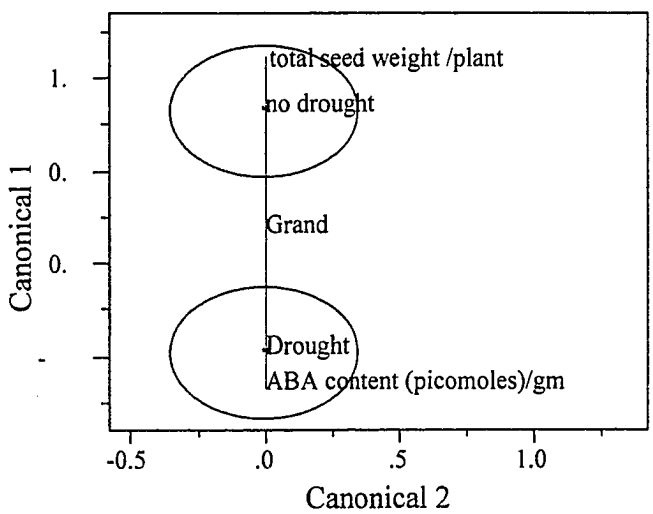

Fig. 5.12. Multivariate means with 95 % confidence interval. for total seed weight (gms) /plant and ABA, at two levels of drought treatments.

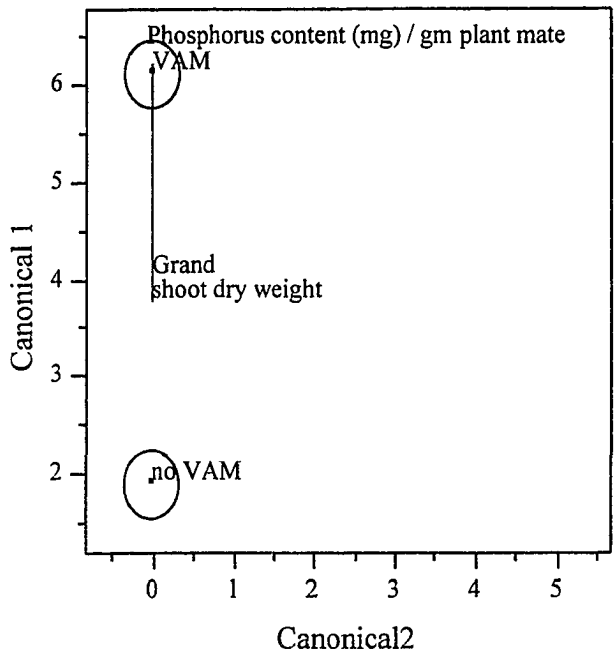

Fig. 5.13. Multivariate means with 95 % confidence interval. for phosphorus (mg) /(gm) and shoot dry weight (gms) at two levels of VAM treatments.

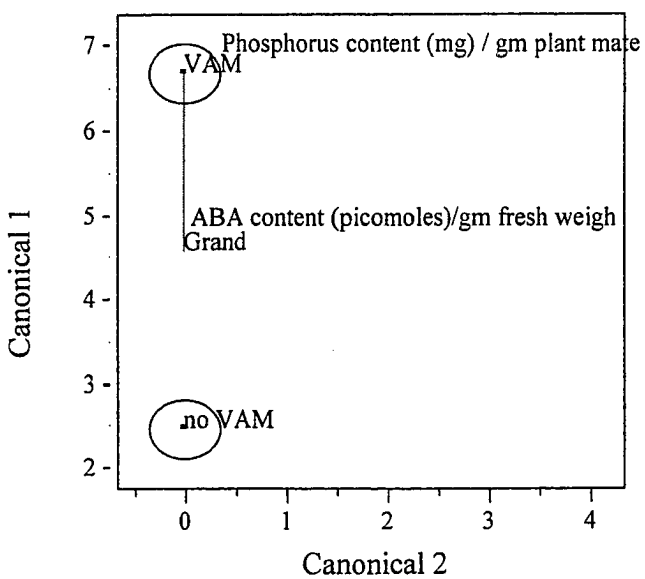

Fig.5.14. Multivariate means with 95 % confidence interval. for ABA and phosphorus at two levels of VAM treatments.

