



Université d'Ottawa - University of Ottawa

**ROLE OF ARBUSCULAR MYCORRHIZAL FUNGUS (*GLOMUS*
INTRARADICES SCHENCK & SMITH) COLONIZATION IN DROUGHT
TOLERANCE OF MAIZE (*ZEA MAYS* L.)**

by

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ABSTRACT

The purpose of this thesis was to study the potential factors involved in mycorrhizae-assisted drought tolerance in maize (*Zea mays* L.). We hypothesized that the AM colonization promotes drought tolerance of the host plant. This may be as a consequence of altered water relations, metabolism or nutritional status of the host plant. These changes enable the host plant to sustain water deficit conditions and recover more rapidly when irrigation is restored. To test these hypotheses, the five objectives were : (i) To measure the physiological responses in maize plants in the absence or presence of AM colonization; (ii) To examine the metabolic changes in these plants; (iii) To determine the host plant nutritional status in order to assess the ability of AM plants to support kernel development; (iv) To evaluate the drought recovery of maize and (v) To examine the effects of AM colonization on nitrogen assimilation in maize as a potential factor in drought tolerance. In order to accomplish these objectives, two greenhouse experiments were conducted with the same set of treatments at two critical stages in maize, the preflowering and tasselling stages. Freshly regenerated seeds of selection cycles C0 (cv. drought-sensitive) and C8 (cv. drought-resistant) of the lowland tropical population 'Tuxpeño sequía' were used for this study. Maize plants were subjected to drought stress for 3 wks at preflowering (45-66 days after sowing) or tasselling stages (75-95 DAS) thereafter the plants were rewatered until the end of the experiment. One half of the maize plants were inoculated with AM fungus (*Glomus intraradices* Schenck & Smith) at the time of sowing.

The AM colonization in maize had a beneficial effect on the water relations and leaf enlargement under water deficit conditions. In comparison to non-AM plants, the AM colonized plants maintained higher (less negative) leaf water potential (LWP) and lower stomatal resistance even after 3 wks of withholding water at the tasselling stage. On rewatering, AM plants took less time (C0, 7 d; C8, 4 d) than non-AM plants (C0, 15 d; C8, 8 d) to attain LWP comparable to well-watered plants. The rapid recovery of AM plants was linked to the increased phosphorus (P) status. The mycorrhizal response was more pronounced in the drought-sensitive (C0) than -resistant (C8) maize cultivars.

Mycorrhizal plants retained significant amounts of sugars and nitrogenous compounds under drought conditions. Higher sugar concentrations accompanying decreasing LWP with the progression of drought stress appeared to be physiologically important for host plants. Soluble protein concentrations were also higher in AM plants may be due to the lesser extent of protein degradation as indicated by lower amino acid concentrations.

Mycorrhizal colonization improved the nutritional status of maize through the enhanced uptake of N, P and other micronutrients. This indirectly helps the AM plants to utilize the soil available moisture more effectively. Our data indicated that the total N content in drought-stressed maize plants were nearly doubled in the presence of AM association. This study suggests that AM colonization is a crucial factor in the host plant N acquisition under drought conditions. The AM colonization significantly affected the maize reproductive behaviour by reducing the days to silking and anthesis-silking interval (ASI) in the drought-sensitive cultivar under well-watered and drought-stressed

conditions. As a result of improved nutritional status and shortened ASI in the drought-sensitive cultivar (C0), grain yield loss due to drought was declined from 55% to 31% with AM association.

Our data indicated that mycorrhizae assist the host plant to enhance N assimilation under water limited environment. The activities of key enzymes involved in N assimilation such as nitrate reductase, glutamine synthetase and glutamate synthase, significantly increased in AM plants. This suggests that AM association helped the plants to transport substantial amounts of NO_3^- from the roots to the shoots for further reduction and assimilation under drought conditions.

The overall results support the hypothesis that the AM colonization assists the two tropical maize cultivars to withstand under moderate drought conditions. The drought tolerance was achieved due to the physiological, metabolic and nutritional modifications in the host plant. These changes can be primarily related to the improved host plant water relations and the nutritional status, especially N and P. This thesis has provided new insights into the changes in N acquisition and assimilation of mycorrhizal plants under drought conditions. The findings of this thesis support the idea that AM fungi are one of the major biological components in the rhizosphere needed to accomplish the goal of sustainable agriculture in arid and semiarid areas.

RÉSUMÉ

L'objectif de cette thèse était d'étudier des facteurs potentiellement impliqués dans la tolérance à la sécheresse du maïs (*Zea mays* L.) mycorhizé. Nous avons postulé que la colonisation MA favorise la tolérance à la sécheresse des plantes hôtes. Ceci peut résulter de modifications au niveau des relations hydriques, du métabolisme ou de l'état nutritionnel de la plante hôte. Ces modifications améliorent la tolérance au déficit hydrique et favorisent le recouvrement suivant une irrigation. Pour vérifier ces hypothèses, les cinq objectifs suivants ont été établis: (i) déterminer les réponses physiologiques chez des plants de maïs en absence ou en présence de la colonisation MA; (ii) évaluer les changements métaboliques chez ces plants; (iii) déterminer l'état nutritionnel des plantes hôtes en relation avec le développement des grains; (iv) évaluer le recouvrement à la sécheresse du maïs et (v) étudier l'effet de la colonisation MA sur l'assimilation de l'azote en tant que facteur potentiel de la tolérance à la sécheresse chez le maïs. Afin d'accomplir ces objectifs, deux expériences en serre ont été réalisées avec des traitements similaires, et ce à deux stades critiques pour le maïs, soient à la pré-floraison et à la floraison. Des grains fraîchement régénérés à partir des cycles de sélection C0 (cv. sensible à sécheresse) et C8 (cv. résistant) de la population tropicale 'Tuxpeño sequía' ont été utilisés pour cette étude. Les plants de maïs ont été soumis à la sécheresse pendant trois semaines à la pré-floraison (45-66 jours après l'ensemencement) ou à la floraison (75-95 jours) suivie d'une irrigation jusqu'à la fin de l'expérience. La

moitié des plants de maïs ont été inoculés avec le champignon MA (*Glomus intratadices* Schenck & Smith) à l'ensemencement.

La colonisation MA a bénéficié aux plants de maïs au niveau des relations hydriques et de l'agrandissement foliaire dans des conditions de déficit hydrique.

Les plants colonisés, par comparaison aux plants témoins non-mycorhizés, maintenaient des potentiels hydriques (PHy) foliaires plus élevés (moins négatifs) et une résistance des stomates plus basse, et ce même après trois semaines d'irrigation au stade de la floraison. Au moment de l'irrigation, les plants MA nécessitaient moins de temps (C0, 7 jours; C8, 4 j) que les témoins (C0, 15 j; C8, 8 j) pour atteindre des valeurs PHy similaires aux témoins qui avaient constamment été irrigués. Le recouvrement plus rapide des plants MA a été relié à une augmentation en phosphore (P). La réponse mycorhizienne était plus prononcée chez le cultivar sensible (C0) que chez le cultivar résistant (C8) à la sécheresse.

Les plants MA retenaient des quantités significativement plus élevées en sucres et en composés azotés en conditions de sécheresse. La mise en parallèle des concentrations plus élevées en sucres avec des valeurs PHy décroissantes, suivant la progression du stress hydrique, est physiologiquement importante pour les plantes hôtes. Des concentrations plus élevées en protéines chez les plants MA pouvaient être causées par une dégradation moindre de protéines tel qu'indiqué par des concentrations plus basses d'acides aminés.

La colonisation MA a amélioré l'état nutritionnel du maïs via une absorption accrue en P, en N et en micro-éléments. Ceci a pu aider indirectement les plants MA à

utiliser l'humidité disponible du sol. Nos résultats indiquaient que la quantité totale en azote chez les plants de maïs doublait presque en présence de l'association MA. Cette étude suggère que la colonisation MA est un facteur clé dans l'acquisition de l'azote en conditions de sécheresse. La colonisation MA a influencé de façon significative le comportement reproductif, en réduisant le nombre de jours de l'émergence des soies ainsi que l'intervalle entre l'émergence de la panicule et celle des soies (ASI), chez le cultivar sensible sous des conditions d'irrigation et de sécheresse. Comme conséquence d'un état nutritionnel accru et d'un ASI raccourci chez le cultivar sensible (C0), la perte en grains était réduite de 55 % à 31 % avec l'association MA.

Nos résultats indiquaient que les mycorhizes contribuent à accroître l'assimilation en azote chez le maïs, et ce dans un environnement limité en eau. Les activités d'enzymes clés de l'assimilation de l'azote, soient la nitrate réductase, la synthétase de la glutamine et la synthase du glutamate, augmentaient significativement chez les plants MA. Ceci suggère que l'association MA a aidé les plantes à transporter de plus grandes quantités de NO_3^- des racines aux parties aériennes pour sa réduction et son assimilation en conditions de sécheresse.

Les résultats de cette étude confirment l'hypothèse à l'effet que la colonisation MA aide les deux cultivars tropicaux de maïs à tolérer des conditions modérées de sécheresse. La tolérance à la sécheresse a été favorisée par des modifications physiologiques, métaboliques et nutritionnelles chez la plante hôte. Ces modifications sont reliées à l'amélioration des relations hydriques et de l'état nutritionnel, particulièrement en N et en P. Cette étude a soulevé de nouveaux aspects au niveau de

l'acquisition et de l'assimilation de l'azote chez les plants de maïs mycorhizés sous des conditions de sécheresse. Cette thèse supporte l'idée que les champignons MA sont l'une des composantes biologiques majeures de la rhizosphère requises dans la réalisation d'une agriculture durable dans les régions arides et semi-arides.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
ADP	Adenosine diphosphate
AET	Actual evapotranspiration
AM	Arbuscular mycorrhizae
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ASI	Anthesis-silking interval
BSA	Bovine serum albumin
C0	Drought-sensitive cultivar
C8	Drought-resistant cultivar
Chl	Chlorophyll
CIMMYT	International wheat and maize improvement centre
DAOM	Department Agriculture Ottawa Mycology
DAS	Days after sowing
DM	Dry mass
DM+	Drought-stressed mycorrhizal plants
DM-	Drought-stressed non-mycorrhizal plants
DMSO	Dimethyl sulfoxide
DSE	Days to silk emergence
DTE	Days to tassel emergence
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EMF	Ectomycorrhizal fungus
FAD	Flavin adenine dinucleotide
FAO	Food and Agricultural Organization
GLA	Green leaf area
Gln	Glutamine
Glu	Glutamate
GOGAT	Glutamate synthase (glutamine:2 oxoglutarate amino transferase)
GS	Glutamine synthetase
GSH	Glutathione reduced
HEPES	N-2-hydroxypiperazine-ethanesulfonic acid
HI	Harvest index
HSD	Honest significant difference
ICAP	Inductively coupled argon plasma spectrophotometer
kDa	kilo Dalton
LWP	Leaf water potential
M+	With AM inoculation
M-	Without AM inoculation
MD	Mycorrhizal dependency
MES	(2-[N-morpholino] ethanesulfonic acid
MPa	Mega pascal
NADH	Nicotinamide adenine dinucleotide (reduced)

NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NED	N-1 naphthylethylene-diamine-dihydrochloride
NiR	Nitrite reductase
NR	Nitrate reductase
NS	Not significant
OD	Optical density
PET	Potential evapotranspiration
PMSF	Phenyl methyl sulfonyl fluoride
PVLG	Polyvinlyl-alcohol-lactic acid-glycerol medium
PVP	Polyvinyl pyrrolidone
RM+	Recovered mycorrhizal plants
RM-	Recovered non-mycorrhizal plants
rpm	Revolutions per minute
RS	Reducing sugars
RWC	Relative water content
S+	Drought-stressed plants
S-	Well-watered plants
SE	Standard error
SMC	Soil moisture content
SMD	Shoot mass after drought
SMH	Shoot mass at harvest
SR	Stomatal resistance
TCA	Trichloroacetic acid
TR	Transpiration rate
TS	Total sugars
USDA	United States Department of Agriculture

CHAPTER 1

GENERAL INTRODUCTION

1.1. Importance of drought in maize production

Drought occurs virtually in all climatic zones but its characteristics vary considerably from one region to another. Drought is, generally, a temporary event while aridity is restricted to low rainfall regions where it exists as a regular feature of climate. From the agricultural perspective, drought can be defined as limited water availability for crop production (Blum, 1996). The growing concern for sustainability of agricultural resources and food security underlies the urgency and importance of tackling drought, which remains one of the most important factors threatening the survival of people in the developing world (Austin, 1990). The occurrence of major droughts in many parts of Sub-Saharan Africa, in much of Asia (especially India) and North America in recent years, has caused serious drawdown in cereal stocks almost below the level (300 million tonnes) that FAO (Food and Agriculture Organization) considers necessary to safeguard world food security (FAO, 1997). Despite many decades of agricultural research, drought continues to be a challenge to agricultural scientists. The development of cultivars with optimal grain yields under drought is a major breakthrough in plant breeding programs (Bolaños and Edmeades, 1993a). From the genetic point of view, drought resistance is an ambiguous trait because the performance of a cultivar depends on the severity, timing and duration of drought that may vary from one year to the other. To make matters more complex, drought generally interacts with other abiotic (Fernandez et al., 1996) and biotic stresses (Edmeades and Deitsch, 1994).

Of the world's major cereals, maize (*Zea mays* L.) is the third most important after rice and wheat. Currently, maize is being planted on over 140 million hectares in the world, producing 575 million tonnes of grain every year (USDA, 1997). About 60 million hectares of maize grown annually in developing countries are often exposed to drought (Edmeades and Deitsch, 1994). An estimated 80% of the maize planted in lowland tropical environments is reported to suffer yield reductions ranging from 10 to 75% because of drought stress (Bolaños et al., 1993). The effects of water deficit differ depending on the severity of the deficit and developmental stage of the crop. Drought stress occurrence at the early vegetative phase causes uneven crop stand, and the yield may be improved by scheduling irrigation optimally at the later stages of crop growth (Squire, 1990). Water deficit during flowering or grain filling contributes to considerable yield loss due to reduction in grain number (Bolaños and Edmeades, 1993b; Edmeades et al., 1993).

1.2. The maize plant

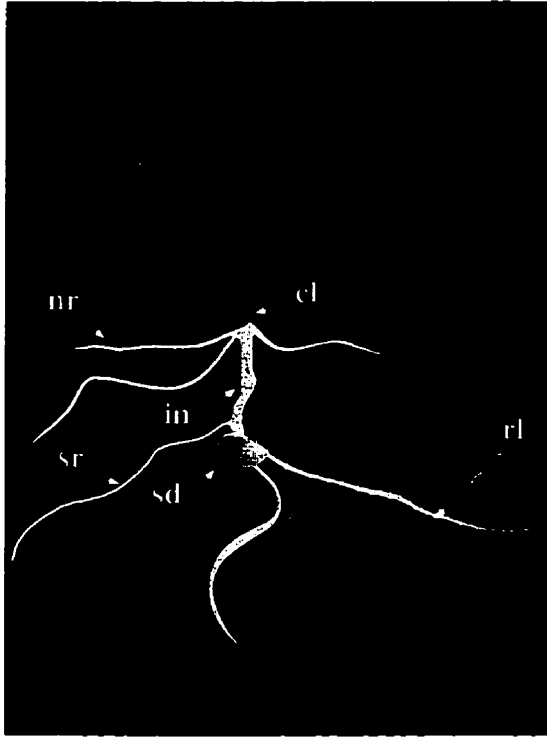
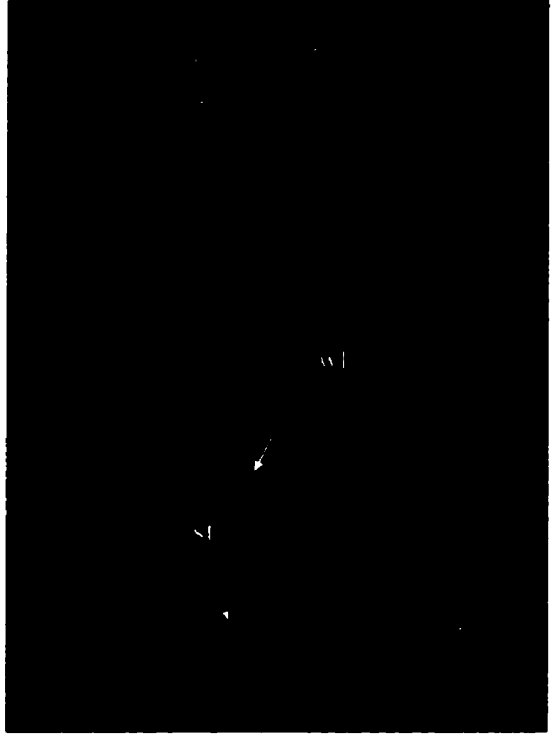
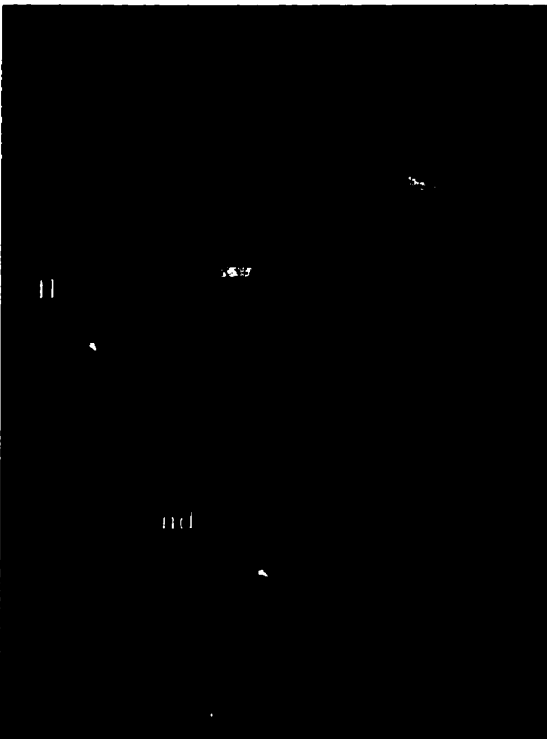
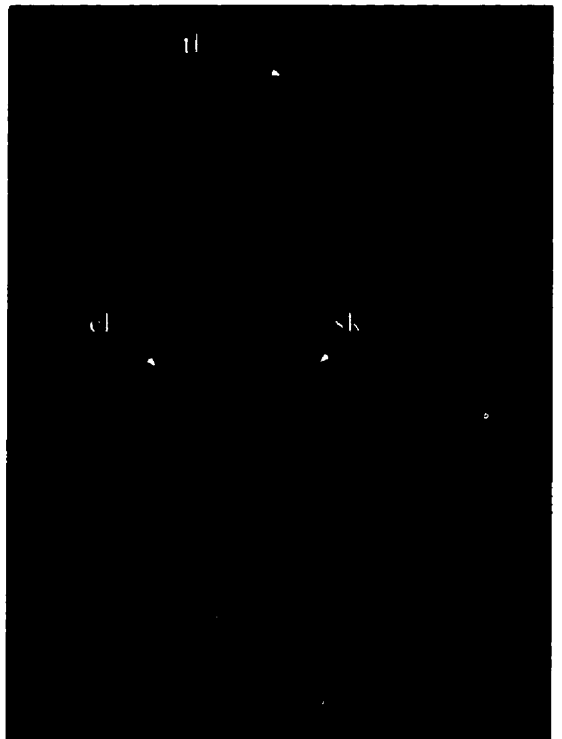
Maize is a large member of the class of Monocotyledones, in the grass family Poaceae. Maize is an annual crop characterized by a fibrous, woody stalk with conspicuous nodes, long narrow leaves spaced alternatively on the stem, a fibrous root system and separate male (tassel) and female (silk) flowers on the same plant (monoecious). Maize is believed to originate from its wild ancestor teosinte, *Zea mexicana* (Schrad.) Kuntze (Galinat, 1985). Molecular evidence provided by Doebley and Stec (1993) suggests that one of the annual teosinte, *Z. mays* subsp. *parviglumis*, was

the most probable ancestral teosinte taxon. Maize and its probable wild ancestor differ dramatically in inflorescence morphology despite the fact that they are the members of the same biological species (Doebley, 1990). It has been proposed that maize is simply a domesticated form of teosinte and the morphological differences between these taxa are the result of human selection under domestication (Doebley, 1990). The main climatic factors of importance for maize production are temperature and rainfall. Maize is considered to be a warm-season plant, requiring temperatures higher than 20°C during the day and 15°C during the night with abundant sunlight for optimum yield. It requires 40-60 cm of water to meet the evapotranspiration demand during the developmental stages. The crop water requirement varies considerably with the amount of water available, climate, soil and water management practices.

1.3. Maize growth and development

Germination and seedling development

Under favourable conditions, maize germination occurs within 2-3 days after planting. Shortly after the appearance of the radicle, the shoot emerges and begins to grow towards the soil surface. The tip of the shoot is protected by the coleoptile and is able to penetrate through the soil without damage to the leaf inside (Fig. 1.1). Once the coleoptile is exposed to light, it stops growing and splits open allowing the leaves to emerge.

A**B****C****D**

Vegetative development

During the vegetative period, the root system, stalk and leaves develop. Leaves are produced at the growing point which remains below the soil surface for the first 3 or 4 weeks following emergence. All the leaves are formed by the time the seedling reaches the 5th to 6th leaf stage. After this stage, the increase in plant size is the result of elongation of the stalk and expansion of the existing leaves.

Tassel and ear development

Shortly after all the leaves have been produced, the tassel and ears are initiated. By the time the maize is knee-high, the developing tassel can be found inside the stem. Over the next several weeks, the tassel is pushed upwards as the stem elongates. Most of the growth of the tassel itself occurs in a rapid burst a few days prior to its emergence from the whorl of leaves. Ears are located 6 to 8 nodes below the tassel. As the plant approaches its full height, the ear and tassel grow rapidly. The two weeks preceding the shedding of pollen represent a very critical period in the determination of the potential size of the ear. The number of ovules (kernels) that produce silk is determined during this period. Adverse growing conditions, such as drought, nutrient deficiency or very high temperature can limit the number of ovules formed (Edmeades et al., 1993).

Flowering

Pollen shed normally begins 2-3 days after the tassel has fully emerged from the whorl. Shedding of pollen continues intermittently for several days. The silk emerging from the ear is pollinated within 4 to 10 days after tassel emergence. When a pollen grain lands on a silk, the pollen germinates to produce a pollen tube which grows down the

entire length of the silk before fertilization can occur. This is the most critical phase of growth in determining yield. This period represents a peak in demand for all growth factors. The maize plant devotes all of its energy to producing an ear. Plant exposed to drought stress during this phase may not be able to supply enough materials to support kernel growth (Westgate, 1994).

Grain filling

Soon after pollination, a period of rapid accumulation of dry matter begins, lasting for 30–40 days. Kernels are usually completely filled within 50–60 days after silking, though this varies with maturities.

1.4. Drought effects on maize development

Drought may occur at any crop growth stage. At the vegetative phase, drought reduces stem and leaf expansion and results in reduced plant height and lower leaf area (Squire, 1990). Drought inhibits leaf expansion in maize well before the photosynthetic rates are affected (Muchow, 1989). Despite the decreased leaf area expansion under drought conditions, the final leaf number produced is generally unaffected (Albrecht and Carberry, 1993).

Maize, a cross-pollinated crop, appeared to be more sensitive than other cereals to water deficit at flowering. This is likely because anthers and silks (female flowers) are about 1 m apart on the plant, and pollen and stigmas are fully exposed to the environment (Bolaños and Edmeades, 1993b). Drought that occurs just before or during the flowering period has been shown to delay silking (Edmeades et al., 1993; Byrne et al., 1995). This

has been associated with a grain yield loss of up to 90% (Bolaños and Edmeades, 1993a). Bassetti and Westgate (1993) reported that drought causes reproductive failure due to the loss of silk receptivity. Receptive silks provide water and nutrients for pollen germination, support pollen tube growth and conduct the pollen tube from germinated grain to the carpel wall of the ovary. Delayed silking has also resulted in barrenness which may be related to the reduced partitioning of assimilates to the developing ear (Edmeades et al., 1993).

The period of grain filling is important to the overall economic yield of grain crops. Under normal conditions, kernel growth is mainly supported by photosynthates and nitrogenous compounds (Westgate, 1994). When maize plants are exposed to drought at the grain filling stage, kernel development is suppressed as a consequence of declining photosynthesis and nutritional status (Schussler and Westgate, 1991; Schussler and Westgate, 1994). Boyle et al. (1991) showed that sucrose infused at the grain filling stage to the stems of drought-stressed maize plants greatly alleviated the reproductive failure. Ta and Weiland (1992) estimated that nearly 60-80% of the total N present at anthesis is remobilized to the developing ear in maize indicating the importance of the stalk in providing N for kernel growth. Such remobilization of N may be impeded under drought conditions. These studies suggested that the reproductive success of maize subjected to drought stress at the grain filling stage is strongly related to the growth and nutritional status of the plant.

1.5. Drought management strategies

Maize production under dryland conditions poses considerable management challenges to the producer. In order to maximize yields of grain crops grown under such circumstances, management strategies might be adopted to conserve soil moisture. Conventionally, agronomic practices such as mulching, organic manuring, excessive potash fertilization and use of anti-transpirant chemicals (KCl, CaCl₂, kaolinite) were recommended. Mulching reduces moisture loss by shading the soil surface. Organic manuring improves water holding capacity of the soil. Potash fertilization assists the maize plants to accumulate K⁺ ions that contributes to the osmotic adjustment and maintenance of photosynthetic activity under drought conditions (Premachandra et al., 1993). These methods are cumbersome and expensive and thus are not all being adopted by the farmers. In the context of sustainable agriculture and to assess the suitability of an integrated drought management strategy, four criteria might be considered: environmental safety, sustainable productivity, economic viability and social acceptance. One of the innovative approaches to mitigate drought stress is by using naturally occurring microbial communities such as mycorrhizal fungi.

1.6. Mycorrhizal symbiosis

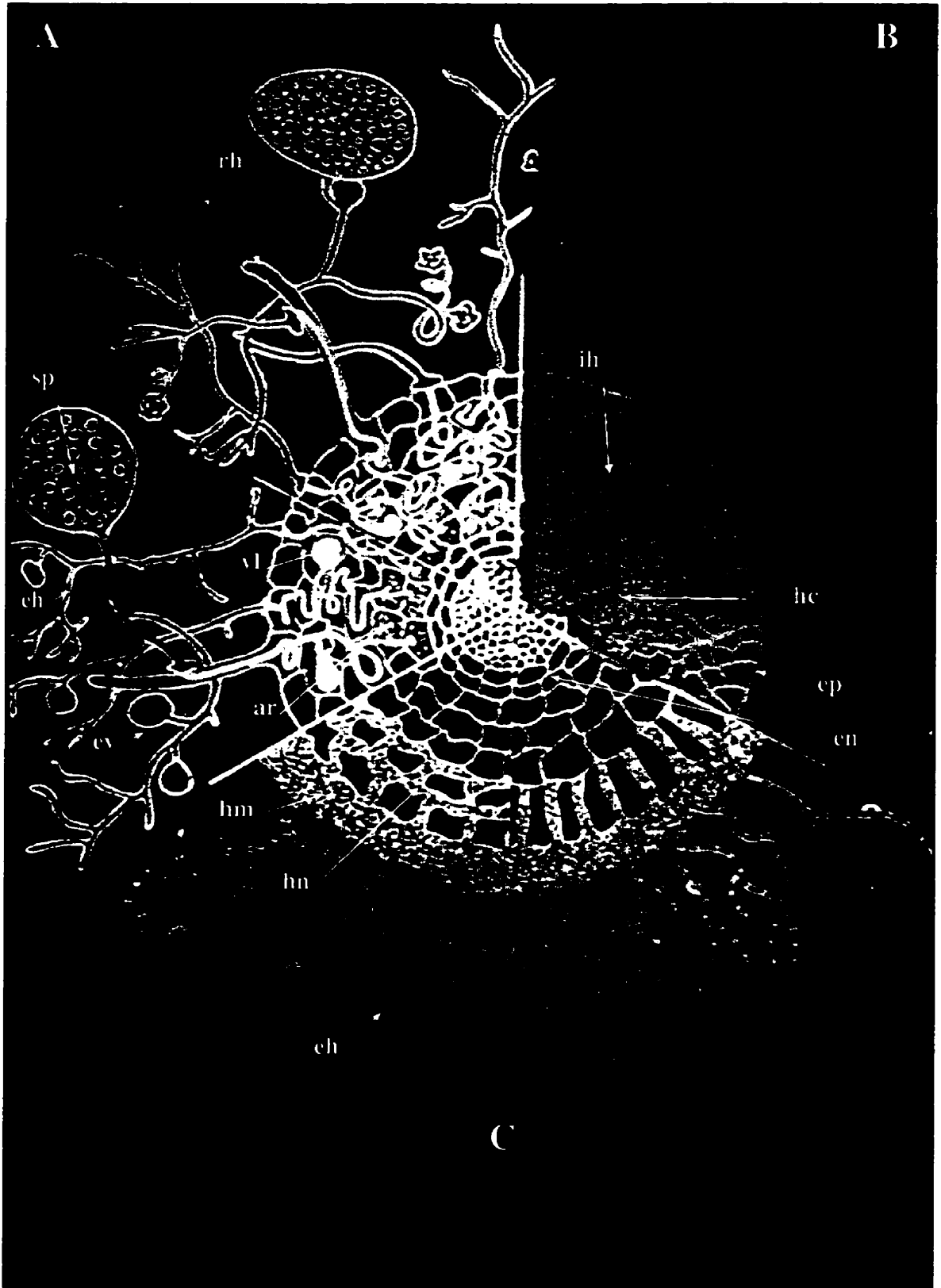
Terrestrial plants have developed numerous strategies to cope with diverse edapho-climatic conditions. One of the most successful strategies is the ability of root systems to establish symbiotic relationships with mycorrhizal fungi (Gianinazzi-Pearson, 1996). The term 'mycorrhiza' (literally 'fungus root') was first used by Frank (1885) to describe the long-lived association between plant roots and fungal mycelium. Mycorrhizae are

ubiquitous soil borne fungi that form symbiotic association with roots of higher plants. Ecto- and endomycorrhizae are the two major types in the natural ecosystem (Sieverding, 1991). Ectomycorrhizal fungi are usually associated with woody angiosperms and gymnosperms, and develop intercellular hyphae in the root cortex (Hartig net) from a mycelial sheath (hyphal mantle) covering the surface of short lateral roots (Fig. 1.2). In the endomycorrhizal association, the fungus grows inter- and intracellularly and forms specific fungal structures (arbuscules and vesicles) within cortical cells. A third group of mycorrhizal fungi that are associated with orchidaceous plants, form hyphal coils in the cortical cells. Among these types, the arbuscular mycorrhizal (AM) symbiotic association is exceptionally common among terrestrial flowering plants. About 80% of the approximately 231 000 species of flowering plants can form endomycorrhizal association with a relatively small number of zygomycetes genera (120 described spp.) belonging to the Endogonaceae family (Schenck and Perez, 1987). The Endogonaceae family has seven identified genera: *Acaulospora*, *Endogone*, *Entrophosphora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. All species of these genera reproduce asexually and form endomycorrhizae, except *Endogone* which has sexual reproduction and forms ectomycorrhizae. *Glomus* is the genus with the largest number of known species (67 spp.) and its occurrence is common in a wide range of natural and cultivated soils (Sieverding, 1991). Fossil evidence suggests that mycorrhizal symbioses existed since the Devonian

Figure 1.2. Diagrammatic representation of morphological features of three types of mycorrhizae

- A** Endomycorrhizae (*ar* arbuscule, *eh* extraradical hyphae, *ev* external vesicle, *rh* root hair, *sp* spore, *vl* vesicle)
- B** Orchidaceous mycorrhizae (*en* endodermis, *ep* epidermis, *ih* intercellular hyphae, *hc* hyphal coil)
- C** Ectomycorrhizae (*eh* extraradical hyphae, *hm* hyphal mantle, *hn* Hartig net).

Adopted and reproduced with permission obtained from the authors (Fortin et al., 1995). This diagram was originally drawn by Dr. Valentin Furlan.



period (> 400 million years ago) in the tissues of the first land plants (Pirozynski and Dalpé, 1989; Remy et al., 1994). In this symbiosis, plants supply a carbon source to the fungi and, in turn, plants are being helped to overcome nutritional and edaphic stresses by the fungi (Smith and Read, 1997).

1.7. Multi-step colonization process

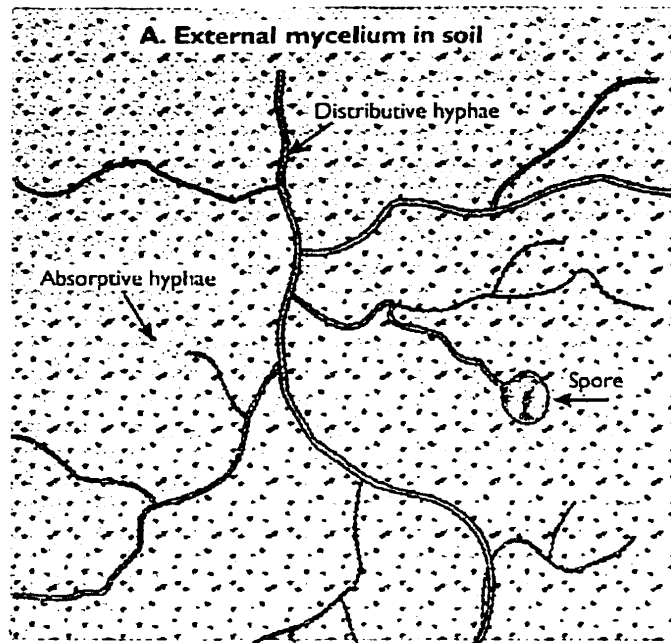
Arbuscular mycorrhizal (AM) fungi in soil exist as thick-walled chlamyospores or as vegetative propagules in roots (Fig. 1.3). **Spore germination** may be induced either by root exudates containing signal molecules, primarily phenolic compounds such as flavonoids and isoflavonoids (Koide and Schreiner, 1992) or organic acids (malic and citric acids) produced by mycorrhization-helper-bacteria (Garbaye, 1994). After germination, the germ tube grows towards the root. Only the perception of the right signals coming from the roots of the host plants promote a differential morphogenesis, consisting of profuse hyphal branching and proliferation (Gianinazzi, 1991). Immediately after contacting their hosts, fungi form **appressoria** indicating that some kind of recognition occurs at this stage (Giovanetti et al., 1994). From the appressoria, a hypha penetrates into the root cortex, where inter- and intracellular proliferation of mycelium takes place (Gianinazzi-Pearson, 1996). The intercellular hyphae branched into the paranchymal host cells as intracellular haustorial structures called **arbuscules**. During the arbuscule development, the plant plasma membrane (peri-arbuscular membrane) is not breached but grows so that the invading hyphae and all their branches remain

Figure 1.3. Principal components of vesicular-arbuscular mycorrhizal association

- A** Hyphae and other structures produced by mycorrhizal fungi in soil.
- B** Storage structures (spores, vesicles and auxiliary bodies) produced by AM fungi in roots or soil.
- C** Structures formed by AM fungi in colonized roots.

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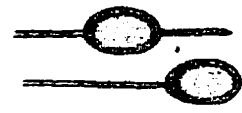
GLOMALEAN MYCORRHIZAL ASSOCIATIONS



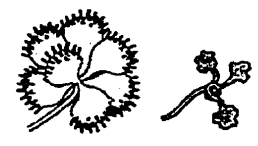
B. Storage structures



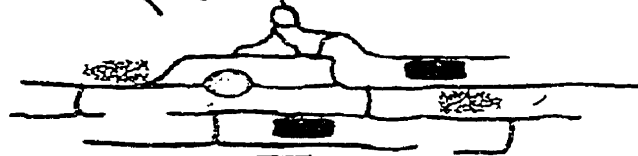
Spores produced in soil



Vesicles in roots

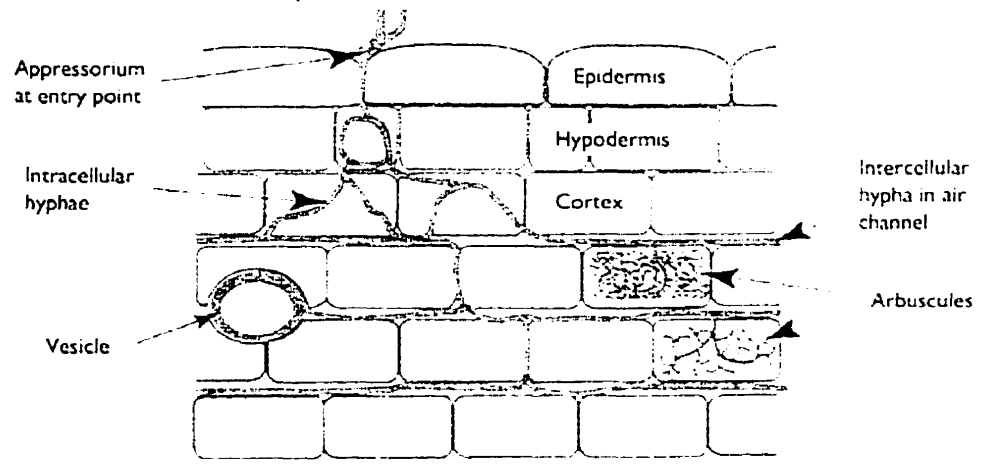


Auxiliary bodies on soil hyphae



95^B

C. Mycorrhizal structures in roots



surrounded by it (Smith and Read, 1997). The arbuscule formation increases the metabolic activity of the host cell due to the bidirectional transfer of metabolites and minerals between the plant cells and the AM fungal symbiont (Smith and Smith, 1990). Arbuscules are ephemeral structures that live for 4-15 days, after which they begin to senesce (Alexander et al., 1988). At the time of arbuscule formation, or often shortly thereafter, AM fungi form inter- and intracellular hyphal swellings called **vesicles**. These structures contain lipids and serve as the reserve food material for the fungus. After the AM fungus is established in the roots, hyphae grow out of the root and in the rhizospheric soil. **Extraradical hyphae** of the AM fungi play a key role in nutrient acquisition from the soil and for the transport of nutrients to the roots. Reproductive structures of the AM fungi (chlamydospores) are formed within 6-8 weeks after the colonization depending on the species. This multi-step colonization process is dynamic and spore germination or sporulation may occur simultaneously.

1.8. Role of mycorrhizae in sustainable agriculture

During the past two decades, mycorrhizal research gained interest among soil and plant scientists. The interest continues unabated with ever-increasing numbers of published papers, books, symposium volumes, interested scientists and research programs (Klironomos and Kendrick, 1993; Varma, 1995). These organisms attracted the interest of scientists from the perspective of plant nutrition, especially phosphorus and nitrogen (McArthur and Knowles, 1993; Tobar et al., 1994a,b; Smith and Read, 1997; Subramanian and Charest, 1997a), drought tolerance (Fitter, 1988; Augé et al., 1994;

Subramanian et al., 1995; Ruiz-Lozano et al., 1996), chilling tolerance (Charest et al., 1993; Paradis et al., 1995), biological control of root pathogens (Benhamou et al., 1994; Linderman, 1994) and alleviation of heavy metal toxicity (Weissenhorn et al., 1995). Colonization of roots by AM fungi has been shown to improve productivity of several crops including maize (Sylvia et al., 1993; Subramanian and Charest, 1997), sorghum (Raju et al., 1990), soybean (Bethlenfalvay et al., 1988) and potato (McArthur and Knowles, 1993). The degree of plant response to AM fungi increases with decreasing soil fertility (Jeffries, 1987) and increasing intensity of drought stress (Sylvia et al., 1993).

The plant response to AM fungi is particularly significant in arid and semi-arid tropics where crop production is usually limited by nutritional deficiencies and drought (Jeffries, 1987). It has been suggested that AM symbiotic association can be used as a biofertilizer to improve nutrient cycling and crop productivity by reducing the fertilizer inputs, thereby conserving soil fertility and reducing production and environmental costs (Hooker and Black, 1995). Mycorrhizal colonization, by helping plants to become established in eroded and degraded habitats, may enhance productivity in afforestation programmes. The use of mycorrhizae can be regarded as an important alternative strategy for a more rationale and sustainable agriculture.

1.9. Plant-water-relations

1.9.1. Leaf water potential

Leaf water potential (LWP) is a measure of the free energy status of water in the plant and constitutes the driving force for the water movement. The LWP is one of the

most useful indicators of plant water status and its measurement has provided valuable data for studying plant responses to drought (Boyer, 1995). When several plant species were exposed to drought, LWP values declined relative to soil water content (Hanson and Hitz, 1982). As the LWP progressively decreases in field and lab grown plants, stomata generally do not respond until a threshold value (critical LWP) is reached, and this value varies widely with plant species (Turner, 1974). Lorens et al. (1987) reported that maize cultivars which maintained higher (less negative) LWP under drought conditions produced more biomass and grain yield.

Under drought conditions, arbuscular mycorrhizal (AM) colonization appears to promote beneficial water relation state of the host plants as a result of enhanced water uptake (direct effect) or stimulated plant nutrition (indirect effect). It is difficult to distinguish direct mycorrhizal effects from those that could be mediated via improved plant nutrition. Some studies have shown that AM hyphae transported water directly to their host plants (Allen, 1982; Faber et al., 1991). On the other hand, it is widely believed that AM association alters host plant water relations as a result of improved phosphorus nutrition (Nelsen and Safir, 1982; Nelsen, 1987; Fitter, 1988). The symbiosis provides AM plants more access to exploit the available soil moisture than non-AM plants (Bethlenfalvay et al., 1988). Conversely, others have indicated that the improvement in water relations of AM plants under drought conditions was unlikely, due to their higher carbon cost (Graham et al., 1987) or larger plant size (Levy et al., 1983). As such, AM association seems to be more advantageous to plants which are subjected to brief periods

of drought stress but the mechanism that makes the plant more drought tolerant is still poorly understood (Smith and Read, 1997).

1.9.2. Stomatal resistance

Stomatal resistance (SR) is the measure of the resistance to water vapour transfer from inside plant tissue to the atmosphere (Nelsen, 1987). This is due to the presence of the surface layer of the plant, including the epidermis, cuticle and stomata. There is generally a large gradient of vapour from the inside of the leaf to the atmosphere. Loss of water vapour concentration from the plant tissue is partially controlled by this resistance. Begg and Turner (1976) showed a sharp increase in SR values at a point where leaves attained their critical water potential. However, Hsiao et al. (1976) observed no unique relationship between SR and LWP. In some studies, SR increased gradually with a declining LWP (Passioura and Stirzaker, 1993). Others suggested that SR is regulated by hormonal signals especially abscisic acid (ABA) originating from the roots (Zhang and Davies, 1990; Davies et al., 1994).

Mycorrhizal colonization of roots can influence the stomatal behaviour of the host plant leaves. The major effect of AM colonization was a decrease in host plant stomatal resistance both during drought and recovery periods (Fitter, 1988; Ruiz-Lozano et al., 1995; Duan et al., 1996). Some studies have shown that the lower SR values in AM plants were mainly due to the P nutritional effect (Koide, 1985; Fitter, 1988). There have been suggestions that stomatal behaviour is influenced by the altered hormonal changes in AM plants (Allen, 1982; Danneberg et al., 1992; Druge and Schonbeck, 1993). Kothari et al. (1990) indicated that increased branching of AM roots may lead to substantial

increase in surface area which helps the plants to fully explore a particular soil volume, extending soil water depletion zones and providing AM roots more access to available water. Duan et al. (1996) also suggested that AM fungi increased the capability of root systems to scavenge water in drier soil resulting in less strain to foliage and hence low SR under drought conditions. These reports suggested that AM association assists the plants to maintain lower SR during the period of drought.

The transpiration rate (TR) is inversely proportional to the SR. It is well documented that stomatal closure is the main cause for TR declining during the progression of drought stress (Hsiao, 1974). Mycorrhizal association appeared to increase TR in several plant species such as red clover (Hardie and Leyton, 1981), rose (Augé et al., 1987b), maize (Kothari et al., 1990) and lettuce (Ruiz-Lozano et al., 1995, 1996).

Water relations of plants appear to be modified by mycorrhizal interactions. The mechanisms are difficult to determine, but most of the effects can be related to the secondary consequence of nutritional status, especially P. In addition, direct water transport through extraradical mycelium of the AM fungi may alter host plant water relations (Smith and Read, 1997).

1.10. Metabolic changes

1.10.1. Osmotic adjustment

Solute accumulation under stress (osmotic adjustment) is one of the most distinctive features of an adaptive response in plants to adverse environmental conditions

such as drought, freezing and salinity. Osmotic adjustment involves the net accumulation of organic and inorganic solutes in the cell in response to a fall in water potential of the cell environment. As a consequence of this net accumulation, the osmotic potential of the cell is lowered, which in turn attracts water into the cell, and tends to maintain turgor pressure (Blum et al., 1996). The organic solutes that accumulate during osmotic adjustment include sugars (Kameli and Lösel, 1995, 1996), amino acids (Good and Zaplachinski, 1994; Girousse et al., 1996) and organic acids (Timpa et al., 1986). Among the inorganic solutes, potassium is known to be involved in osmoregulatory phenomena (Premachandra et al., 1993). The accumulation of ions during osmotic adjustment appears to occur mainly within the vacuoles, where the ions are kept out of contact with enzymes in the cytosol or subcellular organelles. Because of this compartmentalization of ions, some compatible solutes (eg. proline, glycine betaine) accumulate in the cytoplasm to maintain water potential equilibrium within the cell (Hanson and Hitz, 1982; Ludlow and Muchow, 1990). Osmotic adjustment has been considered as an important component of drought tolerance (Morgan, 1984) and used as a direct selection criterion in maize improvement for drought tolerance (Bolaños and Edmeades, 1991). Elmi and West (1995) indicated that the presence of a mycorrhizal fungus (*Acremonium coenophialum* Morgan-Jones and Gams) in tall fescue (*Festuca arundinaceae* Schreb.) enhances host plant persistence in drought-prone environments by promoting osmotic adjustment in leaf blade and tiller survival rate.

1.10.2. Sugars

Sugars have been known to increase in a wide range of plants grown in water deficit conditions (Kameli and Lösel, 1993). The rate and extent of increase in sugars depend on the environmental conditions, species or cultivars (Hanson and Hitz, 1982). Sugar accumulation is widely regarded as an adaptive response to drought conditions (Munns, 1988). Factors which have been suggested to contribute to this increase under drought conditions include reduced translocation of sugars out of the leaves, slower utilization because of reduced growth and other changes such as starch hydrolysis (Van Volkenberg and Boyer, 1985; Munns, 1988; Schubert et al., 1995; Kameli and Lösel, 1996). Soluble sugars that accumulate in plants under severe drought conditions were primarily glucose (Kameli and Lösel, 1996). In another study, sugars accumulated more rapidly in the drought-resistant than drought-sensitive wheat cultivar during the progression of drought stress (Kameli and Lösel, 1993). Mycorrhizal association appears to stimulate starch increase in the host plant under drought (Augé et al., 1987b) or sugar accumulation under cold conditions (Charest et al., 1993). The accumulation of carbohydrates may help the host plant to sustain adverse environmental conditions.

1.10.3. Proteins

Proteins play a vital role in catalytic reactions and are a source of reduced N for vegetative and reproductive growth (Hanson and Hitz, 1982). Inhibition of protein synthesis in plants is an early response to drought stress (Hsiao, 1976). Good and Zaplachinski (1994) observed a decrease in protein synthesis in the leaves of a *Brassica* sp. subjected to drought stress followed by a resumption of synthesis upon rehydration.

Some plant species synthesize new proteins to cope with water deficit conditions (Bartels et al., 1993). Pelah et al. (1997) showed that the drought tolerance of a resistant species, *Populus popularis*, compared to a less tolerant one, *P. tomentosa*, was positively correlated with the accumulation of a set of induced water deficit proteins.

During endomycorrhizal symbiosis, new proteins called mycorrhizins, of fungal and host plant origins were detected (Dumas et al., 1990; Hilbert et al., 1991) These mycorrhizins have been detected in soybean (Pacovsky, 1989), onion (Dumas et al., 1990), tomato (Simoneau et al., 1994) and red clover (Arines et al., 1993). Their molecular weights range from 16 to 78 kDa. Although these studies showed the synthesis of mycorrhizins in AM roots, there is still no information to support the precise function of these proteins. Cliquet and Stewart (1993) found that soluble protein concentrations in maize roots and shoots increased when the roots were colonized with *Glomus fasciculatum*. Higher soluble protein concentrations in shoots of AM plants were also detected under drought (Ruiz-Lozano and Azcón, 1996) and chilling (Charest et al., 1993; Paradis et al., 1995) conditions. Ruiz-Lozano et al. (1996) showed that SOD (superoxide dismutase) activities were stimulated in AM colonized *Lactuca sativa* plants exposed to water deficit. These studies suggested that AM colonization induces the host plants to produce soluble proteins that may play a role in stress tolerance.

1.10.4. Amino acids

Drought may cause metabolic damages in plants which include enhanced proteolysis, depressed protein synthesis or reduced incorporation of amino acids into proteins (Drossopoulos et al., 1985). All these changes, tending to increase amino acid

concentrations in the stressed tissues of several plant species, are regarded as general responses to water deficit (Singh et al., 1973; Venekamp, 1989). One of the most striking responses in plants to water deficit is proline content which may increase as much as 60-100 fold (Good and Zaplachinski, 1994; Gironse et al., 1996). The accumulated proline may play a role in osmotic adjustment or serve as a source of N for recovering drought-stressed tissues (Hanson and Hitz, 1982; Sivaramakrishnan et al., 1988). The level and nature of amino acids may also be altered in the presence of AM association (Pacovsky, 1989; Cliquet and Stewart, 1993). Recently, Johansen et al. (1996) detected substantial amounts of free amino acids in the extraradical mycelium of AM fungus indicating the significance of hyphal contribution to the host plant amino acid pool.

1.11. Nutritional changes

1.11.1. Nitrogen

Nitrogen is considered to be a 'kingpin' among the essential nutrients required for plant growth. The fact that the amount of N available in soil is small while the quantity of N withdrawn by crops is large, renders most arable soils deficient in N (Brady, 1984). In the tropics, soils are generally poor in N due to the excessive loss either through drainage or by volatilization (gaseous loss). Nitrogen can occur in soil in both organic (amino acids) and inorganic (NO_3^- and NH_4^+) forms but NO_3^- is the most abundant form of N in tropical soils (Oaks, 1994). Drought stress impedes the mobility of NO_3^- ions in soils due to its low concentration and diffusion rate (Azcón et al., 1996). Under such environmental

conditions, AM fungi may play a crucial role in transporting N from the soil to the root surface, thereby contributing to plant growth and nutrition (Tobar et al., 1994a,b).

In the past, much focus has been given to AM fungi assisted P nutrition due to the extremely slow diffusion rate of PO_4^- ions and the high level of fixation of the phosphate fertilizer added to soils. Relatively few studies were carried out to assess the role of such plant-fungal symbiosis on N nutrition in crops. Radioisotopic studies have revealed that the extraradical mycelium in AM fungi can derive ^{15}N from the soil (Frey and Schüepp, 1993; Johansen et al., 1993; Johansen et al., 1994). These studies have also indicated the ability of AM hyphae to transport N about 10-30 centimeters from the soil to the host plant roots and any disruption to the hyphal network has implications for N acquisition. Thus the AM plants have access to use the forms of N that are unavailable to non-AM plants (Azcón-Aguilar et al., 1993; Tobar et al., 1994a,b). Owing to the greater demand for N by plants and considering that drought may interfere with the mobility of NO_3^- to the root surface, hyphal transport by AM fungi could be a key factor in sustainable agriculture.

1.11.2. Phosphorus

Soils of the tropics are generally very poor in phosphate and fix much of the phosphorus fertilizer added (Subramanian and Kumaraswamy, 1989). The available portion of total soil P is commonly less than 1% and is mainly controlled by chemical reactions and to a lesser extent by biological processes. Furthermore, the rate of diffusion of PO_4^- ions in soil is extremely low (10^{-8} - 10^{-11} $\text{cm}^2 \text{s}^{-1}$) and varies with P and soil moisture contents (Smith and Read, 1997).

It has been shown that AM association enhances P uptake by plant roots under drought (Nelsen and Safir, 1982; Fitter, 1988; Sylvia et al., 1993; Ruiz-Lozano et al., 1995) or non-drought conditions (McArthur and Knowles, 1993; George et al., 1995; Hetrick et al., 1996). The enhanced P uptake by AM plants has been found to be mainly due to the extraradical mycelium which can absorb P from the soil solution and translocate it to the roots. These processes together with the transfer from the fungus to the plant are much faster than diffusion through the soil (Jakobsen et al., 1992). The extraradical mycelium can contribute to the host plant P status up to 70% (George et al., 1994). Thus, AM hyphae have the capacity to almost completely meet the P demand of the plant by supplying P from the part of the soil that is undepleted by roots. Rapid absorption of soluble form of P by the extraradical mycelium leads to a shift in the equilibrium towards the release of bound P from soil reserves (Smith and Read, 1997). In the rhizosphere, acid phosphatases catalyze the hydrolysis of insoluble P containing compounds in the soil and increase the soluble forms of phosphorus. Dodd et al. (1987) indicated that AM colonization in onion roots increases the acid phosphatase activity by 20-40 times and this may enhance the availability of soil phosphorus.