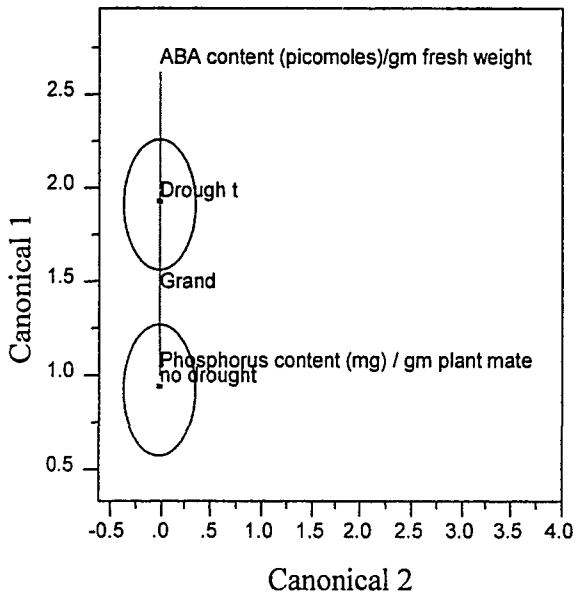

Fig. 5.15. Multivariate means with 95 % confidence interval. for ABA and phosphorus at two levels of drought treatments.

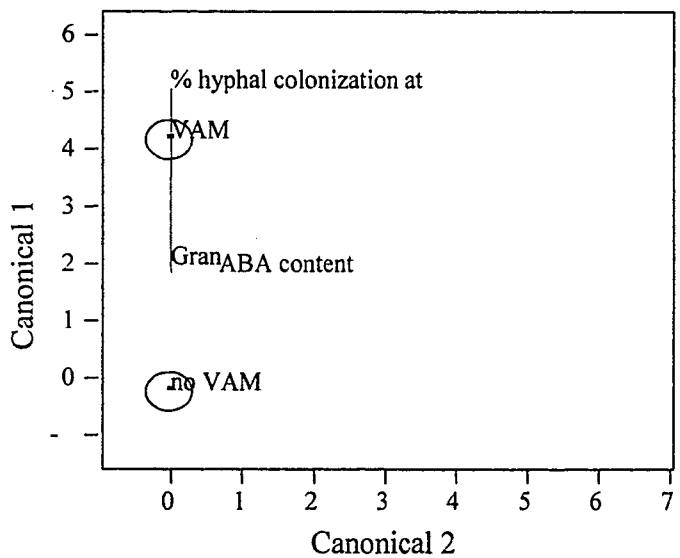

Fig. 5.16. Multivariate means with 95 % confidence interval. for % hyphal colonization at harvest and ABA at two levels of VAM treatments.

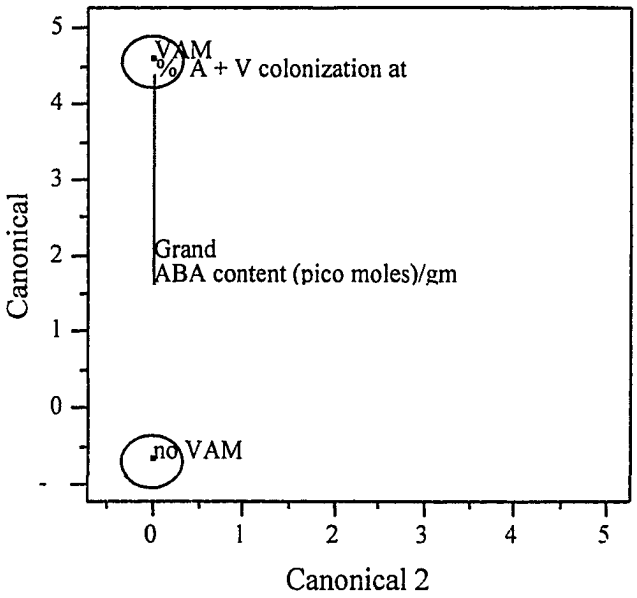

Fig. 5.17. Multivariate means with 95 % confidence interval. for A+V colonization at harvest and ABA at two levels of VAM treatments.

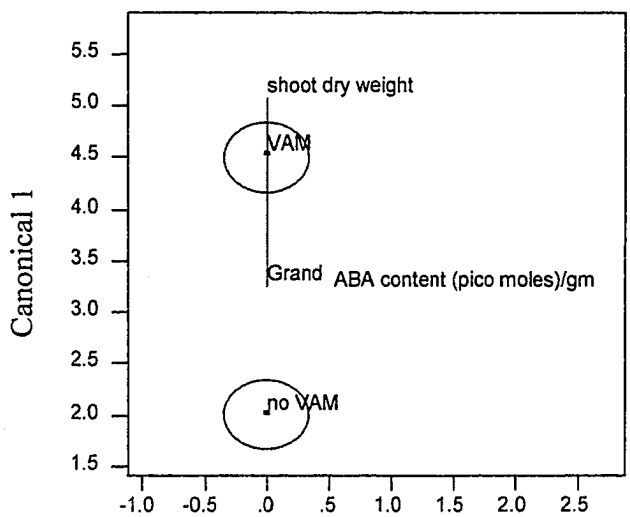

Fig 5.18. Multivariate means with 95 % confidence interval. For shoot dry weight and ABA at two levels of VAM treatments.

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