As a result of the increased uptake of P in roots, AM plants frequently produce higher yields than those without mycorrhizae (Smith and Read, 1997). Improved P nutrition by AM fungi during the periods of water deficit has been postulated as a mechanism for enhancing host plant drought tolerance (Nelsen and Safir, 1982; Bethlenfalvay et al., 1988; Fitter, 1988). In contrast, others believe that host plant drought tolerance is independent of P uptake stimulated by AM fungi (Davies et al., 1993; Augé

et al., 1994). The effects of mycorrhizal associations on agroecosystems are generally beneficial, with only a very few reports of growth depression in field situations (Modjo and Hendrix, 1986; Modjo et al., 1987). Fitter (1991) suggested that the carbon used by the fungus (about 10% of the carbon transported to the roots) represents a considerable cost to the plant which may or may not offset a benefit in terms of nutrient uptake.

1.11.3. Potassium

Potassium plays a key role in drought tolerance of plants and has been found to be the cationic solute which is responsible for stomatal movement (Premachandra et al., 1993). Mycorrhizal effect on K nutrition has not yet been studied extensively. Smith et al. (1981) observed elevated concentrations of K in roots (but not shoots) of AM *Trifolium subterraneum* L. when plants were grown on P-deficient soils. When the non-AM plants were supplied with sufficient P, the K concentrations in AM and non-AM plants were similar, suggesting that the increase in K was due to the indirect effect of P. Ruiz-Lozano et al. (1995) reported that the protection of AM plants against drought stress was partly related to the increased K uptake.

1.11.4. Other nutrients

Continuous fertilization of cultivated crops without the inclusion of micronutrients rendered the arable soils deficient in these nutrients. Colonization of the roots by AM fungi has been shown to improve the productivity of such soils by enhancing the uptake of slowly diffusing ions such as Cu and Zn (Sylvia et al., 1993). Li et al. (1991) demonstrated hyphal uptake and translocation of Cu to *Trifolium repens* L. This contributed about 62% of the total Cu uptake and the mycorrhizal response was

independent of the effects of P nutrition in this study. A number of studies have clearly shown that Zn uptake via mycorrhizae is important for the alleviation of Zn deficiency in several plant species (Evans and Miller, 1988; Sylvia et al., 1993).

The effects of AM colonization on drought tolerance are not necessarily related to water relations. Under drought conditions, nutrient availability to the plant is highly restricted primarily due to the impeded mobility of mineral ions. Consequently, the growth of non-AM plants is likely to be increasingly limited by nutrient availability and reduced root growth would limit the accessibility of water. Under these conditions, the mycorrhizal contribution to nutrient uptake would be of importance.

1.12. Drought recovery

On rewatering, stressed plants recover from drought effects and return to near normal levels of physiological functions depending on the degree of water limitation and their level of resistance. It has been shown that AM colonization assists the plants recovery from short-term drought stress events by increasing the hydraulic conductivity (Safir et al., 1971; Hardie and Leyton, 1981) which is related to the P status of the host plant (Nelsen and Safir, 1982). Levy and Krikun (1980) found that the faster recovery of AM plants of rough lemon (*Citrus jambhiri*) from drought stress was related to stomatal regulation. The recovery of AM plants may also differ depending on the functional compatibility of fungal association. Ruiz-Lozano et al. (1995) showed that drought recovery is related to a particular physiological trend in the host plant according to the AM fungal species involved and to the intrinsic capacity of these to resist stress. Elmi and

West (1995) reported that enhanced osmotic adjustment helped the AM tall fescue plants to recover from drought.

1.13. Nitrogen assimilation

Nitrate is the most abundant form of N available to the plant and hence its reduction and further assimilation represent major metabolic functions (Oaks, 1994a). The global rate of NO_3^- assimilation by plants is roughly 2×10^{13} kg N per year (Guerrero et al., 1981) which is about 10 times greater than that of biological N_2 fixation. Thus NO_3^- assimilation is of fundamental biological importance. Nitrate assimilation by plants involves the uptake of NO_3^- , its reduction to NO_2^- , the conversion of NO_2^- to NH_4^+ and its incorporation into amino acids (Fig. 1.4). The rate of NO_3^- uptake by the plant is the major determinant of the extent of NO_3^- assimilation (Crawford, 1995; Glass and Siddiqi, 1995). Once NO_3^- is absorbed by the root, it can be assimilated in the root itself, transported to the shoot or stored in the vacuole in either root or shoot tissues. The stored NO_3^- can be released into a metabolic pool in the cytoplasm for assimilation, when the external source of NO_3^- has been exhausted (King et al., 1992). The NO_3^- entering the plant cell is assimilated in a series of steps involving the action of four major enzymes; nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (glutamine: 2-oxoglutarate amino transferase, GOGAT).

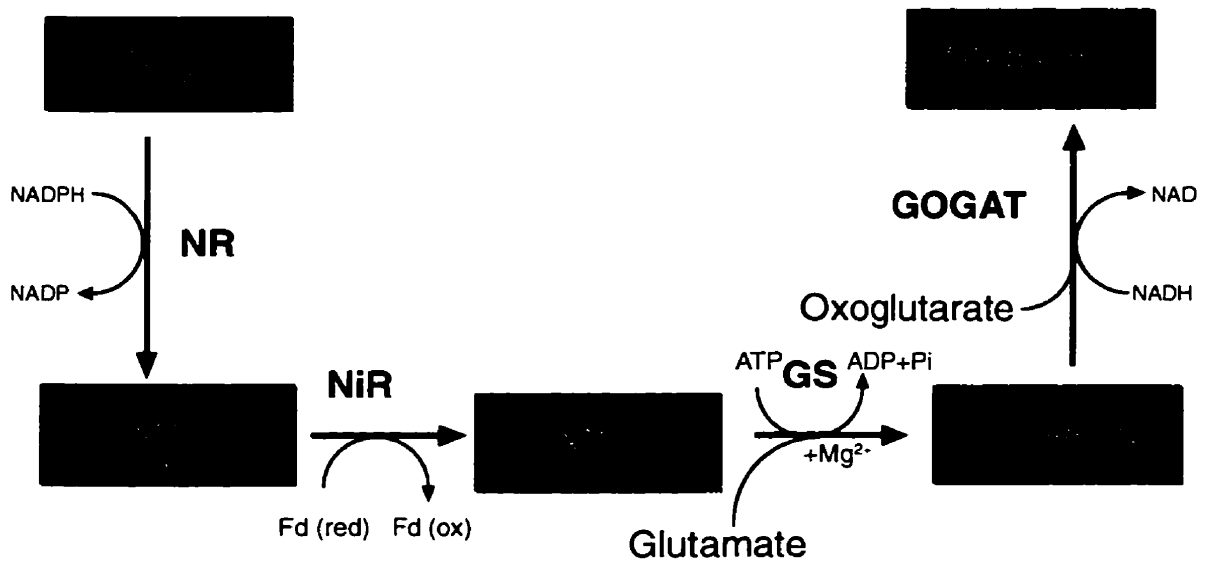
1.13.1. Enzymes of N assimilation

Nitrate reductase (NR)

Nitrate reductase catalyses the reduction of NO_3^- to NO_2^- . This is the first enzyme in the

Figure 1.4. Nitrogen assimilation pathway: The major enzymes involved in nitrogen assimilation include nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT).

Nitrogen assimilation pathway



N assimilation pathway induced by its substrate NO_3^- . It is one of the most intensively studied enzymes and can be used as a marker for the capacity of roots and shoots to assimilate external nitrogen. NR utilizes NADH or NADPH as an electron donor for NO_3^- reduction. In maize, an NADH-specific NR (EC 1.6.6.1) is present in both roots and shoots, while an NAD(P)H-bispecific NR (EC 1.6.6.2) was detected only in roots (Warner et al., 1987). The molecular weight of the NR protein was about 100-120 kDa (Campbell, 1996). In higher plants, NR contains three functional domains: FAD (flavin adenine dinucleotide), haem, and a molybdenum cofactor, present in a stoichiometry of 1:1:1 (Campbell, 1996). In the cytosol of leaf cells, the NADH required for the functioning of NR is supplied by a malate/oxaloacetate shuttle that operates between the chloroplast and cytoplasm (House and Anderson, 1980). In root cells, NR can utilize both NADH and NADPH as reductants (Bowsher et al., 1993).

Nitrite reductase (NiR)

The second enzyme in the sequence, NiR (EC 1.7.7.1) catalyzes the six electron reduction of NO_2^- to NH_4^+ , localized within chloroplasts in leaf and in plastids in root tissues (Oaks, 1994). Ferredoxin is the reductant source in shoots and roots. The molecular weight of the NiR protein is 63 kDa containing a specialized heme (sirohaem) and a Fe_4S_4 centre as prosthetic groups (Siegel and Wikerson, 1989).

Glutamine synthetase (GS)

The NH_4^+ produced by NiR is incorporated into the amide-N of glutamine by GS (EC 6.3.1.2). This enzyme catalyzes the conversion of the amino acid, glutamate, into glutamine, using NH_4^+ , ATP and a covalent cation Mg^{2+} as a cofactor. The native GS

protein has a molecular weight of 350 kDa and is composed of eight identical subunits (Sechley et al., 1992). In leaves, GS is present both in cytosol (GS₁) and chloroplasts (GS₂), while it is found in roots only as a cytosolic (GS_r) form (Mc Nally et al., 1983). In maize leaves, GS₁ and GS₂ were found in both bundle sheath and mesophyll cells (Yamaya and Oaks, 1988).

Glutamate synthase (GOGAT)

GOGAT (glutamine: 2 oxoglutarate aminotransferase) catalyzes the reductive transfer of the amide group of glutamine formed by GS to 2-oxoglutarate to yield two molecules of glutamate. One of the glutamate molecules can be cycled back as a substrate for the GS reaction. This is the GS-GOGAT cycle (Lea and Mifflin, 1974). Two types of GOGAT are known to occur based on the nature of the electron donor, ferredoxin-GOGAT (EC 1.4.7.1) and the NAD(P)H-GOGAT (EC 1.4.1.14), both localized in the chloroplasts of leaves and the plastids in roots. The molecular weight of GOGAT protein is 165 kDa (Sakakibara et al., 1991). All these steps take place primarily in shoots (Campbell et al., 1988; Merlo et al., 1994; Sivasankar and Oaks, 1995) and to a lesser extent in roots (Oaks and Hirel, 1985; Oaks, 1994a).

1.13.2. Role of mycorrhizal fungi in N assimilation

There is a vast body of literature concerning the regulation of N assimilating enzymes by NO₃⁻, light and drought (Sivaramakrishnan et al., 1988; Hoff et al., 1992; Kenis et al., 1994; Oaks, 1994a). Among these factors, NO₃⁻ uptake by the plant is the major factor that determines the extent of N assimilation. The NO₃⁻ ion mobility in soil is

severely restricted under drought conditions due to its low concentration and diffusion rate (Azcón et al., 1996). In addition, drought-stressed plants suffer from a reduction in photosynthesis and this appears to limit the supply of reductants and energy for NO_3^- reduction (Warner and Huffaker, 1989; Oaks, 1994b).

In forest ecosystems, Fogel (1980) estimated that ectomycorrhizal fungi (EMF) account for 43% of the N annual turnover. Through the EMF association, the tree becomes partially heterotrophic for N, competes with other microbes and assimilates N into glutamine. The glutamine produced within EMF is stored as a large pool of soluble N and released to the host plant on demand (Attiwill and Adams, 1994). A number of studies have shown that the formation of EMF association alters the characteristics of N acquisition and assimilation depending on the fungus and host plant species (Vézina et al., 1989; Botton and Cholat, 1995). However, relatively few studies have been reported on N assimilation in plants colonized by AM fungi (Cliquet and Stewart, 1993; Ruiz-Lozano and Azcón, 1996). These studies suggested that the AM association appears to modify the N assimilation pathway of the host plant which may be a relevant factor in drought tolerance.

1.14. Rationale, Hypotheses and Objectives

1.14.1. Rationale

The mechanisms involved in mycorrhizae-assisted host plant drought tolerance are quite complex. It is widely believed that the effects are mainly related to the host plant nutritional status. The understanding of the functionality of AM fungi on the ability of the

host plant to sustain drought conditions requires the determination of physiological, metabolic and nutritional changes. Drought impedes the mobility of water and nutrient ions due to their low concentration and diffusion rate. Under such environmental conditions, AM fungi may play a crucial role in transporting mineral ions and water from the soil to the root surface, therefore contributing to plant growth, nutrition and drought tolerance. The impact of AM colonization on host plant drought tolerance may vary depending on the sensitivity of a cultivar and the developmental stage that coincides with drought.

1.14.2. Hypotheses

1. In the present study, the hypotheses are that under drought conditions, AM colonization promotes beneficial water relations of the host plant and this may be a consequence of enhanced water uptake (direct effect) and/or stimulated nutrition (indirect effect). This enables the host plant to maintain higher water status and to carry out “normal” physiological functions under limited water conditions and recover from drought effect rapidly when irrigation is restored.
2. The AM colonization in the host plant facilitates metabolic modifications that result in accumulation of organic osmolytes which may enable the plants to survive drought conditions.
3. The AM colonization enhances host plant nutritional status and thus plant growth which may alter the reproductive behaviour of tropical maize cultivars. Improved nutritional status of AM plants may supply essential minerals and metabolites to the sink organs (developing ovules) to support kernel growth under water-deficit conditions.

4. The AM colonization provides the host plant access to otherwise unavailable forms of N under limited water conditions. This may modify the activities of key enzymes involved in N assimilation in maize which may be a potential factor involved in host plant drought tolerance.

1.14.3. Objectives

The overall objective of this study was to determine the effects of AM colonization on drought tolerance of two tropical maize cultivars having differential sensitivity when exposed to drought at critical stages for water requirements.

Objective 1: To evaluate the effects of AM colonization on water relations in drought-sensitive and -resistant tropical maize cultivars based on their physiological responses (leaf water potential, stomatal resistance, transpiration rate, green leaf area) during three weeks of drought at the tasselling stage (**Chapter 2**).

Objective 2: To examine whether the improved water relations in the host plant are related to metabolic modifications in the presence of AM association. The biochemical parameters were determined using the levels of metabolic indicators of drought tolerance such as sugars, soluble proteins, amino acids and chlorophyll (**Chapter 3**).

Objective 3: (i) To determine the nutritional status of maize plants in order to assess whether mycorrhizal colonization enables the host plant to supply enough minerals for kernel growth under drought conditions. (ii) To examine the changes in growth and reproductive behaviour (anthesis-silking interval) of the tropical maize cultivars as a consequence of nutritional improvement achieved by AM association (**Chapter 4**).

Objective 4: To assess the progression of drought and of drought recovery of these two tropical maize cultivars at the preflowering stage in the presence or absence of AM colonization using reliable physiological indicators (**Chapter 5**).

Objective 5: To examine the effects of AM colonization on the levels of major enzymes (nitrate reductase, nitrite reductase, glutamine synthetase and glutamate synthase) involved in nitrogen assimilation after drought and recovery of maize and to assess these enzyme activities as potential factors in host plant drought tolerance (**Chapter 6**).

Note

This thesis is presented in the form of five published papers. Each chapter, from **Chapter 2 - Chapter 6**, represents the reproduction of one paper. For all these chapters, the published papers were revised in order to provide complete experimental procedure and for better clarity. I accomplished three objectives (1, 2 and 3) using the data collected from the first greenhouse experiment. The second greenhouse experiment was conducted with some modifications, and the data were used to accomplish the other two objectives (4 and 5). In order to avoid repetition, experimental layout and growth conditions used in the greenhouse experiments are explained in detail once in Chapter 2. The references in all the chapters were merged and presented once at the end of this thesis.

CHAPTER 2

ARBUSCULAR MYCORRHIZAE AND WATER RELATIONS IN MAIZE

This chapter is a revised version of a paper published in *New Phytologist* (129: 643-650, 1995) by K.S. Subramanian, C. Charest, L.M. Dwyer and R.I. Hamilton. The results of a mean comparison test (Tukey test) and up-to-date references were incorporated to provide consistency with other chapters. This chapter fulfills the first objective of this thesis: To evaluate the effects of arbuscular mycorrhizal colonization on water relations in two tropical maize cultivars having differential sensitivity to drought based on their physiological responses during three weeks of drought at the tasselling stage.

2.1. Introduction

Drought is considered to be a major factor affecting plant growth and yield in dryland areas (Begg and Turner, 1976) and even in irrigated areas (Tazaki et al., 1980). Plant characteristics associated with improved performance under drought include those which allow plants to gain access to and absorb a greater volume of water, to reduce rates of water loss, or to maintain higher physiological activity at low water status (Ludlow and Muchow, 1990). Several reports have shown that leaf expansion is very sensitive to moisture deficit and responds rapidly to changes in leaf water status (Michelena and Boyer, 1982; Dwyer and Stewart, 1986; Otegui et al., 1995). In tropical maize cultivars, Sobrado (1986) found a strong relationship between leaf expansion rate and pre-dawn leaf turgor potentials; expansion ceased at turgor potentials of less than -2.0 MPa.

Drought stress accelerates the senescence of lower leaves in maize, and cultivars with increased capacity for osmotic adjustment have delayed leaf senescence under drought (Bolaños et al., 1993). Maize cultivars that maintained less negative leaf water potentials under drought conditions had higher dry matter and grain yield (Lorens et al., 1987). Drought that coincides with tasselling in maize causes delayed silking, resulting in an increase in length of the anthesis-silking interval (Edmeades et al., 1993). Water potential values of silks, below -0.8 MPa were shown to inhibit pollination and to decrease the grain set in maize by 20-40% (Bassetti and Westgate, 1993).

Under drought conditions, arbuscular mycorrhizal (AM) colonization improves water relations of host plants (Fitter, 1985). The possible mechanisms are by (i) improving hydraulic conductivity (Hardie and Leyton, 1981; Cooper, 1984); (ii) allowing increased transpiration rate and decreased stomatal resistance (Bethlenfalvay et al., 1988); (iii) reducing leaf elasticity (Augé et al., 1987a); (iv) increasing leaf water and turgor potentials (Augé et al., 1987b); and (v) increasing effective rooting length and depth (Davies et al., 1992). In addition, more rapid recovery from drought stress and greater soil moisture extraction at low soil water potential have been observed in AM plants (Hardie and Leyton, 1981). Allen (1982) suggested that AM fungal hyphae absorb and translocate water directly to their hosts, thus acting as a bridge between the dry zone around root and adjacent moist regions. However, George et al. (1992) have observed no direct water transport by AM hyphae to plants. Graham et al. (1987) have also shown that the improvement in water relations of AM citrus plants under drought conditions was unlikely, due to the greater carbon costs and reduced hydraulic conductivity of AM

plants. Radioisotopic studies revealed that the external hyphae in AM fungi can derive N (Frey and Schüepp, 1993) and P (Jakobsen et al., 1992) from soil source and transport it to the host plants. Nitrate uptake by hyphae may be of special significance under drought conditions when root NO_3^- uptake is limited by impaired soil solution mass flow (Tobar et al., 1994a,b). This evidence supports the view that enhanced water use in AM plants was due to the indirect effects of hyphal transport of slowly diffusing mineral ions.

Others suggested that host plant water use is regulated by changes in phytohormones (Augé and Duan, 1991; Duan et al., 1996), or the differences in root morphology (Kothari et al., 1990).

We hypothesized that under drought conditions, AM colonization in maize improves water relations that may play an important role in drought tolerance of the host plant. To test this, we have measured the midday leaf water potential, stomatal resistance, transpiration rate and green leaf area in AM and non-AM plants of the drought-sensitive and -resistant maize cultivars when irrigation was withheld for three weeks following tasselling.

2.2. Materials and Methods

2.2.1. Plant growth conditions

A greenhouse experiment was conducted at the Central Experimental Farm, Ottawa, using freshly regenerated maize seeds of “Tuxpeño sequía” selection cycles 0 (cv. C0, drought-sensitive) and 8 (cv. C8, drought-resistant) obtained from CIMMYT (*Centro Internacional de Mejoramiento de Maíz y Trigo*), Mexico. Treatments consisted

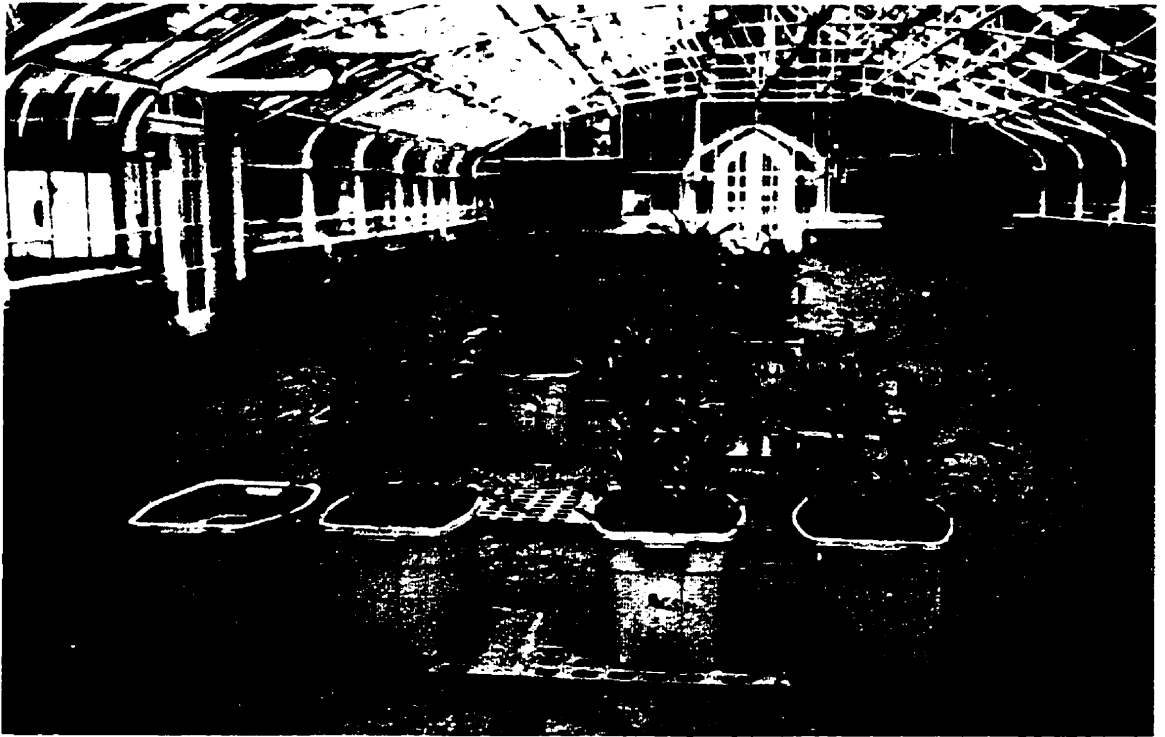
of two tropical maize cultivars (C0 and C8); two moisture regimes, irrigating once a week throughout the crop period (S-) and irrigation withheld for three weeks (75-95 days after sowing) following tasselling (S+); and two mycorrhizal treatments, with (M+) or without (M-) AM fungus inoculation. Thus there were eight treatment combinations replicated four times in a randomized block design (Fig. 2.1). Six maize plants were grown in each plastic container (65 X 40.6 X 42 cm) with bottom drainage holes, in vermiculite at 25°C : 20°C (day : night) with 14 h photoperiod (6 AM - 8 PM), 65-70% relative humidity, irradiance of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by high-power sodium vapour lamps (General Electrics, Lucalox, LU 40073, USA; 475W, 120V, 4.2 amps). The mycorrhizal inoculum (*Glomus intraradices* Schenck & Smith; specimen no. DAOM 181602) used in this study was originally isolated from the rhizosphere of *Fraxinus* sp. and subsequently cultivated on *Coleus* and *Mimulus* spp.. This strain incorporated in peat moss was used as inoculum provided by Premier Tech, Rivière-du-Loup, Québec. One litre (= 300g) of inoculated or non-inoculated peat moss per container was applied at 5 cm depth prior to sowing. All the plants were fertilized with a 500 ml container⁻¹ week⁻¹ Hoagland solution (N, 210 mg; P, 31 mg; K, 235 mg; Ca, 160 mg; Mg, 49 mg; S, 64 mg; Mn, 0.5 mg; Cu, 0.02 mg; Zn, 0.05 mg; B, 0.5 mg; Mo, 0.01 mg; and Fe-chelate, 100 mg, in 1000 ml distilled H₂O; Hoagland and Arnon, 1938) diluted in a 10-20 times volume of irrigation water depending on cumulative evapotranspiration rate. Two maize plants per container remained at the time of the experiment, the other four plants being used for the root colonization studies. At the time of plant sampling, the roots were intact and posed little disruption to the neighbouring ones. Irrigations were withheld from half the M- and M+

Figure 2.1.

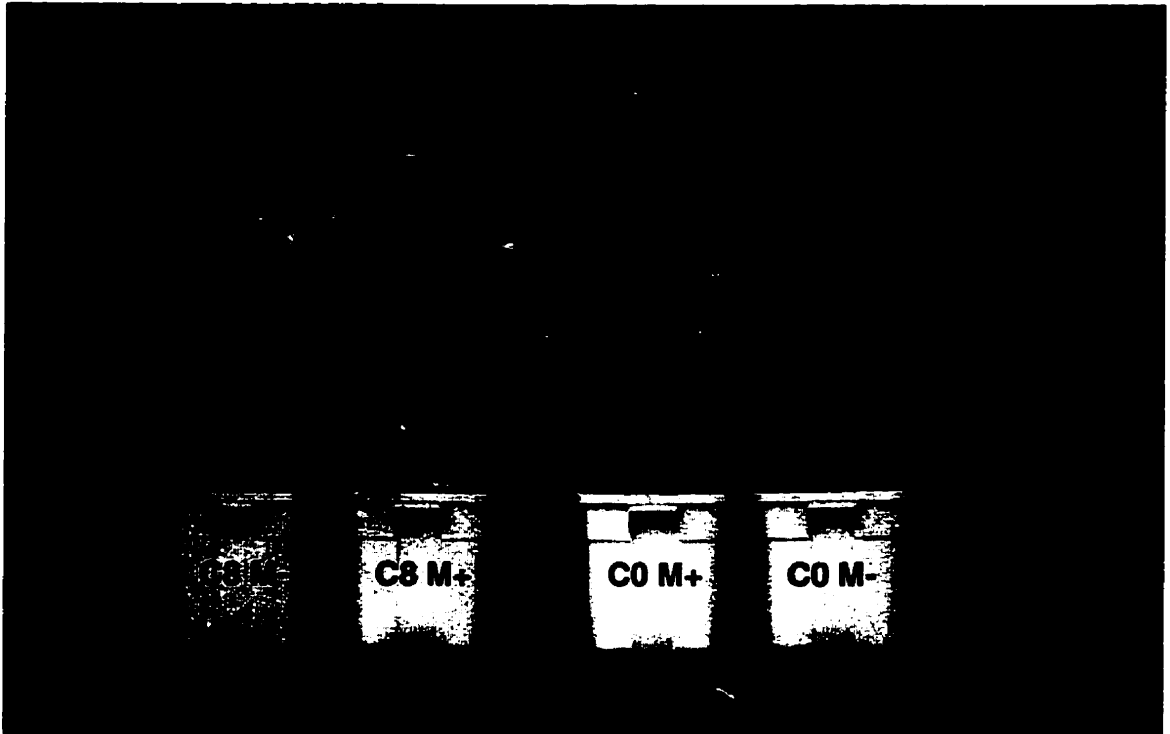
A An overview of the greenhouse experiment, at Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa.

B A picture showing mycorrhizal & non-mycorrhizal plants of the drought-sensitive (C0 M+ & C0 M-) and the drought-resistant (C8 M+ & C8 M-) cultivars at week 6 before the imposition of drought stress.

A



B



plants for three weeks, starting 75 days after sowing. Thereafter, all plants were watered until harvest to compensate for weekly cumulative evapotranspiration.

2.2.2. Determination of mycorrhizal colonization

Roots were washed thoroughly in distilled H₂O, heated in 2.5% KOH on a hot plate for 5 min to bleach the roots under the fumehood, washed with d H₂O to remove the excess KOH, acidified in 1% HCl overnight followed by a washing with d H₂O, stained with 0.05% aniline blue solution (aniline blue, 0.5g; glycerol, 500 ml; d H₂O, 450 ml; HCl 1%, 50 ml) followed by destaining with the solution as in the previous step but without the stain. The stained roots were cut into 1 cm segments before mounting on slides in polyvinyl-alcohol-lactic acid-glycerol (PVLG) medium (Dalpé, 1993). A total of 200 1 cm segments per cultivar were examined under a compound microscope (at 10 X) for the presence of arbuscules, vesicles or both, and the percentages of colonization and arbuscules were determined (Fig. 2.2). These measurements were repeated at 6, 8, 10 and 12 weeks after sowing.

2.2.3. Determination of leaf water potential, stomatal resistance, and transpiration rate

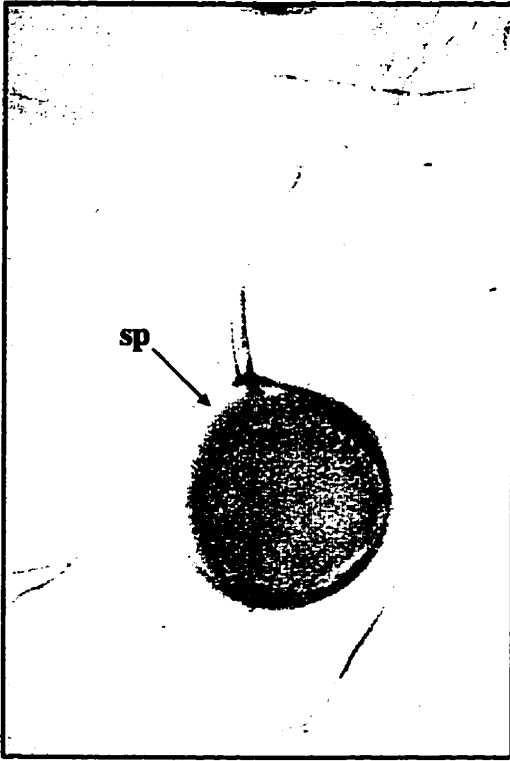
Leaf water potential (LWP) was measured on the fully expanded ear leaf (7th or 8th) between 10:00 - 12:00 h daily during the three week period of drought stress (75-95 days after sowing) using a Scholander pressure chamber (Scholander et al., 1964). To measure the LWP, a fresh leaf section (2 cm long; 1 cm wide) was excised from the plant and partly sealed in a pressure chamber with the cut end of the leaf section protruding out of the chamber. Upon excision, the tension in the leaf xylem is partly released, and the xylem sap retracts into the xylem. Pressure raised in the chamber until the sap just

Figure 2.2. Mycorrhizal structures enumerated during the assessment of root colonization

- A** Chlamydospore (*sp*) of a *Glomus* sp.
- B** Arbuscule (*ar*)
- C** Vesicle (*vt*)
- D** Extraradical hyphae (*eh*)

Courtesy: Figure A from Dr. Y. Dalpé

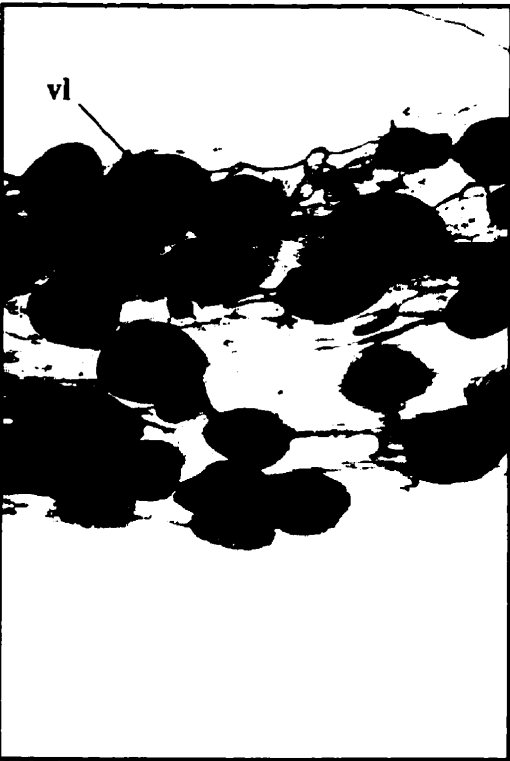
A



B



C



D



appears at the cut end of the xylem. This balance pressure was readily detected as LWP and expressed in MPa. Stomatal resistance and transpiration rate were measured simultaneously using a steady-state diffusion porometer (Licor model 1600) on the fully expanded ear leaf (Dwyer and Stewart, 1985). A small chamber (2 cm diameter) was clamped for 1 or 2 min on to a leaf surface. Water loss from a leaf placed in the Licor chamber is determined by measuring the flow rate of dry air necessary to maintain relative humidity (RH) inside the chamber. Typically, the ambient RH is used as a null point, and the dry air is injected into the chamber at a rate which is just sufficient to balance the transpirational water flux out of the leaf. This maintains the chamber RH at the set point. Stomatal resistance and transpiration rates were measured by a microprocessor directly from the values of RH, leaf and air temperatures, and flow rate.

2.2.4. Potential and actual evapotranspiration

Potential ET (PET) is the evapotranspiration (ET) when there is no limitation in water availability, i.e. when the growing media can supply all the water required by the plant. Actual ET (AET) is the amount of water evapotranspired, and this is less than PET as water is usually limiting. The ET was estimated gravimetrically before each irrigation. Surface evaporation was eliminated by providing a thin layer (2.5 cm) of perlite to all the containers. Water losses from well-watered and drought-stressed plants were recorded as PET and AET, respectively. The AET/PET ratio was also determined to ascertain the relative advantage of mycorrhizal inoculation under drought conditions.

2.2.5. Green leaf area

Total green leaf area (GLA) was estimated by multiplying the product of the length and maximum width of each leaf by a factor of 0.73 (McCree, 1974) and summing overall leaves of the plant at tasselling just before drought stress began. During the stress period, total GLA retained was assessed on the alternate days by subtracting the dried leaf area from the total (Dwyer and Stewart, 1986).

2.2.6. Statistical analysis

A three-way analysis of variance (ANOVA) was done (SAS Institute Inc, 1989) on the data (leaf water potential, stomatal resistance, transpiration rate and green leaf area) obtained at the first, second and third weeks after the drought stress began. Critical differences at the 5% level of significance were tested using Tukey's Studentized Range (HSD) test.

2.3. Results

2.3.1. Mycorrhizal colonization

The mycorrhizal fungal colonization in the cultivars C0 and C8 appeared to increase with the progression of plant growth, and the values were generally higher for C8 than C0 throughout the experiment (Table 2.1). At week 12, C8 had 96.5% colonization which was 25% higher than C0. Arbuscules as a percentage of total colonization declined progressively from 53.2 and 65.3% (6th week) to 13.1 and 13.8% (12th week) in C0 and C8, respectively. Regardless of growth stage, the number of

Table 2.1. Mycorrhizal colonization (%), arbuscules (%) and number of arbuscules or vesicles per 1 cm root segment in the maize cultivars C0 and C8 (n = 200)

Cultivars	Weeks after sowing			
	6	8	10	12
	Mycorrhizal colonization (%)			
C0	55.5	80.9	81.8	71.8
C8	63.5	87.9	85.5	96.5
	Arbuscules (%)			
C0	53.2	31.7	25.3	13.1
C8	65.3	33.7	13.4	13.8
	Number of arbuscules (cm root ⁻¹)			
C0	12.5	12.7	7.2	2.5
C8	20.0	14.2	8.6	5.5
	Number of vesicles (cm root ⁻¹)			
C0	11.0	27.4	21.2	16.8
C8	10.6	28.0	56.1	34.5

arbuscules and vesicles tended to be higher in C8 than C0, but the differences between the cultivars were less pronounced for arbuscules than vesicles.

2.3.2. Leaf water potential

Midday leaf water potentials (LWPs) of drought-stressed M- plants of C0 and C8 were significantly ($P < 0.001$) lower (more negative) at the end of the second and third weeks (Table 2.3; Fig. 2.3) than in well-watered M- or M+ plants (Table 2.2). After two weeks of drought stress, LWPs of M+ plants of C0 (-1.47 MPa) were significantly higher ($P < 0.05$) than LWPs of M- plants (-2.02 MPa). At the end of the third week of drought stress, LWP of M- C0 plants were near wilting (-2.45 MPa) while M+ C0 plants maintained LWP well above the wilting point (-1.74 MPa). A similar trend was observed in C8, but the mycorrhizal effect was significant only at the third week. The LWPs of well-watered M+ and M- plants of both the cultivars declined progressively with time (Table 2.2).

2.3.3. Stomatal resistance

Under well-watered conditions, stomatal resistance (SR) values of M+ and M- plants of C0 and C8 were similar throughout the experiment with an exception of M+ C0 plants which had significantly lower SR than M- C0 plants at day 7 (Table 2.2). Drought stress significantly ($P < 0.001$) increased the SR values of M+ and M- plants of both cultivars at the end of the second and third weeks in comparison to the well-watered plants at the same stage (Tables 2.2 & 2.3; Fig. 2.4). At the end of three weeks of drought, SR increased in M- and M+ plants by 3.0 and 1.8 times in C0 and 2.5 and 1.8 times in C8, respectively, compared with well-watered plants (Table 2.2; Fig. 2.4).

Figure 2.3. Leaf water potential ($n = 4$) of mycorrhizal (solid square) and non-mycorrhizal (empty square) plants of maize cultivars C0 (top) and C8 (bottom) during three weeks of drought stress (75-95 DAS). Statistics were done at 7, 14 and 20 d after the drought treatment began. The data for well-watered plants are presented in Table 2.2. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

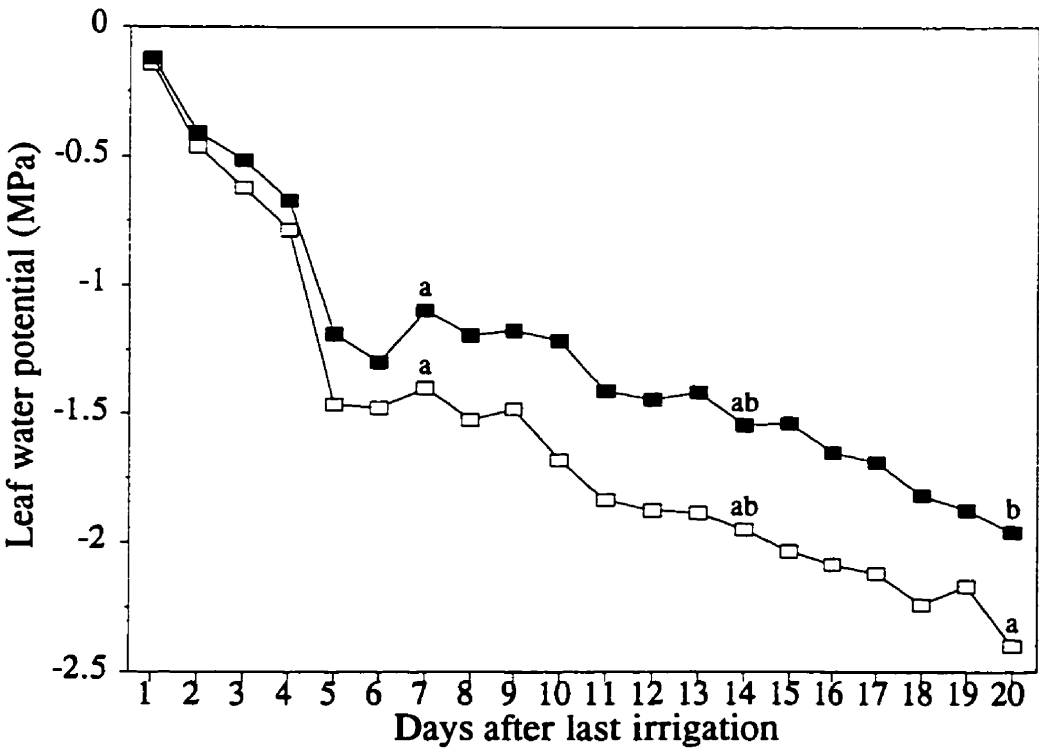
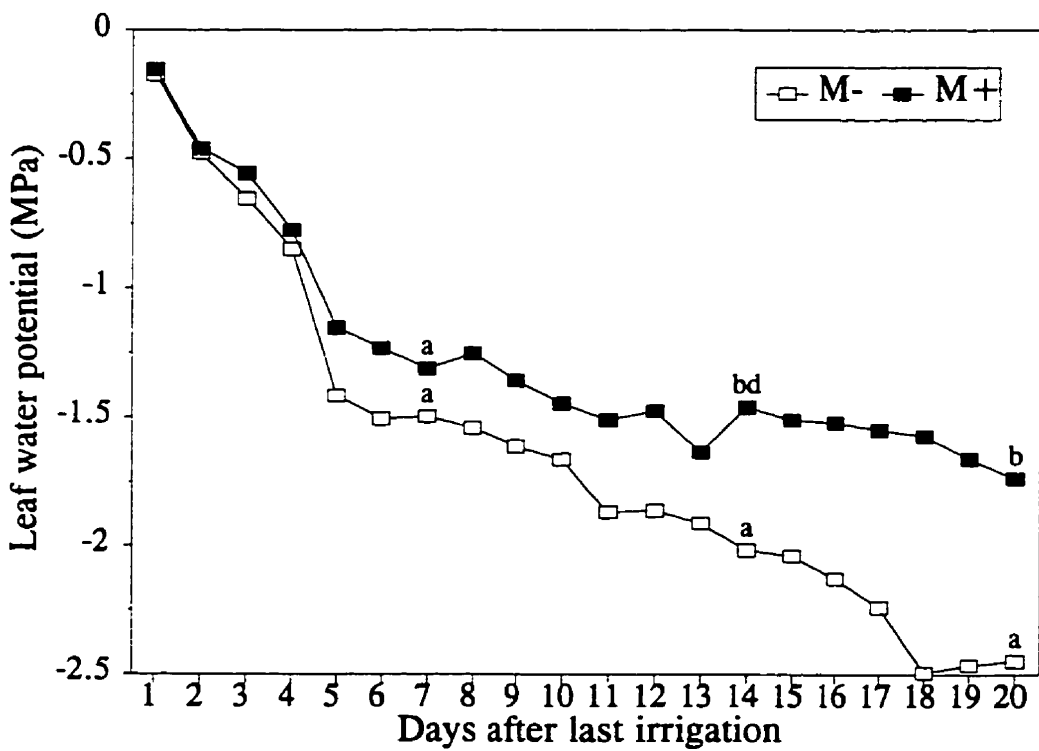


Figure 2.4. Stomatal resistance ($n = 4$) of mycorrhizal (solid square) and non-mycorrhizal (empty square) plants of maize cultivars C0 (top) and C8 (bottom) during three weeks of drought stress (75-95 DAS). Statistics were done at 7, 14 and 20 d after the drought treatment began. The data for well-watered plants are presented in Table 2.2. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

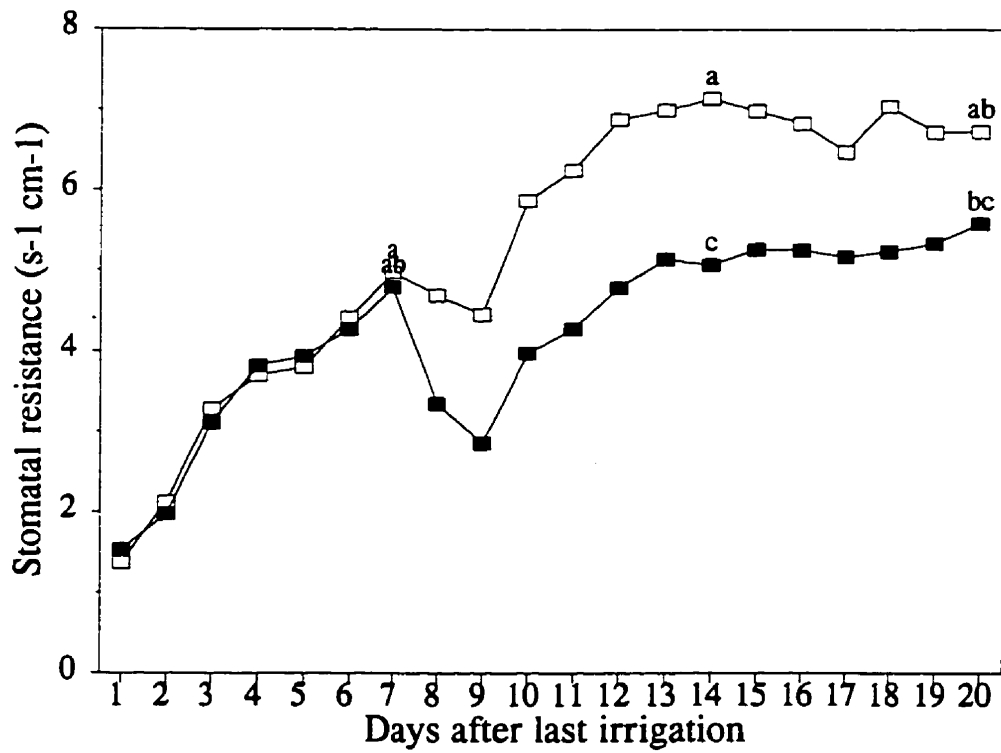
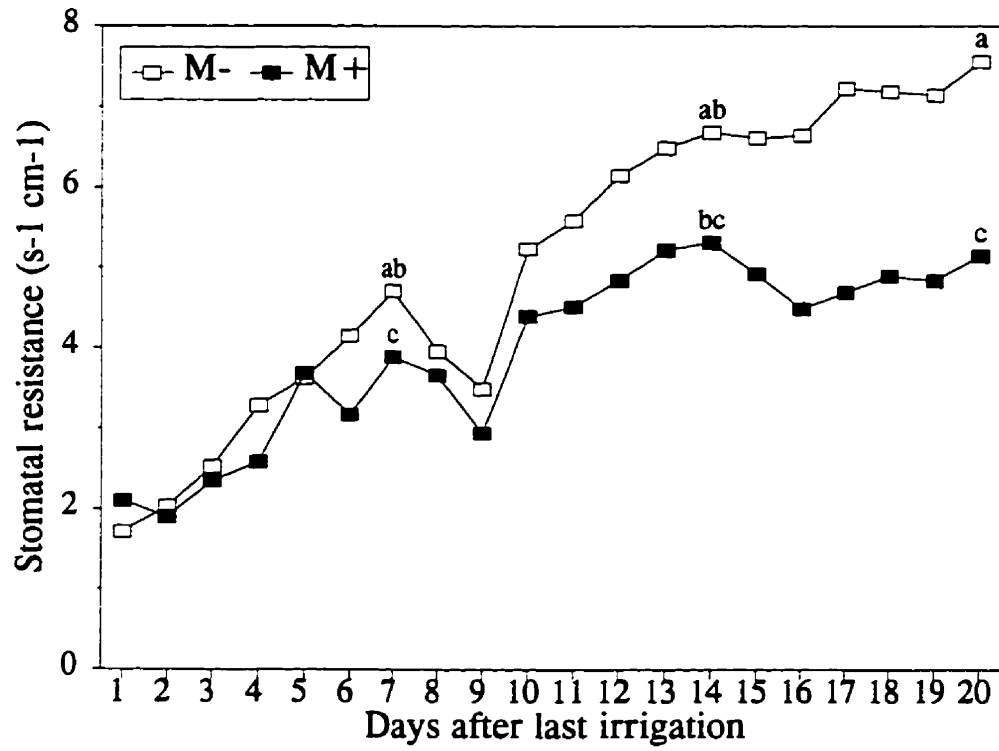


Table 2.2. Means ($n = 4$) and standard error (in parentheses) for leaf water potential (LWP) in MPa, stomatal resistance (SR) in $s^{-1} cm^{-1}$, transpiration rate (TR) in $\mu g cm^{-3} s^{-1}$ and green leaf area (GLA) in cm^2 at 1, 2 and 3 week of the experiment in the drought-sensitive (C0) and -resistant (C8) cultivars under well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within column indicate significant differences ($P < 0.05$) using Tukey's Studentized Range (HSD) test

Week	LWP			SR			TR			GLA		
	1	2	3	1	2	3	1	2	3	1	2	3
<i>Cultivar (C0)</i>												
M+	-1.38 ^d (0.10)	-1.09 ^{cd} (0.05)	-1.25 ^c (0.02)	2.90 ^d (0.10)	3.21 ^d (0.10)	2.89 ^d (0.04)	10.9 ^a (1.00)	9.11 ^{ab} (0.41)	7.73 ^c (0.22)	5082 ^{bc} (33)	4677 ^{bc} (60)	4310 ^c (51)
M-	-1.52 ^a (0.13)	-1.26 ^{cd} (0.04)	-1.28 ^c (0.05)	4.59 ^{ab} (0.15)	3.22 ^d (0.36)	2.56 ^d (0.06)	7.38 ^c (0.20)	8.83 ^{ab} (0.23)	8.05 ^{bc} (0.25)	4934 ^c (56)	4506 ^d (44)	4048 ^{cd} (52)
<i>Cultivar (C8)</i>												
M+	-1.24 ^a (0.03)	-1.07 ^d (0.06)	-1.18 ^c (0.11)	4.28 ^{bc} (0.13)	2.84 ^d (0.41)	3.07 ^d (0.05)	9.86 ^{ab} (0.68)	9.95 ^a (0.78)	9.22 ^{ab} (0.16)	6680 ^a (17)	6280 ^a (24)	5787 ^a (85)
M-	-1.24 ^a (0.02)	-1.18 ^{cd} (0.06)	-1.15 ^c (0.05)	4.68 ^{ab} (0.21)	2.62 ^d (0.26)	2.67 ^d (0.04)	7.51 ^c (0.28)	7.72 ^b (0.31)	9.58 ^a (0.16)	5332 ^a (68)	4870 ^{bd} (89)	4454 ^b (62)

Note: These data were statistically analyzed altogether and respectively with the data that appear on the figures 2.2 to 2.5.

Table 2.3. Levels of significance for ANOVA for leaf water potential (LWP), stomatal resistance (SR), transpiration rate (TR) and green leaf area (GLA) at the end of weeks 1, 2 and 3 after drought

Week	LWP			SR			TR			GLA		
	1	2	3	1	2	3	1	2	3	1	2	3
Cultivars (C)	*	NS	NS	***	NS	NS	NS	NS	**	***	***	***
Stress (S)	NS	***	***	***	***	***	**	***	**	NS	*	***
Mycorrhizae (M)	*	**	***	***	***	***	***	***	**	***	***	***
C X S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C X M	NS	NS	NS	***	NS	NS	NS	NS	NS	***	**	**
S X M	NS	*	*	**	*	***	**	*	***	NS	NS	**
C X S X M	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	**	***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant

Tukey's test showed significantly lower SR values for drought-stressed M+ than M- plants at the end of the first and third weeks for C0 and only at the third week for C8.

There was a drop in SR values on the ninth day of drought stress (Fig. 2.4) that may have been associated with the scheduling of irrigation of well-watered plants, which could have increased the relative humidity of the ambient air.

2.3.4. Transpiration rate

Transpiration rates (TRs) significantly ($P < 0.05$ or $P < 0.001$) decreased in both M+ and M- plants of C0 and C8 at the end of second and third weeks of drought stress in comparison to well-watered plants (Tables 2.2 & 2.3; Fig. 2.5). In the presence of AM association, C0 plants significantly maintained higher transpiration rates at the end of second and third weeks of drought while it was not significant in C8 (Fig. 2.5). Even after three weeks of withheld irrigation, M+ plants had higher TR by 37.4% in C0 and 21.0% in C8 over drought-stressed M- plants. The well-watered M+ plants of C0 and C8 had greater transpiration rates than M- plants after the first week and in C8 at the second week of the experiment (Table 2.2).

2.3.5. Evapotranspiration

Under the drought-stressed conditions, AET/PET ratios appeared to decline in both cultivars either with or without AM association (Fig. 2.6). In C0, the ratios dropped from 1.0 to 0.4 in M- plants and from 1.0 to 0.5 in M+ plants. Despite higher transpiration loss of water in M+ plants of C0, AET/PET ratio tended to be 20% higher in M+ than M- plants. In C8, the ratios between M+ and M- plants were similar.

Figure 2.5. Transpiration rate ($n = 4$) of mycorrhizal (solid square) and non-mycorrhizal (empty square) plants of maize cultivars C0 (top) and C8 (bottom) during three weeks of drought stress (75-95 DAS). Statistics were done at 7, 14 and 20 d after the drought treatment began. The data for well-watered plants are presented in Table 2.2. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

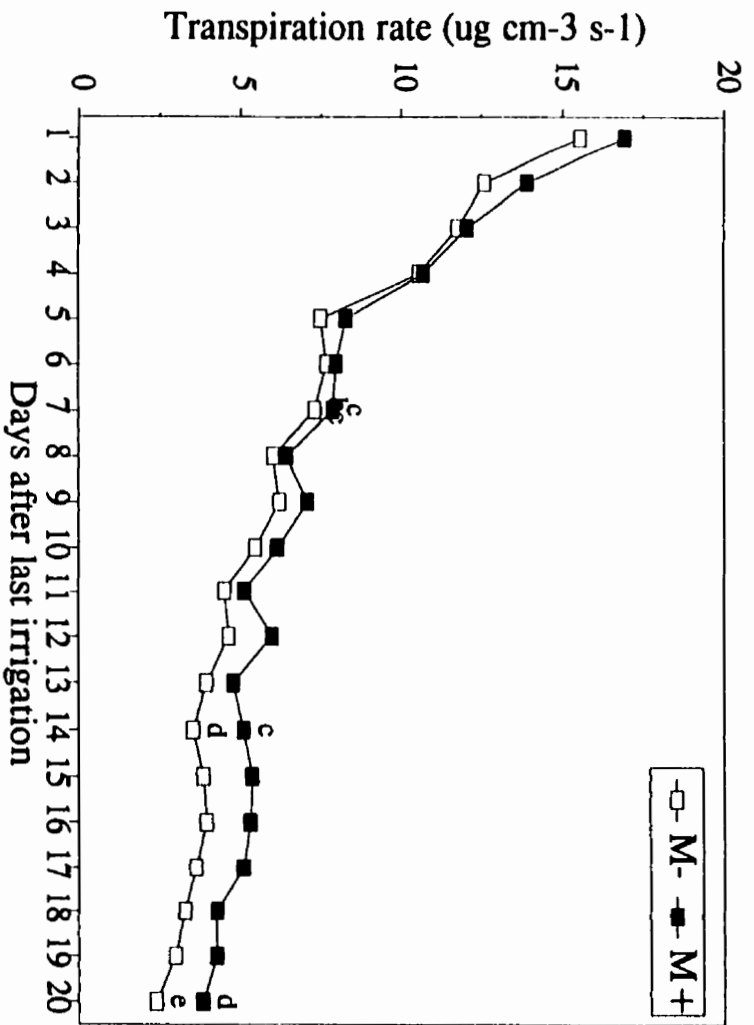
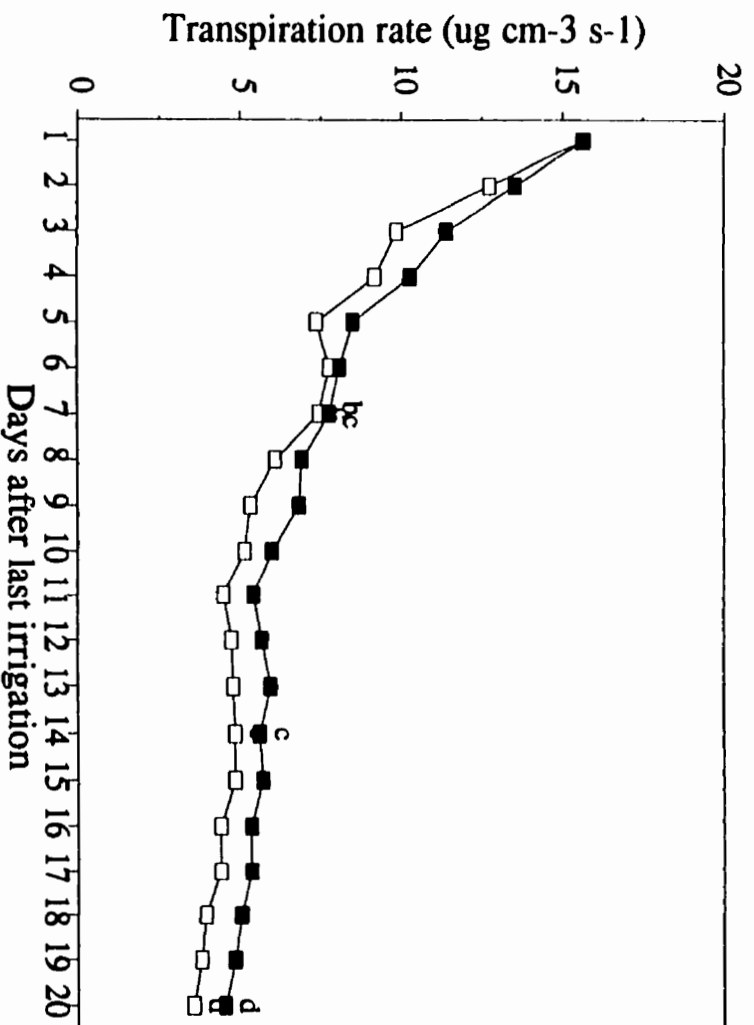
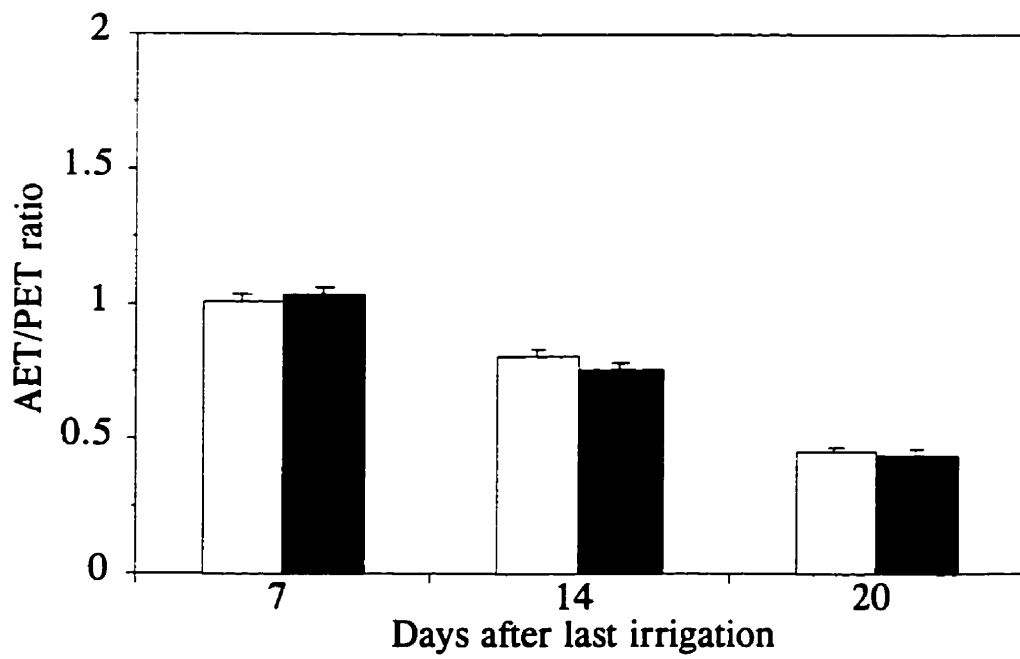
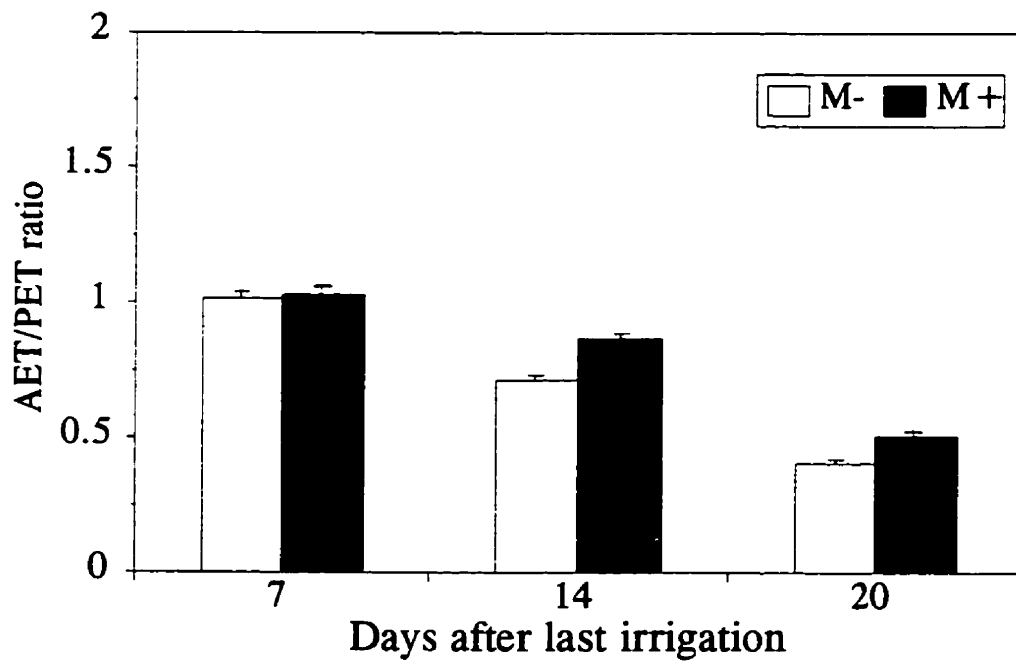


Figure 2.6. Actual ET/ Potential ET ratio (n = 4) of mycorrhizal (solid bar) and non-mycorrhizal (empty bar) plants of maize cultivars C0 (top) and C8 (bottom) during three weeks of drought stress (75-95 DAS). Lines on the top of the bar represent the standard errors of mean.



2.3.6. Green leaf area

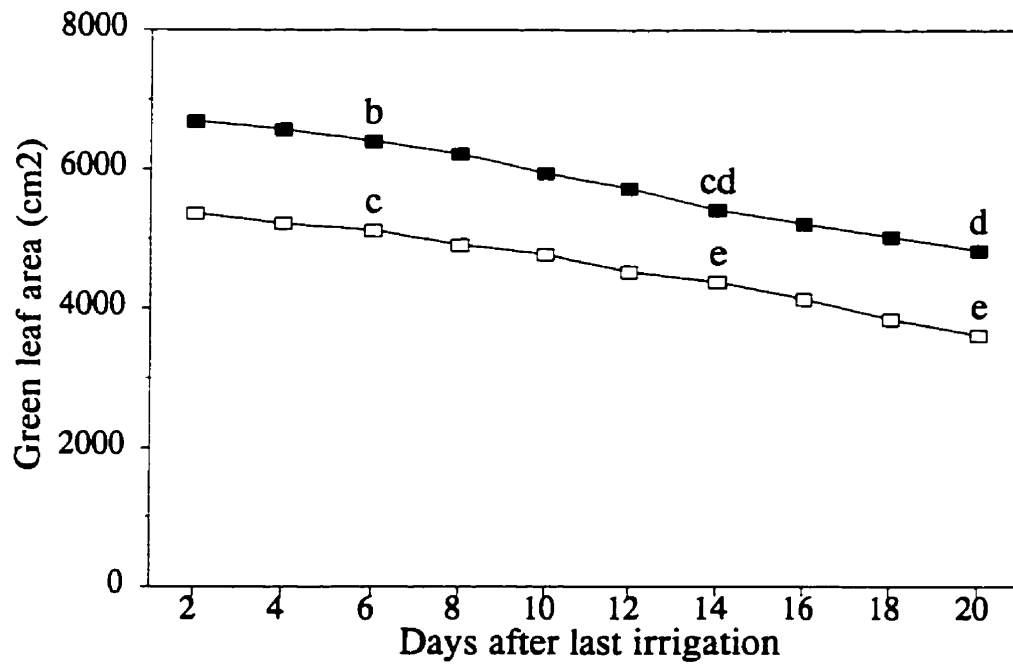
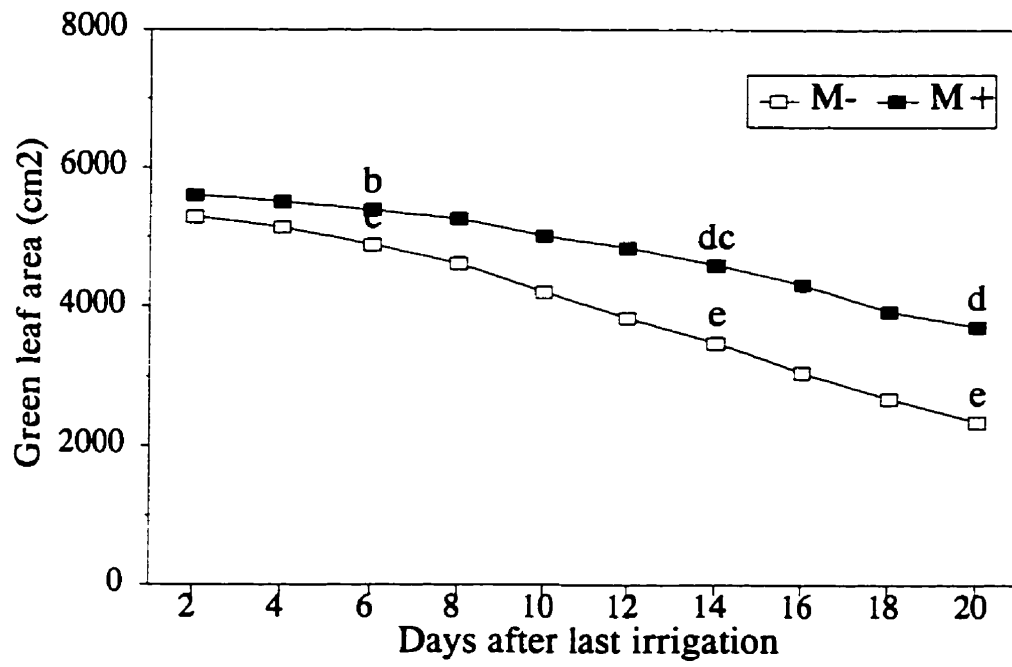
Throughout the drought stress period, M+ plants of C0 and C8 retained significantly ($P < 0.01$ and $P < 0.001$) higher green leaf area (GLA) than M- plants (Table 2.3; Fig. 2.7). At the end of the experiment, the GLA values for M+ plants were higher than M- plants of C0 (M+ 3698 cm²; M- 2335 cm²) and C8 (M+ 4827 cm²; M- 3604 cm²). The M+ and M- plants of C0 and C8 retained 85.5% & 58.0% and 83.4% & 80.9% GLA, respectively, in comparison to well-watered plants (Table 2.2; Fig. 2.7). After three weeks of drought stress, M+ plants of C0 and C8 retained 27.8% and 2.5% higher GLA than M- plants, respectively.

2.4. Discussion

The colonization levels of the cultivars C0 and C8 by *Glomus intraradices* were higher than those previously reported for other maize cultivars with this *Glomus* sp. (Augé et al., 1994) or with *G. mosseae* (Charest et al., 1993). The lower level of colonization in the drought-sensitive C0 cultivar compared to C8 could have been attributed to the reduced carbon availability from the host plant (Nelsen and Safir, 1982; Kehri and Chandra, 1990).

Colonization by arbuscular mycorrhizal (AM) fungi of these two maize cultivars had a consistent effect on the plant water relations and leaf enlargement under moderate drought stress conditions. The present study supports the positive effect of AM in maize by maintaining leaf water potential (LWP) at higher (less negative) values after three weeks of drought stress. These data correspond to the findings of Nelsen and Safir (1982)

Figure 2.7. Green leaf area ($n = 4$) of mycorrhizal (solid square) and non-mycorrhizal (empty square) plants of maize cultivars C0 (top) and C8 (bottom) during three weeks of drought stress (75-95 DAS). Statistics were done at 6, 14 and 20 d after the drought treatment began. The data for well-watered plants are presented in Table 2.2. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).



who reported that LWP of AM onion plants stayed higher despite more negative soil water potential. Bethlenfalvay et al. (1988) indicated that AM soybean plants were able to extract soil moisture down to the permanent wilting point. Mycorrhizal colonization might have stimulated a greater proliferation and surface area that contributed to a better utilization of available water (Kothari et al., 1990). On the contrary, Levy et al. (1983) have shown lower (more negative) LWP in stressed AM plants, suggesting that the greater effective root length and higher transpiration rates by AM plants may have more quickly depleted the available soil moisture. Graham et al. (1987) also indicated that the improvement of water relations in drought-stressed AM plants was unlikely because of the reduced hydraulic conductivity of roots. In the present study, the decline in LWPs with the progression of growth observed in well-watered maize plants is consistent with another study (Wolfe et al., 1988), and was probably due to an increase in resistance to water uptake as the crop aged (Bolaños et al., 1993). The cultivar effect on the increased LWP was significant only during the first week of the experiment. This is in agreement with the study of Bolaños et al. (1993), who observed no difference in LWPs of the same maize cultivars (C0 and C8) grown under field conditions, suggesting that the extent of genetic variability for LWP is small.

In the present study, the stomatal resistance (SR) of AM and non-AM plants increased gradually with the progression of drought stress. This gradual increase in SR with a declining LWP is in contrast with the study of Turner (1974), who reported for maize a sharp increase in SR at a critical LWP of -1.7 MPa. The gradual stomatal closure observed in this study is consistent with a feed-forward theory proposed by Passioura and

Stirzaker (1993). These authors suggested that in pot-cultured plants, roots sense the lack of water and begin to close stomates before LWP drops to a critical level. Under drought conditions, the response of stomatal resistance (SR) to AM was clearly exhibited. The lower SR values in well-watered or drought-stressed AM plants may indicate that these plants were able to keep the stomata open longer than non-AM plants. Augé et al. (1987a) showed that as the mycorrhizal plants had lower SR and higher LWP, the plants fix CO₂ more efficiently. In this regard, in another experiment (Chapter 3), I have shown that AM maize plants under drought conditions had higher total and reducing sugar concentrations than non-AM plants (Subramanian and Charest, 1995). The ability of the AM plants to maintain higher sugar levels is physiologically important in helping the plants to withstand drought stress and to recover after the relief of stress (Kameli and Lösel, 1993). This study suggests that the lower stomatal resistance of the AM maize plants under drought stress could be as a result of accumulation of organic osmolytes such as sugars and amino acids (Subramanian and Charest, 1995).

Throughout the drought stress period, AM plants of C0 and C8 tended to maintain higher transpiration rates (TRs), commensurate with the higher (less negative) leaf water potential and lower stomatal resistance. Kothari et al. (1990) reported that mycorrhizae enhanced TRs of drought-stressed maize plants by about 30%, and this was attributed to a larger leaf area. This study agrees with the findings of Augé et al. (1987b), who observed a positive correlation between mycorrhizal colonization and transpiration rate. Higher AET/PET ratios suggest that mycorrhizal inoculation benefits maize plants under drought conditions by increasing the water availability. Mycorrhizal C0 plants appeared to

maintain an AET/PET ratio 20% higher than non-AM plants. Such a difference was not seen in the drought-resistant C8 cultivar. This implies that AM inoculation could enable the drought-stressed plants of the sensitive C0 cultivar to use the available water efficiently.

The green leaf area (GLA) of maize plants declined progressively with the drought stress period. Reductions in leaf area by 15-20% under different durations of drought stress have been previously reported in maize (Saab and Sharp, 1989). In this study, mycorrhizal colonization led the maize plants to retain higher GLA, especially in the drought-sensitive cultivar (C0). Such a positive response of GLA to mycorrhizae has been reported in maize (Augé et al., 1994), sorghum (Ebel et al., 1994) and wheat (Panwar, 1993). Higher GLA in AM plants may be as a result of enhanced nitrogen acquisition by host plants through the hyphal transport of NO_3^- (Tobar et al., 1994a,b) or nitrogen assimilating enzymes (Cliquet and Stewart, 1993). The retention of GLA with AM inoculation would be beneficial in drought management by maintaining a higher photosynthetic rate (Augé et al., 1987b; Panwar, 1993).

In summary, mycorrhizal colonization had a significant effect in improving water relations and in retaining more green leaf area in drought-stressed maize plants. The response was more pronounced in the drought-sensitive (C0) than the drought-resistant (C8) cultivars. These findings suggest that AM association improves plant water relations and contributes to drought tolerance in maize. This may play an important role in the context of sustainable agriculture.

CHAPTER 3

ARBUSCULAR MYCORRHIZAE ON THE METABOLISM OF MAIZE UNDER DROUGHT

This chapter is a reproduction with minor modifications of a paper published in *Mycorrhiza* (5: 273-278; 1995) by K.S. Subramanian and C. Charest. This chapter fulfills the second objective of this thesis : To examine whether the improved water relations in the two tropical maize cultivars are related to metabolic modifications in the AM colonized plants under drought conditions.

3.1. Introduction

Drought stress affects physiological and biochemical processes in plants (Hsiao et al., 1976; Hanson and Hitz, 1982), resulting in altered metabolic pathways. The major effects are those involving the accumulation of organic osmolytes such as sugars, amino acids and organic acids (Kameli and Lösel, 1995, 1996; Girousse et al., 1996; Timpa et al., 1986). These metabolic changes are believed to promote drought tolerance in plants by maintaining turgor through osmotic adjustment (Morgan, 1984). Kameli and Lösel (1993) reported that glucose accumulated more rapidly in wheat under drought and to a higher concentration in drought-resistant than -sensitive cultivars. The rate and accumulation of sugars accompanying decreasing water potential appear to be physiologically important in helping plants withstand drought and recover after stress is relieved. Direct relationships were observed between sugars and xerophytic features (Iljin, 1957) as well as dehydration tolerance of grass species (Schwab and Gaff, 1986)

and these data support a positive role of sugars during drought stress. The contribution of sugars to osmotic adjustment in sorghum was approximately equal to that of inorganic solutes K and Cl (Jones et al., 1980).

Arbuscular mycorrhizal (AM) fungi appeared to promote host plant drought tolerance which is partly attributed to changes in the host's rate of photosynthesis (Harris et al., 1985), or levels of carbohydrates (Nemec and Guy, 1982) and proteins (Dumas et al., 1990). Higher chlorophyll and leaf starch levels were observed in mycorrhizal rose plants under drought stress (Augé et al., 1987a). Davies et al. (1993) found no correlation between carbohydrates and osmotic adjustment in mycorrhizal *Capsicum annuum* plants. Soluble proteins were increased with AM fungal inoculation in maize (Charest et al., 1993) and tobacco (Dumas et al., 1990) and this enhancement was regarded as an indicator of plant tolerance. Pacovsky (1989) found an increase in aspartate and arginine in mycorrhizal soybean roots, thus demonstrating that N utilization was altered in the symbiotic association.

We hypothesised that under drought conditions AM fungal colonization of maize assists in the accumulation of organic solutes, such as sugars and nitrogenous compounds, which contribute to drought tolerance of the host plant. To test this, I have examined metabolic changes (sugars, proteins and amino acids) in AM and non-AM maize plants of drought-sensitive and drought-resistant when drought stress imposed for three weeks following tasselling.

3.2. Materials and Methods

3.2.1. Plant growth conditions

The details of growth conditions and treatments were presented in Chapter 2.2.1.

3.2.2. Metabolite analysis

Fully expanded 7th or 8th maize leaves were sampled at the beginning, middle (day 10) and end of the drought spell (day 20) and estimated for chlorophyll, sugar, protein and amino acid concentrations.

3.2.2.1. Chlorophyll

Freeze-dried leaf tissues (50 mg) were immersed in 50 ml 95 % ethanol in a conical flask covered with aluminium foil and kept it in the fridge until complete removal of chlorophyll. The optical density (O.D) of the extract was measured at 645 and 663 nm (Bruinsma, 1963). Chl_a , Chl_b , Chl_{a+b} and $Chl_{a/b}$ were estimated using the following formula

$$Chl_a = (O.D_{663} \times 12.7) - (O.D_{645} \times 2.7)$$

$$Chl_b = (O.D_{645} \times 22.9) - (O.D_{663} \times 4.7)$$

$$Chl_{a+b} = Chl_a + Chl_b$$

$$Chl_{a/b} \text{ ratio} = Chl_a / Chl_b$$

3.2.2.2. Sugars

Leaf tissues (100 mg dry mass) were ground with 5-10 ml deionized H₂O on fine sand in a mortar and pestle, the extract transferred into a flask and the volume was made up to 25 ml. The leaf extract was heated on a hot plate for 15 min, cooled, filtered through Whatman #1 paper and the final volume adjusted to 25 ml. One ml of the filtered

extract was transferred into test tubes, added with 1 ml d H₂O (reducing sugars) or invertase enzyme solution (total sugars), homogenized well and the tubes were incubated at room temperature for 30 min. Enzyme solution was prepared by dissolving 10mg invertase in 100 ml sodium acetate 0.01M pH 5.0 buffer (70 ml sodium acetate 0.01 M + 30 ml acetic acid 0.01 M). The enzyme reaction (sucrose hydrolyzed into glucose and fructose) was terminated by adding 1 ml copper solution (**Solution A**: 25g Na₂CO₃, 25g sodium potassium tartrate, 20g NaHCO₃, 200g NaSO₄ dissolved in 800 ml freshly boiled d H₂O then made up to 1000 ml; **Solution B**: 15g CuSO₄ in 100 ml d H₂O; solutions **A** and **B** were mixed at 25:1 at the time of use). The tubes were boiled for 30 min in a water bath, cooled to room temperature, added with 2 ml arsenomolybdate reagent, and the contents were diluted with 5 ml d H₂O. A blank tube was run with all the reagents except the leaf extract. The resultant chromophore was measured at 525 nm. Sugar concentrations in the test solution were detected from a standard curve prepared with different concentrations of glucose (Appendix 1).

3.2.2.3. *Proteins*

Leaf tissues (100 mg freeze-dried) were ground up on ice with 10 ml Tris-HCl buffer (pH 7.8), fine sand and 0.2g PVP (insoluble polyvinylpyrrolidone) in a mortar and pestle. The leaf extract was centrifuged at 12 000 rpm for 20 min at 4°C, filtered through Whatman #1 filter paper. The filtered extract (100 µl) was placed in a test tube, 5 ml diluted Bio-Rad dye reagent (1 volume of Bio-Rad protein assay for 4 volumes of d H₂O) was added, and the contents were homogenized by gentle inversion. The Bio-Rad protein assay is a dye-binding assay in which a differential color change occurs in response to

various concentrations of protein in the leaf extract. The O.D was measured at 595 nm (Bradford, 1976). Protein concentrations in the test solution were detected from the standard curve prepared using BSA (Appendix 2)

3.2.2.4. Amino acids

Amino acids were extracted in 10 ml of 95% ethanol from freeze-dried leaf tissue (100 mg) by grinding with a mortar and pestle on ice. The extract was centrifuged at 5000 rpm for 10 min. Amino acids were screened by automated precolumn phenylthiocarbonyl amino acid analysis using the Applied Biosystems Inc. Model 420A-Boa-92a free amino acid analyzer and expressed as cumulative means and Ses. The amino acid analysis was done by Mrs. Patricia Lanthier in the lab of Dr. Yoguchi, National Research Council of Canada, Ottawa.

3.2.3. Statistical analysis

A three-way analysis of variance (ANOVA) was done (SAS Institute Inc, 1989) on the data (chlorophyll, sugars, proteins) obtained at the beginning, middle and end of the drought stress period. The data for chlorophyll ratio were log-transformed prior to statistical analysis. Critical differences at the 5% level of significance were tested using Tukey's Studentized Range (HSD) test.

3.3. Results

3.3.1. Chlorophyll

Chlorophyll concentration in maize leaves was not altered either by drought stress or mycorrhizal colonization in the two cultivars. The average total chlorophyll

concentrations for M+ and M- plants were 7.85 and 8.09 mg g⁻¹ DM for C0 and 7.87 and 8.18 mg g⁻¹ DM for C8, respectively. At the end of three weeks of drought stress, M- plants of C0 and C8 cultivars had significantly ($P < 0.05$ or $P < 0.001$) lower chlorophyll a/b ratios than well-watered M+ or M- plants (Table 3.1; Fig. 3.1). In the presence of AM association, both cultivars (C0, 16%; C8, 17%) had higher chlorophyll ratios in comparison to M- plants under drought conditions.

3.3.2. Sugars

Total soluble sugar concentrations were significantly ($P < 0.01$) lower in drought-stressed M- plants of C0 and C8 cultivars compared to unstressed M+ or M-plants or stressed M+ plants at the end of three weeks of withheld irrigation (Table 3.1; Fig. 3.2). Continuously withheld irrigation of three weeks led to a reduction in total sugar concentrations by 32.7% & 32.5% in M- and 12.7% & 13.9% in M+ plants of C0 & C8 cultivars, respectively. Reducing sugar concentrations in M+ and M- plants of both cultivars under well-watered and drought-stressed conditions were similar throughout the experiment (Table 3.1; Fig. 3.3). However, at the end of three weeks of drought, M+ plants had higher levels of reducing sugars (C0, 20.0 and C8, 23.4 mg g⁻¹ DM) than M- plants (C0, 12.8 and C8, 16.6).

3.3.3. Proteins

The protein concentrations in maize leaves of C0 and C8, with or without mycorrhizae, in most cases tended to decrease with the age of the plant (Fig. 3.4). At the beginning of the experiment, protein concentration in C8 was significantly ($P < 0.001$) higher than C0 and the difference was diminished at the later stages (Table 3.1). Drought

Figure 3.1. Chlorophyll a/b ratio ($n = 4$) in leaves of well-watered non-mycorrhizal (S-M-) & mycorrhizal (S-M+) and drought-stressed non-mycorrhizal (S+M-) & mycorrhizal (S+M+) plants of maize cultivars C0 (top) and C8 (bottom) at the beginning (empty bar), middle (dotted bar) and end (solid bar) of the experiment (75-95 DAS). Means with different letters are significantly different according to Tukey's test ($P < 0.05$). Three data sets at the beginning (a-c), middle (l) and end (x,y) of the experiment were analyzed separately.

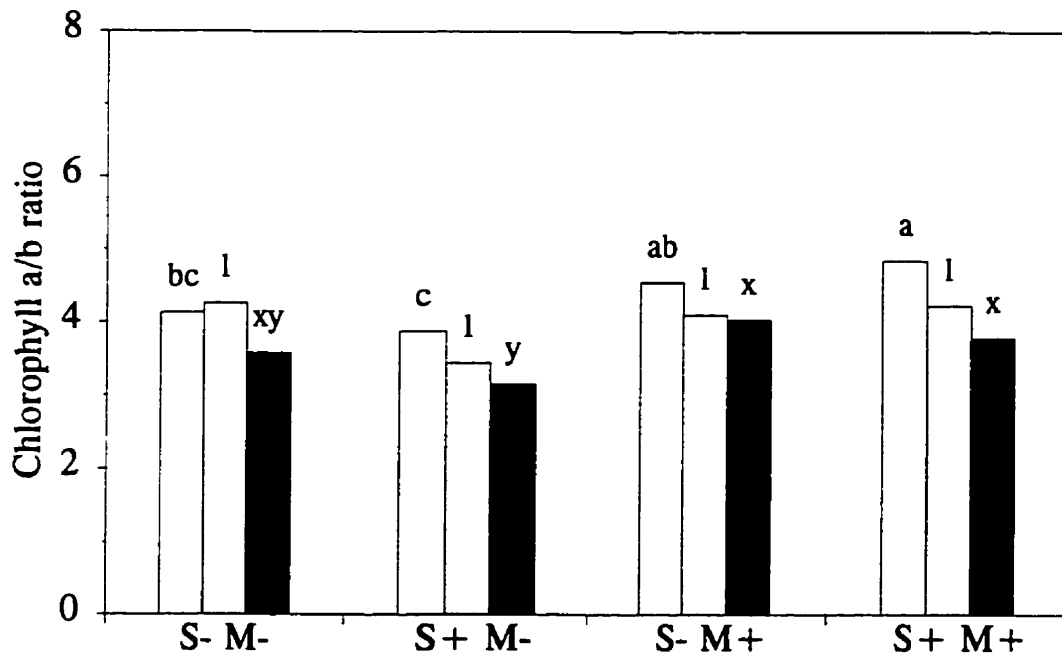
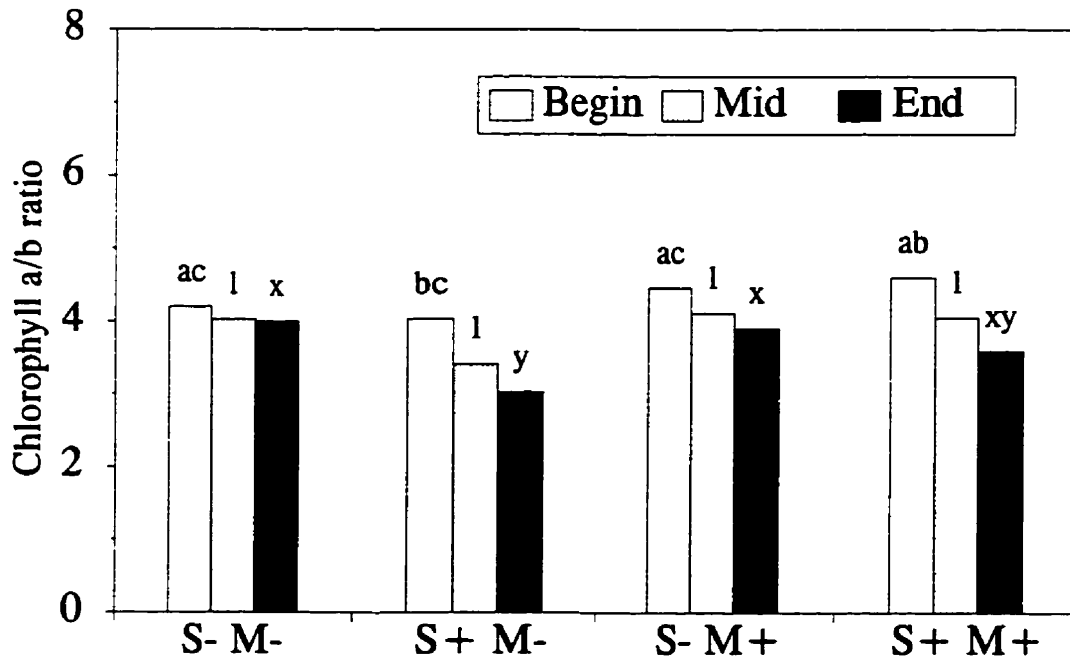


Figure 3.2. Total sugars in mg g^{-1} dry mass ($n = 4$) in leaves of well-watered non-mycorrhizal (S-M-) & mycorrhizal (S-M+) and drought-stressed non-mycorrhizal (S+M-) & mycorrhizal (S+M+) plants of maize cultivars C0 (top) and C8 (bottom) at the beginning (empty bar), middle (dotted bar) and end (solid bar) of the experiment (75-95 DAS). Means with different letters are significantly different according to Tukey's test ($P < 0.05$). Three data sets at the beginning (a), middle (l) and end (w-z) of the experiment were analyzed separately.

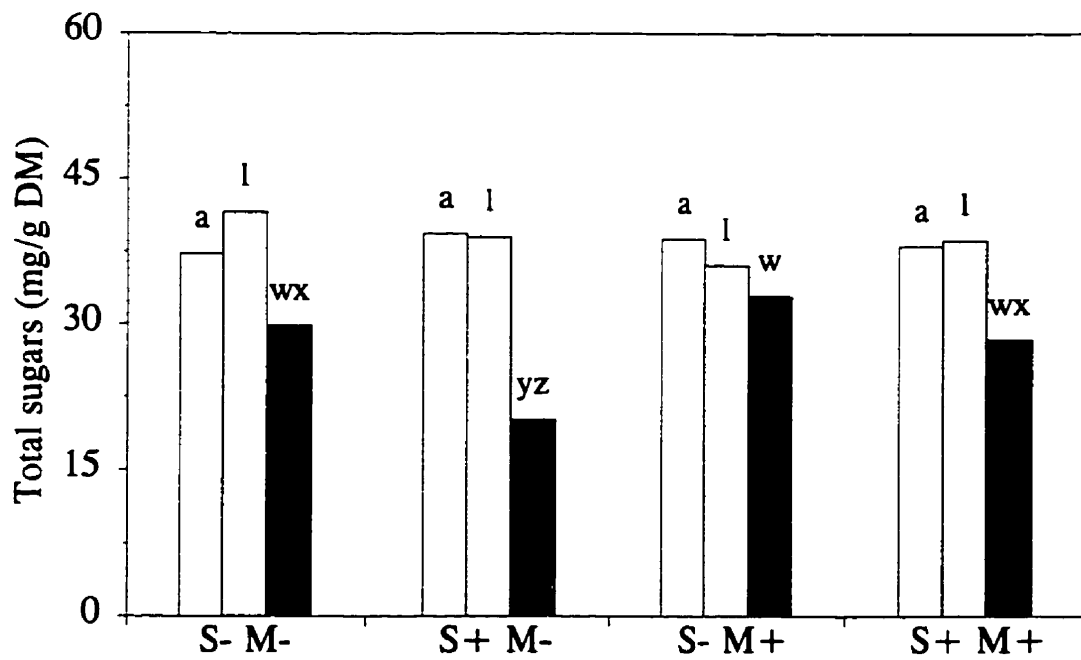
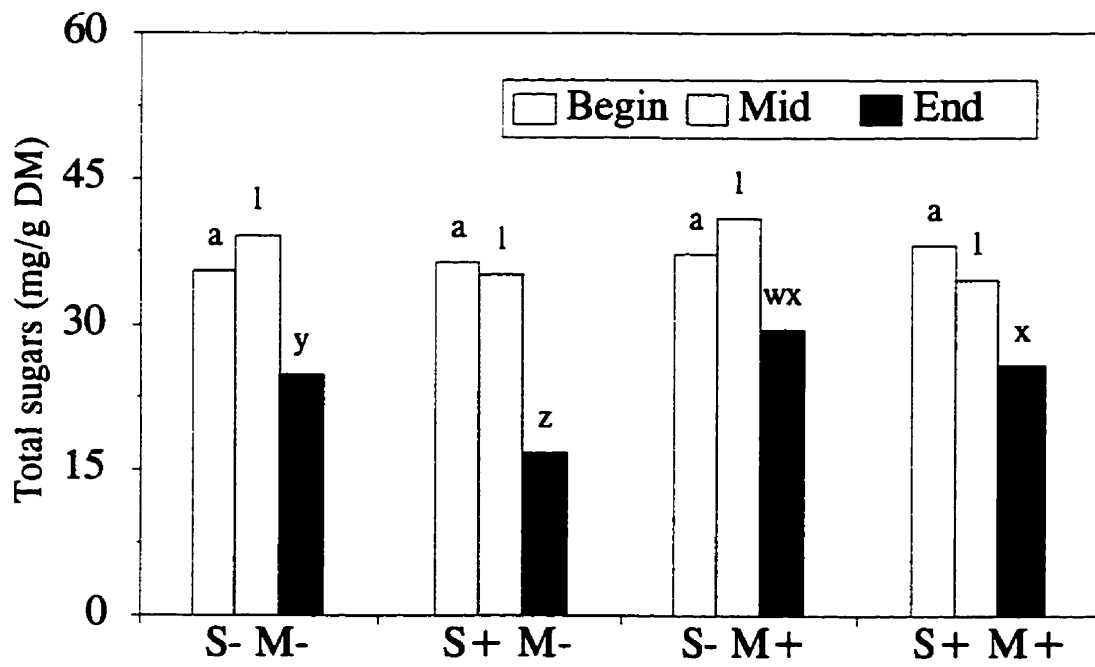


Figure 3.3. Reducing sugars in mg g^{-1} dry mass ($n = 4$) in leaves of well-watered non-mycorrhizal (S-M-) & mycorrhizal (S-M+) and drought-stressed non-mycorrhizal (S+M-) & mycorrhizal (S+M+) plants of maize cultivars C0 (top) and C8 (bottom) at the beginning (empty bar), middle (dotted bar) and end (solid bar) of the experiment (75-95 DAS). Means with different letters are significantly different according to Tukey's test ($P < 0.05$). Three data sets at the beginning (a), middle (l) and end (x-z) of the experiment were analyzed separately.

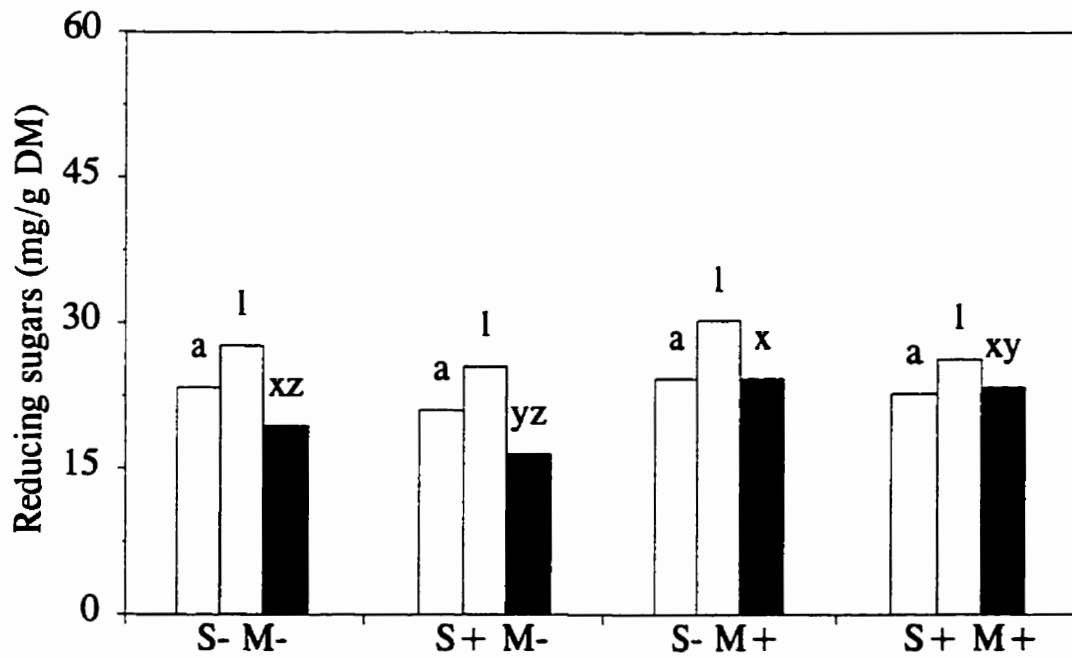
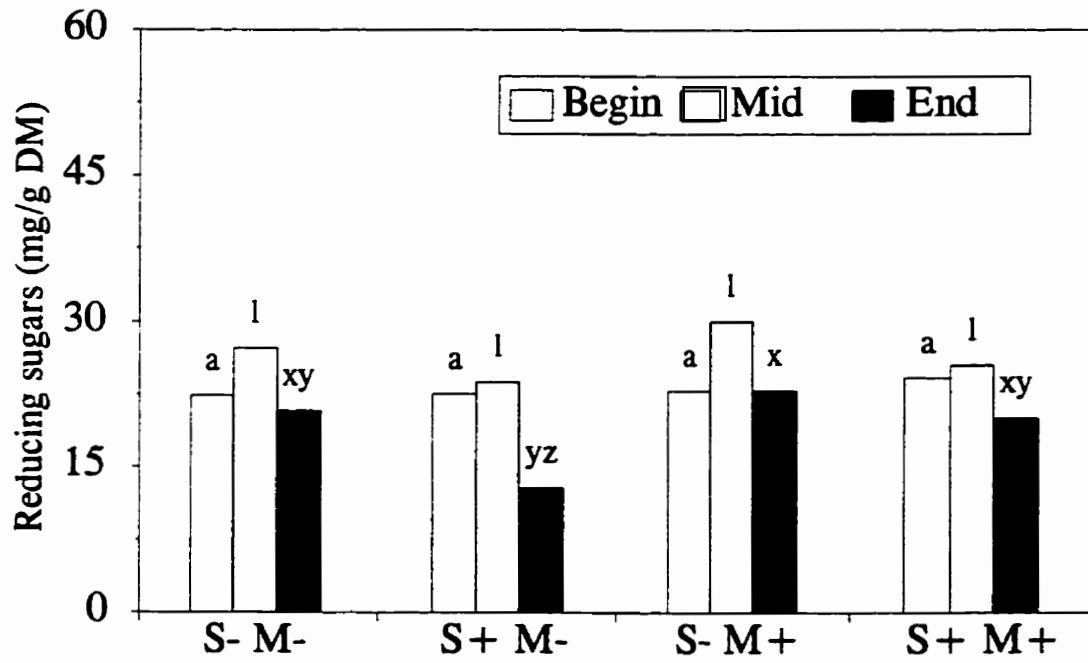


Figure 3.4. Soluble proteins in mg g^{-1} dry mass ($n = 4$) in leaves of well-watered non-mycorrhizal (S-M-) & mycorrhizal (S-M+) and drought-stressed non-mycorrhizal (S+M-) & mycorrhizal (S+M+) plants of maize cultivars C0 (top) and C8 (bottom) at the beginning (empty bar), middle (dotted bar) and end (solid bar) of the experiment (75-95 DAS). Means with different letters are significantly different according to Tukey's test ($P < 0.05$). Three data sets at the beginning (a), middle (l) and end (x-z) of the experiment were analyzed separately.

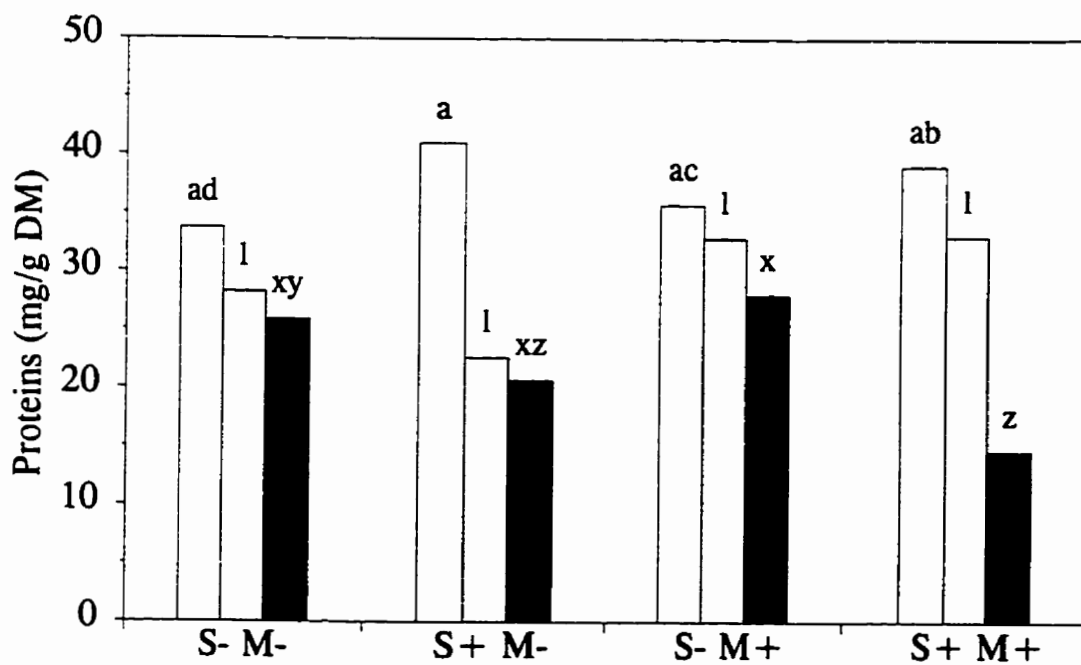
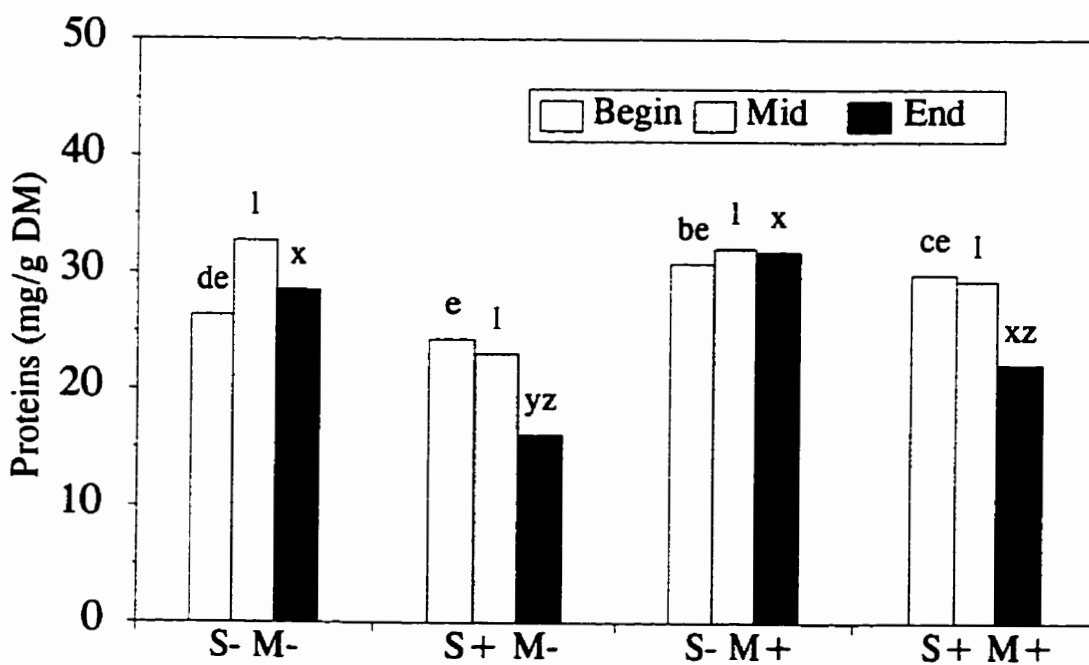


Table 3.1. Levels of significance for ANOVA for chlorophyll a/b ratio (CHL), total sugars (TS), reducing sugars (RS), proteins (PRO) measured at the beginning (B) middle (M) and end (E) of the experiment (75-95 DAS)

	CHL			TS			RS			PRO		
	B	M	E	B	M	E	B	M	E	B	M	E
Cultivar (C)	NS	NS	NS	*	NS	***	NS	NS	NS	***	NS	NS
Stress (S)	NS	*	***	NS	*	***	NS	**	**	NS	*	***
Mycorrhizae (M)	***	*	***	NS	NS	***	NS	NS	***	*	*	*
C X S	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
C X M	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
S X M	NS	*	*	NS	NS	**	NS	NS	NS	NS	NS	NS
C X S X M	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS not significant

stress lowered the protein concentration regardless of cultivar. The protein concentrations in C0 or C8 dropped by 44 % and 20%, respectively, by the end of drought spell, compared with unstressed plants of the same stage. The protein loss was restricted to 30% in C0 in the presence of AM fungus, but such a response was not seen in C8.

3.3.4. Amino acids

The statistical analyses on total amino acid concentrations have shown no significant differences for drought or mycorrhizal treatments. The cultivars C0 and C8 appeared to have increases in the total amino acid concentrations during the drought stress period (Table 3.2), namely by 40.6% and 43.7% in M- C0 and C8 plants, respectively, compared with unstressed M- plants. The total amino acid concentrations seemed to increase by 10.7% and 19.2% in drought-stressed M+ plants of C0 and C8 plants, respectively in comparison to well-watered M+ plants. The drought-resistant cultivars tended to have generally higher amino acid concentrations than C0 regardless of drought or mycorrhizal treatments. The predominant amino acids detected in both cultivars were asparagine, alanine, serine, glutamate, glutamine and glycine, which together accounted for over 70% of the total pool. Under drought conditions, AM plants of C0 and C8 showed an increase in aspartate and glutamine and asparagine and glycine, respectively. No clear differences were observed for other amino acids.

3.4. Discussion

Under drought conditions, arbuscular mycorrhizal (AM) fungal colonization in the tropical maize cultivars assists in metabolic changes that lead to reduced degradation of

Table 3.2. Amino acid concentrations ($\mu\text{mol g}^{-1}$ dry mass) of leaves from well-watered non-AM (S- M-) or AM (S- M+) and drought-stressed non-AM (S+ M-) or AM (S+ M+) maize cultivars C0 and C8. Each value is the mean of 6 data (two replicates each at the beginning, middle and end of the experiment, SE).

	<i>Drought-sensitive (C0)</i>				<i>Drought-resistant (C8)</i>			
	S- M-	S- M+	S+ M-	S+ M+	S-M-	S- M+	S+ M-	S+ M+
Ala	19.0 (1.2)	23.0 (3.3)	25.0 (4.6)	19.4 (2.3)	19.0 (2.7)	19.0 (4.0)	32.0 (5.6)	31.0 (2.5)
Asn	13.0 (2.3)	13.0 (0.2)	29.0 (9.8)	17.0 (12.0)	17.0 (9.7)	45.0 (10.0)	45.0 (10.0)	51.0 (20.0)
Asp	2.4 (0.2)	3.7 (0.5)	2.5 (0.7)	3.1 (1.8)	3.6 (0.8)	3.5 (0.9)	4.5 (1.2)	3.0 (0.6)
Gln	5.1 (2.5)	1.4 (0.4)	14.0 (6.3)	16.0 (7.6)	5.2 (1.9)	7.4 (2.3)	7.7 (2.2)	5.1 (3.1)
Glu	3.5 (0.3)	3.9 (0.7)	4.7 (1.0)	4.6 (0.4)	5.3 (0.8)	4.6 (0.6)	5.6 (0.1)	5.4 (1.5)
Gly	2.9 (0.8)	5.0 (1.0)	5.2 (1.6)	4.8 (0.8)	3.5 (0.7)	4.0 (0.9)	5.4 (1.4)	6.8 (3.5)
Ser	6.5 (1.0)	9.3 (0.2)	9.8 (2.4)	7.3 (1.3)	7.7 (1.3)	7.7 (1.5)	13.0 (4.1)	12.0 (3.6)
Thr	1.9 (0.3)	3.1 (0.6)	2.2 (0.7)	1.5 (0.3)	2.4 (0.5)	2.4 (0.7)	2.1 (0.4)	2.4 (1.0)
Tyr	1.1 (0.5)	1.6 (0.2)	1.6 (0.4)	0.7 (0.3)	1.0 (0.3)	0.7 (0.1)	1.3 (0.4)	1.0 (0.6)
Val	1.1 (0.2)	1.3 (0.2)	1.6 (0.3)	1.2 (0.3)	1.4 (0.3)	1.5 (0.3)	0.1 (0.0)	0.6 (0.1)
Others ^a	3.0 (0.6)	4.9 (1.2)	4.6 (1.1)	3.0 (0.9)	2.8 (0.7)	4.0 (1.1)	5.6 (1.1)	5.2 (2.2)
Total	59.5 (9.9)	70.2 (8.5)	100.2 (28.9)	78.6 (28.0)	68.9 (19.7)	99.8 (22.4)	122.3 (26.5)	123.5 (38.7)

^a Arg, Cys, His, Ile, Leu, Lys, Met, Phe, and Pro

sugars and nitrogenous compounds. The AM colonization alleviated the chlorophyll degradation and maintained its concentrations at levels comparable to unstressed plants. Reduction in chlorophyll content due to drought stress is well-established (Hsiao, 1973; Sung, 1985). This study agrees with the findings of Augé et al. (1987a) who detected higher chlorophyll content in drought-stressed AM than non-AM rose leaves.

Mycorrhizal fungal association, by improving nutritional status (McArthur and Knowles, 1993), can support a higher chlorophyll content (Rachel et al., 1992) and subsequently lead to a higher production of photosynthates (Gianinazzi-Pearson and Gianinazzi, 1983).

The ability of the AM plants to maintain sugar levels during drought stress is physiologically important in assisting the plants to sustain the effects of water deficit conditions. The enhanced sugar concentrations in drought-stressed AM maize plants observed in this study may be related to these plants having higher (less negative) leaf water potential and lower stomatal resistance than non-AM plants (Subramanian et al., 1995; Chapter 2). A direct relationship between reducing sugars and degree of adaptation to drought has been observed in cotton plants (Ackerson, 1981). However, Drossopoulos et al. (1987) found no relationship between either glucose or fructose concentrations and drought stress in wheat. In another study, glucose accumulated in proportion to decreasing leaf water potential more rapidly in drought-resistant than drought-sensitive wheat cultivars (Kameli and Lösel, 1993).

The AM association accentuated the protein content under drought-stressed conditions, especially in the drought-sensitive cultivar. These data agree with the findings of Arines et al. (1993) who reported two to six fold increase in soluble proteins in

mycorrhizal clover roots. The enhanced protein concentrations appear to be an indicator of stress tolerance (Charest et al., 1993). The AM-inducible proteins or polypeptides (endomycorrhizins) identified in some plant species (Dumas et al., 1990; Simoneau et al., 1994) may also play an adaptive role in drought tolerance. When maize plants were subjected to drought stress, the total amino acid concentration increased but to a lesser extent in AM than non-AM plants. This indicates an adaptive role of mycorrhizae in alleviating protein degradation. Moreover, AM fungi seem to play an active role in N nutrition under drought conditions (Tobar et al., 1994a,b). The relative increase in amino acids also demonstrates that N utilization of maize was altered in the presence of mycorrhizal association. Cliquet and Stewart (1993) have shown that AM fungi increase ammonium assimilation, glutamine production and xylem translocation in maize. This illustrates the role of mycorrhizae in regulating and triggering modifications in N metabolism of host plants (Attiwill and Adams, 1993).

In summary, I have demonstrated that AM colonization assists in metabolic changes that play an adaptive role in drought resistance of maize. The increase in organic solutes such as sugars and nitrogenous compounds may contribute to osmotic adjustment, resulting in drought tolerance in the host plant. This may be of agronomic significance, particularly in arid and semi-arid tropics where drought is not uncommon.

CHAPTER 4

NUTRITIONAL, GROWTH AND REPRODUCTIVE RESPONSES OF MAIZE TO ARBUSCULAR MYCORRHIZAL INOCULATION DURING AND AFTER DROUGHT STRESS AT TASSELLING

This chapter is a reproduction of a paper published in *Mycorrhiza* (7: 25-32, 1997) by K.S. Subramanian and C. Charest. This chapter fulfills the third objective of this thesis: (i) To determine the nutritional status of maize plants in order to assess whether mycorrhizal colonization enables the host plant to supply enough minerals for kernel growth under drought conditions; and (ii) To examine the changes in growth and reproductive behaviour (anthesis-silking interval, ASI) of the two tropical maize cultivars as a potential consequence of nutritional improvement related to AM association.

4.1. Introduction

In maize, grain yield reduction caused by drought ranges from 10 to 75 % depending on the severity and stage of occurrence (Bolaños et al., 1993). Drought stress coinciding with flowering delayed silking and resulted in an increase in the anthesis-silking interval (Bolaños and Edmeades, 1993a); this was usually associated with reductions in grain number and yield (Edmeades et al., 1993). Bolaños and Edmeades (1993b) observed a negative exponential relationship between grain yield and ASI when the tropical maize population “Tuxpeño sequía” was subjected to drought at flowering; grain yield declined by 90% as ASI increased from 0 to 10 days. Westgate (1994)

reported that water deficit after anthesis shortened the duration of grain filling by causing premature desiccation of the endosperm and by limiting embryo volume. Zinselmeier et al. (1995) showed an increase in the frequency of zygotic abortion in maize exposed to drought during pollination which could completely eliminate kernel set and result in considerable yield loss.

The arbuscular mycorrhizal (AM) fungal colonization improves productivity of several crops under drought conditions (Bethlenfalvay et al., 1988; Sylvia et al., 1993; Ruiz-Lozano et al., 1995). The responses to AM fungi have been attributed to enhanced uptake and translocation of the slowly diffusing nutrient ions PO_4^- , NH_4^+ , Zn^{2+} and Cu^{2+} (Nelsen, 1987; Kothari et al., 1990; Frey and Schüepp, 1993; Tobar et al., 1994b). The external hyphae of AM fungi play a vital role, especially in host plant P nutrition by exploration of a soil volume extending beyond the depletion zone around the roots by providing access to P, which is otherwise only transported by slow diffusion processes (Jakobsen, 1992). However, AM fungi may not significantly contribute to plant growth in soils of high fertility (Jeffries, 1987). The degree of AM fungal response is generally more pronounced under conditions of edapho-climatic stresses (Jeffries, 1987; Sylvia et al., 1993). Thus AM fungi, as an important factor in nutrient acquisition, may improve drought resistance under suboptimal plant growth conditions (Morgan et al., 1994).

Under drought conditions, mycorrhizal colonization promotes water relations of the host plants through stimulated plant nutrition (an indirect effect) and possibly through enhanced water uptake (Allen, 1982; Faber et al., 1991). Nelsen (1987) reported that drought tolerance of mycorrhizal onion plants was mainly due to improved P nutrition

which contributed to the healthy state of the host plant. Hardie and Leyton (1981) stressed that drought may be relieved by increased rate of root growth and more efficient extraction of water from the soil as a consequence of increased P uptake. Greater P uptake promoted root growth, which in turn enhanced the hydraulic conductivity and transpiration rate in AM soybean plants (Bethlenfalvay et al., 1988). Augé et al. (1994) obtained AM and non-AM plants of comparable size and biomass when the latter received a greater application of inorganic P fertilizer under moderate drought conditions. As with P, the external hyphae of AM fungi also enhanced the uptake of ^{15}N from soil and its transport to host plants (Frey and Schüepp, 1993). Under limited water conditions, when root NO_3^- uptake was restricted by impaired mass flow of the soil solution, Tobar et al. (1994a,b) found that NO_3^- transport through AM hyphae from the soil to lettuce plants resulted in enhanced shoot mass and N uptake in AM plants. AM inoculation has also been shown to enhance uptake of K and Mg (Hall et al., 1977; Hall, 1978; Azcón and Ocampo, 1981), Ca (Pai et al., 1994), and Cu and Mn (Sylvia et al., 1993), and this may indirectly have an impact on drought resistance of the host plant. Kothari et al. (1990) showed that rates of water uptake per unit root length and per unit time by AM maize plants were about twice that of non-AM plants and attributed this to hyphal transport. However, others reported that improvement of water relations of AM plants under drought conditions was unlikely, due to the greater carbon cost and reduced hydraulic conductivity (Graham et al., 1987).

We hypothesized that inoculation of maize with AM fungi would improve the host plant nutritional status and thus plant growth, which may alter the reproductive

behaviour and yield of maize cultivars sensitive to drought. To test this, we determined the effects on nutrient uptake, shoot mass, grain yield, days to anthesis and days to silking in mycorrhizal and non-mycorrhizal plants of drought-sensitive (C0) and -resistant (C8) maize cultivars exposed to three weeks of withheld irrigation following tasselling.

4.2. Materials and Methods

4.2.1. Plant growth conditions

The details of growth conditions and treatments were presented in Chapter 2.2.1.

4.2.2. Nutrient analysis

Shoots (after three weeks of drought stress and at harvest) and grains were sampled for nutrient analysis. Tissues were dried at 70°C, weighed, and digested in a sealed chamber method (Anderson and Henderson, 1986). Briefly, 200 mg of powdered tissue was placed into a glass centrifuge tube, 1-2 ml of a 7:3 (v:v) mixture of HClO₃ and H₂O₂ was added and the tube was tightly capped. After 2 h or overnight predigestion at ambient temperature, 1 ml of H₂O₂ was added and the tube was again tightly sealed and placed under the fumehood on a hot plate for 10-30 min until the acid extract turned to colorless. The digested samples were diluted to 25 ml with d H₂O. All the minerals except nitrogen were determined with an inductively coupled argon plasma spectrophotometer (Model 9000, Thermal Jarrel Ash, Waltham, Mass., USA). The N content was estimated (Sivasankar and Oaks, 1995) using an Elemental Analyzer (Perkin Elmer Series II 2400, USA). The term “nutrient content” refers to the total quantity of nutrients present in the shoot or grain masses.

4.2.3. Shoot mass, grain yield and harvest index

At the end of three weeks of drought stress and at harvest, shoots (stem, leaves, and tassel) and grains were dried at 70°C for 48 h. The ratio of grain yield to shoot mass is termed harvest index (HI). Mycorrhizal dependency (MD) or response to mycorrhizal colonization was calculated using the following formula (Plenchette et al., 1983):

$$\text{MD (\%)} = \frac{\text{Grain yield (M+)} - \text{Grain yield (M-)}}{\text{Grain yield (M+)}} \times 100$$

4.2.4. Reproductive behaviour

During the three weeks of drought stress, the day of emergence of male (tasselling) and female (silking) inflorescences was noted. A plant was considered to have flowered or silked if at least one extruded anther or one strand of silk was visible. The difference in days between anthesis and silking is referred to as anthesis-silking interval, ASI (Edmeades et al., 1993).

4.2.5. Statistical analysis

A three-way analysis of variance (ANOVA) was applied (SAS Institute Inc, 1989) to all the data, which was also examined using Tukey's Studentized Range (HSD) test. The data for harvest index was log-transformed prior to statistical analysis.

4.3. Results

4.3.1. Nutrient content

The drought treatment at tasselling appeared to decrease contents of N, P, K, Ca and Cu in the shoots of M- and M+ plants of C0 and C8 (Table 4.1). However, drought-

stressed M+ plants of C0 had significantly ($P < 0.05$ or $P < 0.01$) higher contents of N, Ca, Mn and Cu than M- plants (Table 4.1). The increases in mineral content due to AM colonization in C8 in drought conditions were significant for N, Ca and Cu and there was a significant decrease in Fe. Regardless of drought treatment, Fe uptake significantly decreased in M+ plants of C8.

Even after five weeks of recovery, drought had a significant ($P < 0.05$ or $P < 0.01$) negative effect on shoot for most of the minerals analyzed (Table 4.2). According to ANOVA, mycorrhizae had a significant effect by increasing nutrient content, except for Cu and Zn. During this period of recovery, AM fungi significantly increased the shoot contents of N, Cu and Zn in C0, and N, P and S in C8.

Cultivar, drought treatment and mycorrhizal colonization had significant effects on the grain contents of most of the minerals examined (Table 4.3). The drought treatment in general lowered the grain nutrient contents of M+ and M- plants. However, in drought conditions, significantly higher nutrient contents were found in M+ than M- plants for N, Mg and Mn in C0, and for N, P, K, Mn and Zn in C8.

4.3.2. Shoot mass

Shoot mass measured after three weeks of drought stress following tasselling was significantly ($P < 0.001$) higher in M+ than M- plants of C0 (Table 4.4; Figure 4.1). Drought stress reduced the shoot mass of M- C0 plants by 23% but only by 12% in M+ plants. A similar trend was also found at harvest (Table 4.4; Figure 4.2). Drought-stressed M+ C0 plants produced a shoot mass comparable to well-watered M- plants.

Table 4.1. Means (n=3) and standard errors (in parentheses) for nutrient content (mg/plant) in shoots of drought-sensitive and -resistant maize cultivars after three weeks of drought-stressed or well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within a column indicate significant differences ($P < 0.05$) using the Tukey's Studentized Range (HSD) test

	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn
<i>Drought-sensitive (C0) cultivar</i>										
Drought-stressed										
M+	3437 ^{cd} (119)	709 ^{ab} (77)	4589 ^{bc} (457)	2830 ^{ab} (232)	1203 ^a (68.1)	315 ^a (9.0)	62.1 ^b (2.8)	17.5 ^{ab} (0.6)	2.43 ^b (0.12)	307 ^{abc} (40.6)
M-	2649 ^f (268)	492 ^b (9.9)	3507 ^c (644)	1795 ^c (271)	953 ^a (140)	302 ^a (45.3)	58.7 ^b (4.7)	12.7 ^a (1.4)	1.83 ^c (0.24)	184 ^c (33.5)
Well-watered										
M+	4435 ^{bc} (89)	964 ^a (62)	6162 ^{ab} (539)	3523 ^a (161)	1397 ^a (114)	406 ^a (71.7)	54.8 ^b (3.9)	20.6 ^{ab} (2.6)	3.59 ^a (0.23)	420 ^a (40.5)
M-	3954 ^{cd} (43)	647 ^{ab} (56.8)	5223 ^{abc} (247)	3150 ^{ab} (282)	1250 ^a (145)	315 ^a (28.3)	90.0 ^{ab} (15.5)	14.0 ^{cd} (0.5)	1.86 ^c (0.04)	243 ^{bc} (12.8)
<i>Drought-resistant (C8) cultivar</i>										
Drought-stressed										
M+	4875 ^{ab} (127)	967 ^a (129)	5732 ^{ab} (272)	2904 ^{ab} (147)	1338 ^a (12.5)	365 ^a (42.3)	62.4 ^b (6.4)	22.6 ^a (0.5)	2.71 ^b (0.23)	306 ^{abc} (32.2)
M-	3038 ^{ef} (192)	736 ^{ab} (102)	4089 ^{bc} (232)	2154 ^c (187)	1343 ^a (253)	310 ^a (21.9)	147 ^a (20.4)	20.4 ^{ab} (1.4)	1.91 ^c (0.19)	310 ^{abc} (38.2)
Well-watered										
M+	5571 ^a (199)	1194 ^a (338)	6414 ^{ab} (359)	3572 ^a (415)	1242 ^a (160)	367 ^a (35.4)	66.9 ^b (8.8)	25.8 ^a (0.6)	2.60 ^b (0.05)	339 ^{ab} (25.9)
M-	4889 ^{ab} (210)	778 ^{ab} (47)	7197 ^a (768)	4113 ^a (182)	1204 ^a (75.6)	376 ^a (43.1)	144 ^a (26.7)	19.3 ^{ab} (2.0)	2.67 ^b (0.23)	395 ^{ab} (36.1)
ANOVA : C (cultivars), S (drought treatment), M (mycorrhizal treatment)										
C	***	*	**	*	NS	NS	**	**	**	*
S	***	**	***	***	NS	NS	NS	NS	***	**
M	***	**	**	*	NS	NS	***	*	**	*
C X S	NS	NS	NS	NS	NS	NS	NS	**	*	NS
C X M	*	NS	NS	NS	NS	NS	**	**	NS	**
S X M	**	NS	*	**	NS	NS	NS	NS	NS	NS
C X S X M*	NS	NS	NS	NS	NS	NS	*	*	*	NS

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS not significant

Table 4.2. Means (n = 3) and standard errors (in parentheses) for nutrient content (mg/plant) in shoots of drought-sensitive and -resistant maize cultivars at the harvest stage after drought-stressed or well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within a column indicate significant differences ($P < 0.05$) using the Tukey's Studentized Range (HSD) test

	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn
<i>Drought-sensitive (C0) cultivar</i>										
Drought-stressed										
M+	4340 ^{bc} (315)	803 ^{ab} (38)	5476 ^{ab} (295)	1840 ^{cd} (137)	1735 ^{ab} (215)	383 ^b (100) _b	129 ^b (21)	27.1 ^b (4.8)	3.18 ^b (0.25)	152 ^b (11)
M-	3085 ^d (152)	633 ^{ab} (107)	3584 ^b (877)	1354 ^d (159)	1496 ^b (83)	364 ^b (25)	127 ^b (3)	27.6 ^b (2.2)	2.47 ^c (0.20)	55.9 ^c (8.1)
Well-watered										
M+	5578 ^{ab} (418)	910 ^a (54)	6563 ^a (895)	3763 ^{abc} (831)	1250 ^a (45)	572 ^{ab} (14)	228 ^a (4)	46.0 ^a (2.8)	4.19 ^{bc} (0.62)	363 ^a (48)
M-	4995 ^{ab} (381)	882 ^a (63)	5678 ^{ab} (1087)	2147 ^{bcd} (349)	871 ^{ab} (166)	491 ^{ab} (67)	119 ^b (9)	41.5 ^{ab} (4.8)	4.97 ^a (0.52)	180 ^b (18)
<i>Drought-resistant (C8) cultivar</i>										
Drought-stressed										
M+	4547 ^{bc} (255)	892 ^a (100)	5978 ^{ab} (429)	2772 ^{ad} (528)	1876 ^a (64)	642 ^a (17)	110 ^b (12)	31.5 ^{ab} (3.9)	2.75 ^{bc} (0.10)	54.5 ^c (7)
M-	3223 ^d (212)	489 ^b (78)	5052 ^{ab} (136)	1543 ^d (140)	1734 ^{ab} (73)	343 ^b (15)	232 ^a (14)	26.6 ^b (1.9)	2.50 ^{bc} (0.09)	47.5 ^c (6.6)
Well-watered										
M+	5944 ^a (287)	889 ^a (67)	7735 ^a (786)	4509 ^a (393)	2270 ^a (58)	563 ^{ab} (23.1)	242 ^a (14)	40.1 ^{ab} (3.1)	3.50 ^{abc} (0.22)	157 ^b (15)
M-	4002 ^{bc} (218)	748 ^{ab} (56)	6567 ^a (538)	4219 ^{ab} (626)	2050 ^a (127)	529 ^{ab} (25)	141 ^b (18)	30.1 ^{ab} (1.7)	2.94 ^{bc} (0.31)	456 ^a (18)
ANOVA : C (cultivar), S (drought treatment), M (mycorrhizal treatment)										
C	NS	NS	*	**	*	*	**	NS	**	*
S	***	***	***	***	***	**	**	***	***	***
M	***	*	**	**	**	**	*	*	NS	NS
C X S	NS	NS	NS	NS	NS	NS	NS	*	*	**
C X M	NS	*	NS	NS	NS	NS	**	NS	NS	***
S X M	NS	NS	NS	NS	NS	NS	***	NS	NS	**
C X S X M	NS	NS	NS	NS	NS	*	**	NS	NS	***

* $P < 0.05$; ** $P < 0.01$; $P < 0.001$; NS not significant

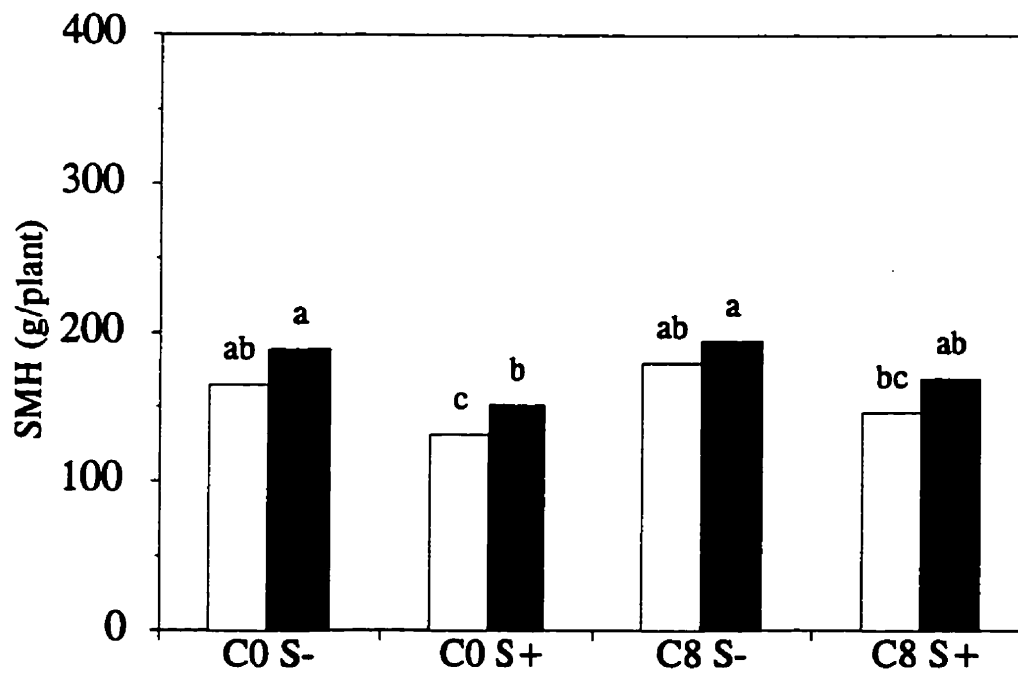
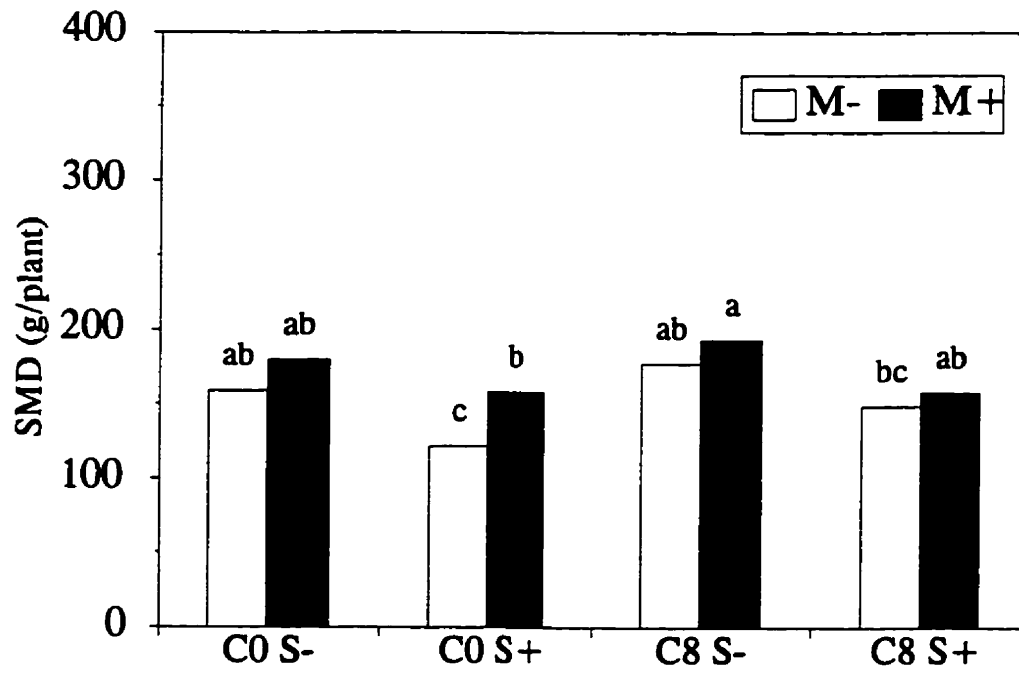
Table 4.3. Means ($n = 3$) and standard errors (in parentheses) for nutrient content (mg/plant) in grains of drought-sensitive and -resistant maize cultivars at the harvest stage after drought-stressed or well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within a column indicate significant differences ($P < 0.05$) using Tukey's Studentized Range (HSD) test

	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn
<i>Drought-sensitive (C0) cultivar</i>										
Drought-stressed										
M+	1589 ^a (58)	263 ^{cd} (26.0)	464 ^b (47.2)	484 ^{cd} (102)	280 ^b (8.5)	92.1 ^{bc} (9.5)	8.62 ^{bc} (1.09)	4.10 ^{bc} (0.17)	0.57 ^{cd} (0.02)	44.1 ^c (5.7)
M-	688 ^f (48)	169 ^d (23)	331 ^b (2)	347 ^d (24.3)	159 ^c (21.1)	60.5 ^c (12.1)	5.20 ^c (0.79)	2.09 ^{cd} (0.22)	0.31 ^d (0.07)	47.7 ^d (0.4)
Well-watered										
M+	2108 ^{cd} (134)	472 ^a (36)	671 ^a (110)	1591 ^a (71.0)	433 ^a (49.9)	176 ^a (9.2)	19.8 ^a (0.30)	6.34 ^a (0.38)	0.70 ^a (0.18)	40.9 ^{cd} (4.3)
M-	1720 ^{de} (45.5)	427 ^{ab} (64)	739 ^a (37.9)	985 ^b (48.5)	489 ^a (18.6)	125 ^{ab} (11.5)	17.8 ^a (3.57)	5.01 ^{ab} (0.40)	0.51 ^{cd} (0.04)	102 ^a (2.3)
<i>Drought-resistant (C8) cultivar</i>										
Drought-stressed										
M+	2673 ^b (41)	427 ^{ab} (47)	894 ^a (27.7) _b	721 ^{bc} (78.5)	229 ^{bc} (10.4)	160 ^a (13.9)	8.19 ^{bc} (0.94)	1.76 ^f (0.13)	0.34 ^{cd} (0.01)	56.4 ^{bc} (4.6)
M-	1583 ^a (135)	244 ^{cd} (32)	475 ^a (23.3)	432 ^{cd} (38.9)	246 ^{bc} (15.5)	135 ^{ab} (14.8)	12.7 ^{ab} (0.72)	2.73 ^{cd} (0.19)	0.59 ^{cd} (0.03)	26.5 ^d (3.1)
Well-watered										
M+	3604 ^a (163)	391 ^{abc} (24.5)	836 ^a (13.9)	1659 ^a (229)	559 ^a (53.1)	190 ^a (24.4)	18.9 ^a (1.36)	3.69 ^c (0.14)	1.06 ^a (0.11)	75.7 ^b (4.70)
M-	2532 ^{bc} (43)	509 ^a (18)	905 ^a (54.0)	830 ^{bc} (92.4)	414 ^a (22.2)	189 ^a (13.4)	20.0 ^a (0.68)	1.63 ^f (0.08)	0.85 ^{ab} (0.09)	37.2 ^b (2.8)
ANOVA : C (cultivar), S (drought treatment), M (mycorrhizal treatment)										
C	***	*	***	NS	NS	***	*	***	**	*
S	***	***	***	***	***	***	***	***	***	***
M	***	*	***	***	*	*	NS	***	NS	NS
C X S	NS	*	**	NS	NS	NS	NS	***	*	NS
C X M	**	NS	NS	NS	NS	NS	*	**	*	***
S X M	*	**	**	**	NS	NS	NS	**	NS	***
C X S X MNS	*	**	**	NS	***	NS	NS	***	*	***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS not significant

Figure 4.1. Shoot mass ($n = 4$) after drought (SMD) of maize cultivars C0 and C8 under well-watered (S-) or drought-stressed (S+) conditions with (filled bars) or without (empty bars) arbuscular mycorrhizal (AM) colonization . Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

Figure 4.2. Shoot mass ($n = 4$) at harvest (SMH) of maize cultivars C0 and C8 under well-watered (S-) or drought-stressed (S+) conditions with (filled bars) or without (empty bars) AM colonization. Means with different letters are significantly different according to Tukey's test at ($P < 0.05$).



4.3.3. Grain yield

The effect of AM colonization was only significant in drought-stressed C0 plants (Table 4.4; Figure 4.3). Drought stress decreased the final grain yield of C0 by 55 % compared to well-watered M- plants, and the reduction was only by 31 % in the presence of AM colonization. After the drought treatment, AM C0 plants had a grain yield comparable to that of C8 plants with or without AM colonization. The mycorrhizal dependencies of grain yield in C0 and C8 were 14.7% and 8.0% under well-watered and 42.9% and 14.4% under drought-stressed conditions, respectively.

4.3.4. Harvest index

The drought treatment significantly ($P < 0.05$) decreased the HI values (Table 4.4; Fig. 4.4). The HI values were significantly higher in M+ than M- plants of C0 under drought conditions. The HI values for drought-stressed AM plants of C0 were comparable to those for drought-stressed C8 plants, either with or without AM inoculation.

4.3.5. Reproductive behaviour

The days to tassel emergence (DTE), days to silk emergence (DSE) and anthesis-silking interval (ASI) were significantly ($P < 0.001$) lower for the drought-resistant (C8) than the drought-sensitive (C0) cultivars (Tables 4.4 & 4.5). However, drought stress had little impact on DTE (Table 4.5) as the plants were only exposed at the beginning of drought treatment. The lowest DTE value was obtained for the well-watered M+ plants of C8. The AM colonization significantly reduced the DSE of C0 under both conditions (Table 4.5). The DSE values of M+ plants of C0 were significantly lower than M- plants

Figure 4.3. Grain yields ($n = 4$) of maize cultivars C0 and C8 under well-watered (S-) or drought-stressed (S+) conditions with (filled bars) or without (empty bars) AM colonization. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

Figure 4.4. Harvest index ($n = 4$) of maize cultivars C0 and C8 under well-watered (S-) or drought-stressed (S+) conditions with (filled bars) or without (empty bars) AM colonization. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

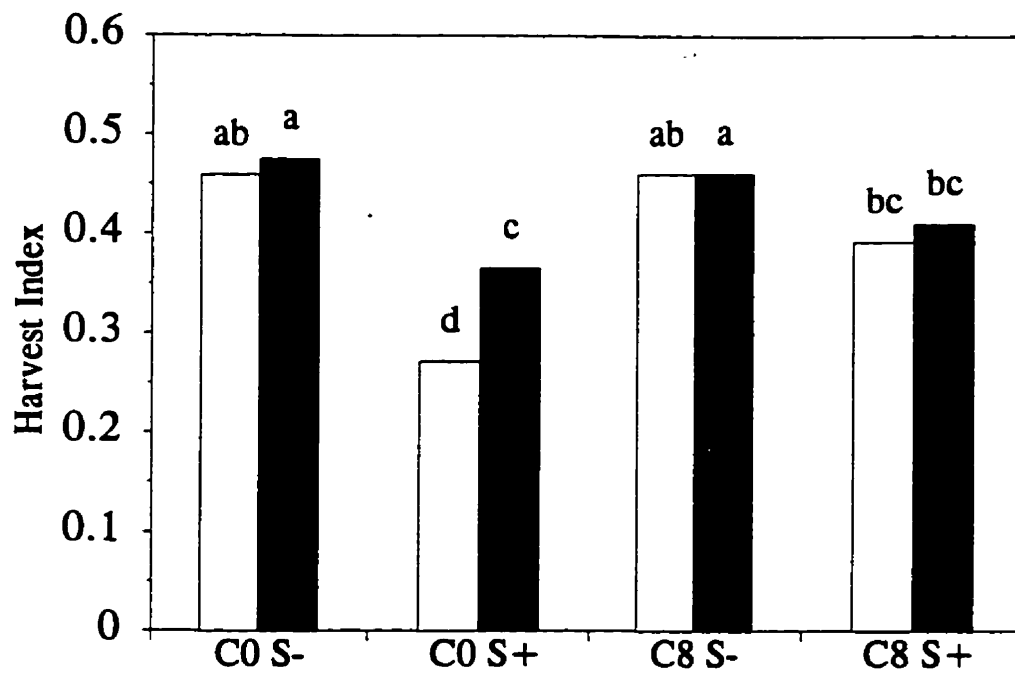
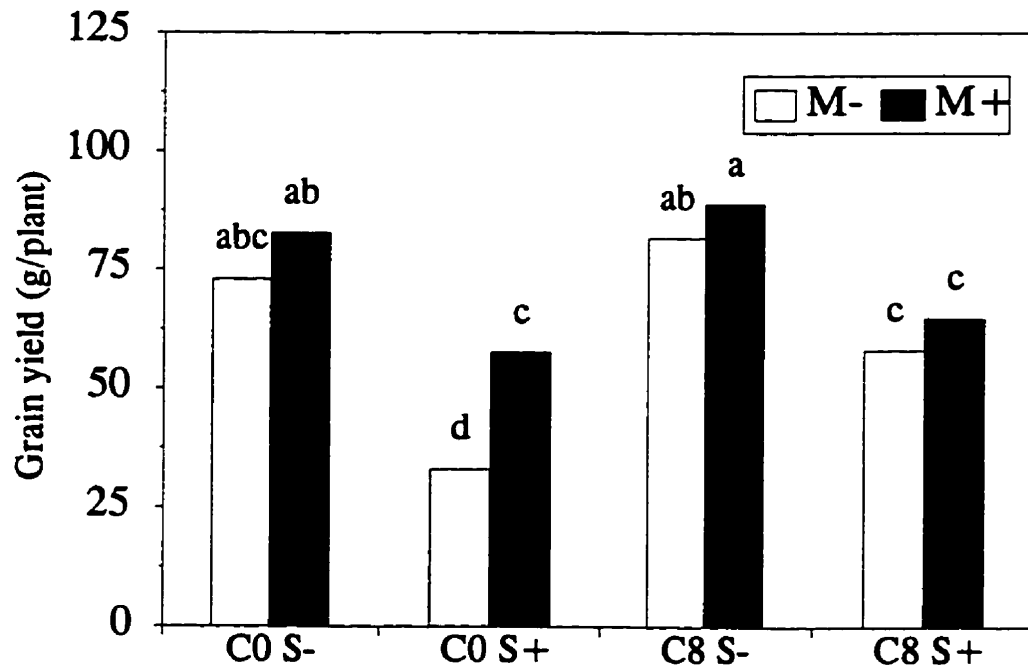


Table 4.4. Levels for ANOVA for shoot mass after drought (SMD) and at harvest (SMH), grains (GRN), harvest index (HI), days to tassel emergence (DTE), silk emergence (DSE), and anthesis-silking interval (ASI)

	SMD	SMH	GRN	HI	DTE	DSE	ASI
Cultivars (C)	*	*	***	***	***	***	***
Stress (S)	***	***	***	***	NS	NS	NS
Mycorrhizae (M)	***	***	***	***	***	***	***
C X S	NS	NS	NS	***	NS	NS	NS
C X M	NS	NS	NS	*	*	NS	NS
S X M	NS	NS	NS	*	NS	NS	NS
C X S X M	NS	NS	NS	NS	NS	NS	NS

* $P < 0.05$; *** $P < 0.001$; NS not significant

Table 4.5. Means ($n = 4$) and standard errors (in parentheses) of DTE, DSE, and ASI for drought-sensitive and -resistant maize cultivars after drought-stressed or well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within a column indicate significant differences ($P < 0.05$) using Tukey's Studentized Range (HSD) test

	DTE	DSE	ASI
<i>Drought-sensitive (C0) cultivar</i>			
Drought-stressed			
M+	73.8 ^a (1.0)	85.5 ^b (0.6)	11.8 ^b (1.3)
M-	74.0 ^a (0.8)	88.0 ^a (1.8)	14.0 ^a (2.3)
Well-watered			
M+	74.0 ^a (0.8)	84.8 ^b (1.7)	10.8 ^b (1.0)
M-	74.0 ^a (1.2)	87.5 ^a (0.6)	13.5 ^a (1.3)
<i>Drought-resistant (C8) cultivar</i>			
Drought-stressed			
M+	70.5 ^{ab} (0.6)	74.3 ^c (0.5)	3.80 ^c (0.5)
M-	72.3 ^{ab} (0.5)	76.0 ^c (0.0)	3.80 ^c (0.5)
Well-watered			
M+	70.0 ^c (0.8)	73.8 ^c (1.3)	3.80 ^c (0.5)
M-	71.5 ^{bc} (0.6)	72.3 ^c (1.5)	3.80 ^c (1.5)

with or without drought treatment. As a result, ASI values of M+ C0 plants were significantly lower than those of M- plants. In comparison to C8 plants, ASI values for C0 were 3.2 and 3.8 times higher in M+ and M- plants, respectively.

4.4. Discussion

The inoculation of the two tropical maize cultivars having differential sensitivity to drought with the AM fungus, *Glomus intraradices*, had a beneficial effect on plant nutrition, growth, grain yield and reproductive behaviour during and after moderate drought stress conditions. The results of this study suggest that AM colonization improves drought tolerance of maize cultivars through the enhanced uptake of slowly diffusing mineral ions such as PO_4^- , Ca^{2+} , Cu^{2+} and Zn^{2+} . Our results agree with the findings of Sylvia et al. (1993), who reported that AM colonization with *G. fasciculatum* increased the concentrations of P and Cu in both shoots and grains of field-grown maize under increasing intensities of drought stress. According to the studies of Kothari et al. (1990) on maize and of Raju et al. (1990) on sorghum, the enhanced host plant nutrition resulting from AM colonization may be explained by a greater absorption surface area due to the extraradical mycelium or proliferated root growth.

Numerous greenhouse and field experiments have shown conclusively that plants colonized by AM fungi are much more efficient in taking up soil P than non-AM plants (Smith and Gianinazzi-Pearson, 1988; McGonigle and Miller, 1993; Augé et al., 1994; Asmah, 1995). In our study, AM colonization increased grain P content under drought conditions. Our earlier study (Subramanian et al., 1995, Chapter 2) indicated that AM

maize plants maintained higher (less negative) leaf water potentials than non-AM plants even after three weeks of drought stress. The results here agree with the findings of Nelsen and Safir (1982), who observed that improved P nutrition enabled AM onion plants to maintain higher leaf water potentials despite a more negative soil water potential. I have shown that the root colonization with AM fungi enhanced the uptake of N and P in shoots and grains under drought conditions. Tobar et al. (1994a,b) showed a direct effect of AM fungus on N acquisition by lettuce plants grown in drought-stressed soil. The increased P status of AM plants may have allowed the host plant to absorb more Zn^{2+} and Cu^{2+} (Jarrell and Beverly, 1981). Pai et al. (1994) indicated an increase in Ca uptake by AM-inoculated cowpea plants, which in turn helped the plants to withstand drought by improving host plant water relations.

In the present study, the higher shoot mass of AM plants may be related to increased nutrient content of immobile elements such as P, Cu and Zn. Medeiros et al. (1994) observed a significant positive correlation between biomass and nutrient content in sorghum. Subramanian et al. (1995) showed that AM maize plants under drought conditions had higher leaf water potentials and lower stomatal resistances indicating that the stomata of these plants remained open longer than those of non-AM plants. We previously indicated that AM plants under drought conditions retained more sugars than non-AM plants (Subramanian and Charest, 1995, Chapter 3), which is physiologically important for tolerance of drought and recovery after drought stress (Kameli and Lösel, 1993). Consequently, AM-colonized maize plants retained a 27.5% higher green leaf area

than non-AM plants, especially in the sensitive cultivar under drought conditions, and thus contributed to enhanced shoot mass (Subramanian et al. 1995; Chapter 2).

In our study, the beneficial effect of AM inoculation was more pronounced in the drought-sensitive C0 cultivar, as indicated by grain yield and mycorrhizal dependency. The increased HI values of AM plants thus suggest that significant amounts of nutrients, especially N, P, and assimilates, were translocated from the source to the sink to support kernel development and grain yield. Schussler and Westgate (1994) observed that decreasing the amount of reserve assimilates at flowering increased the vulnerability of kernel set to lowered water potential in field-grown maize plants. Zinselmeier et al. (1995) showed that the assimilate supply in water-deficit maize plants is not sufficient to newly formed zygotes, and that this leads to zygotic abortion and kernel set. With AM colonization, maize plants were able to supply nutrients and assimilates for kernel growth, thus mitigating reduction of grain yield under water-deficit conditions.

The ASI is one of the most important parameters considered during the evaluation of drought-resistant strains for water-deficit environments (Fischer et al., 1989). The observed differences here in ASI values between C0 and C8 are consistent with the study of Bolaños and Edmeades (1993b), who reported that ASI is a heritable trait which decreases as selection progresses. These authors stressed that maize yield was reduced as much as 90% as the ASI increased from 0 to 10 days. In our study, shortening of the ASI by 2 days in the mycorrhizal drought-sensitive C0 cultivar may have contributed to its higher grain yield.

In summary, AM colonization appears to improve host plant nutrition under drought conditions. The AM response was more pronounced in the drought-sensitive than -resistant cultivars. Improved plant nutrition due to AM colonization promoted plant growth, which in turn shortened the ASI of the drought-sensitive C0 cultivar, and thus produced higher grain yield under drought conditions. This study reveals that AM inoculation enhances the nutritional status of tropical maize and enables these host plants to sustain moderate drought conditions.

CHAPTER 5

EFFECT OF ARBUSCULAR MYCORRHIZAE ON DROUGHT AND RECOVERY OF MAIZE AT THE PREFLOWERING STAGE

This chapter is a reproduction of a paper published in *Canadian Journal of Botany* (75: 1582-1591, 1997) by K.S. Subramanian, C. Charest, L.M. Dwyer and R.I. Hamilton.

This chapter fulfills the fourth objective of this thesis: To determine the progression of drought stress and drought recovery of the two tropical maize cultivars at the preflowering stage in the presence or absence of arbuscular mycorrhizal colonization.

5.1. Introduction

Drought is the primary constraint to plant growth and productivity over much of the land surface (Austin, 1990). Lack of water has been a selective force on plant species to evolve certain physiological mechanisms that confer adaptation to cope with drought (Hanson and Hitz, 1982). One such physiological change is the accumulation of sugars (Kameli and Lösel, 1993, 1995; Subramanian and Charest, 1995; Chapter 3). Several authors proposed that accumulation of sugars in drought-stressed plants results from rates of photosynthesis that exceed rates of photosynthate utilization (Munns and Weir, 1981; Van Volkenburgh and Boyer, 1985; Kameli and Lösel, 1996).

The role of arbuscular mycorrhizal (AM) fungi in drought tolerance is due to complex interactions of several mechanisms such as (i) direct water uptake by the fungal mycelium (Hardie, 1985; Faber et al., 1991); (ii) enhanced leaf water potential

(Subramanian et al., 1995, Chapter 2); (iii) improved host plant nutritional status (Nelsen and Safir, 1982; Tobar et al., 1994a,b; Subramanian and Charest, 1997, Chapter 4); and (iv) altered metabolism (Subramanian and Charest, 1995, Chapter 3). Among these mechanisms, the ability of AM fungi to maintain adequate P nutrition in plants under drought conditions has been postulated as a major factor in improved drought tolerance (Fitter, 1988; Bethlenfalvay et al., 1988). In relation to improved P nutrition, AM colonization has been shown to enhance several aspects of water relations such as hydraulic conductivity (Hardie and Leyton, 1981), leaf water potential (Nelsen and Safir, 1982) and stomatal conductance (Fitter, 1988). In contrast, Davies et al. (1993) and Augé et al. (1994) found that mycorrhizal symbiosis in helping host plant drought tolerance acted independently of P nutrition. Ruiz-Lozano et al. (1995) reported that net photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency were increased during the course of mycorrhizal colonization of lettuce plants under drought conditions. Subramanian et al. (1995) showed that AM-inoculated maize plants of drought-sensitive cultivar exposed to drought stress at tasselling were able to maintain LWP well above the wilting point (-1.74 MPa) while in non-AM plants, LWP reached a critical point (-2.45 MPa). Hardie and Leyton (1981) indicated that mycorrhizal red clover plants recovered turgor more rapidly than non-AM plants when soil was rewetted.

The AM colonization is believed to stimulate the accumulation of soluble sugars of the host plant under drought conditions. Richardson et al. (1992) showed that drought-stressed AM-colonized tall fescue plants accumulate glucose and fructose in leaf sheaths at levels that could contribute significantly to osmotic adjustment. Elmi and West (1995)

reported that the presence of AM association in tall fescue (*Festuca arundinacea* Schreb.) promotes host persistence in drought-prone environments by assisting osmotic adjustment in leaf blade and tiller survival rate. In contrast, White et al. (1992) found no differences in osmotic adjustment between AM-colonized and non-colonized *Festuca* plants. Subramanian and Charest (1995) reported that AM colonization favours retention of metabolites in maize plants when subjected to drought stress at tasselling.

We hypothesized that AM colonization in maize stimulates accumulation of phosphorus and sugar contents. This may enable the host plant to enhance LWP during drought and recover rapidly when irrigation is restored. To test this, we examined LWP, leaf RWC, P and sugar contents and biomass in AM and non-AM plants of the drought-sensitive (C0) and -resistant (C8) maize cultivars when exposed to three weeks of drought followed by three weeks of recovery at the preflowering stage.

5.2. Materials and Methods

5.2.1. Plant growth conditions

This is the second greenhouse experiment carried out with a modification in the stage of drought treatment. A 2 X 2 X 2 factorial randomized block design included two cultivars (C0 & C8); two moisture regimes [well-watered (S-) & irrigation withheld (S+) for three weeks (45-65 days after sowing, DAS) followed by three weeks (66-86 DAS) of rewatering], and two mycorrhizal treatments [with (M+) or without (M-) AM inoculation]. There were eight treatment combinations replicated four times. The growth conditions maintained in the greenhouse is as described in Chapter 2.2.1. Six plants per

container were grown at the start of the experiment. From each container two plants were sampled for the root colonization studies and the other four plants remained at the beginning of the drought treatment. During the course of the experiment (45-86 DAS), volumetric soil moisture content was determined daily in all the treatments using Time Domain Reflectometry (TDR) technique (Topp et al., 1980) to assess the loss of moisture from the growing medium. Irrigation was withheld from half of the plants for three weeks starting 45 DAS. Thereafter, drought-stressed plants were rewatered for three weeks to examine the drought recovery. Two plants from each container, harvested after the drought and the recovery periods, were analyzed for sugar and P contents.

5.2.2. Root colonization studies

The AM-colonized roots were stained with the aniline blue dye (Dalpé, 1993) before mounting on slides in polyvinyl-alcohol-lactic acid-glycerol medium (Chapter 2.2.2). Samples of 100 one cm root segments per treatment were examined for the presence of arbuscules or vesicles or hyphae. The percentage of the AM-colonized segments were determined at the end of the drought (65 DAS) and the recovery (86 DAS) periods. The mycorrhizal colonization in the drought-stressed and well-watered plants were 67% and 75%, respectively, for both cultivars. After the three weeks of rewatering, colonization tended to be higher in previously C0 drought-stressed plants (89%) than C0 well-watered plants (69%) while the percentage colonization remained constant in C8 (76% and 74% for drought-stressed and well-watered, respectively).

5.2.3. Determination of soil moisture, leaf water potential and relative water content

Volumetric soil moisture content (SMC) was measured using the non-destructive TDR technique (Topp et al., 1980). The SMC is determined based on the electrical properties of soil. During the measurement, the TDR (Trase System1 Model 6050 X1, Soil Moisture Equipment Corp. CA, USA) coaxial transmission line was buried into the soil column of each container at 20 cm depth. The TDR generates a high-frequency electromagnetic pulse and sends it at the speed of light down a transmission line. The velocity of propagation of the high-frequency wave in soils is determined primarily by the water content. The wave is reflected from the open ends of the wave guides and returns along the original path. By microprocessor, the travel time of the wave is used to directly calculate the dielectric constant of the soil. From the readings, volumetric SMC was determined using a conversion table (Appendix 3). During the drought and recovery periods, the measurements were made daily at the centre of the container to compare the soil moisture depletion in M+ and M- treatments under well-watered and drought-stressed conditions. Leaf water potential was measured daily (as described in Chapter 2.2.3) on the fully expanded leaf (6th or 7th) between 10 AM and 2 PM during the six weeks of the experiment using Scholander pressure chamber (Scholander et al., 1964). The relative water content (RWC) is the water status of a plant expressed on the basis of the fully rehydrated state. The RWC was calculated weekly on the 7th or 8th leaf during the drought period using the following formula (Turner, 1986):

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where FW is leaf fresh weight., DW is leaf dry weight after 24 h drying at 70°C and TW is leaf turgid weight after submergence in distilled H₂O for 4 h.

5.2.4. Sugar and phosphorus analyses

Shoot and root samples taken at the end of the drought and recovery periods were analyzed for P and sugar contents. Sugars were determined by the classical method (Chapter 3.2.2.2) of Nelson adapted by Potvin and Charest (1991). Tissues were dried (70°C for 24 h), weighed, digested in HClO₃ : H₂O₂ (v/v 7:3) mixture for 30 min in a sealed chamber (Subramanian and Charest, 1997; Chapter 4.2.2). The digested samples were diluted to 25 ml with deionized H₂O. Phosphorus concentration was determined by a colorimetric assay using the ascorbic acid method (Walsh and Beaton, 1973). Phosphate ions in the solution complexed with molybdate ions in the ascorbic acid reagent producing a blue color. The P concentration was determined by measuring the intensity of the coloration at 820 nm. The assay mixture contained 1.0 ml diluted leaf or root extract, 1.0 ml 0.5 M NaHCO₃ pH 8.5, 0.5 ml 1 N HCl, 1.7 ml d H₂O and 0.8 ml ascorbic acid reagent. The volume of the contents were made up to 10 ml with d H₂O. Phosphorus concentration was measured after allowing the solutions to stand for 30 min. The P concentrations were detected in the test solution from a standard curve prepared with different levels of P (Appendix 4). P content reported in this thesis was calculated by multiplying P concentration with root or shoot dry masses. The coloring reagent was prepared by mixing ammonium molybdate (8 g in 250 ml d H₂O) and antimony potassium tartrate (0.2908 g in 100 ml d H₂O). Both solutions were added to a 1000 ml

HCl (431 ml concentrated HCl per litre) and the volume made up to 2000 ml with d H₂O, stored in a dark place. Ascorbic acid reagent was made daily by dissolving 1.056 g ascorbic acid in 200 ml molybdate stock solution.

5.2.5. Root and shoot dry masses

At the end of three weeks of drought and recovery periods, harvested roots and shoots were dried at 70°C for 48 h and dry masses were determined.

5.2.6. Statistical analysis

A three-way analysis of variance (ANOVA) was done (SAS Institute Inc, 1989) on the data of all parameters obtained on 7, 14 and 21 days after the drought and recovery treatments began. The data on soil moisture content and leaf relative water content were arcsin transformed prior to statistical analyses. Critical differences at the 5% level of significance were tested using Tukey's Studentized Range (HSD) test.

5.3. Results

5.3.1. Soil moisture content

Throughout the experiment, under well-watered conditions (Table 5.1), the volumetric soil moisture contents (SMCs) tended to be higher in M+ than M- treatments. During the three weeks of drought (Fig. 5.1), SMCs decreased progressively with the advancement of drought stress in M+ and M- treatments of both cultivars, but the SMC values were higher for M+ than M- treatments. Even after three weeks of continuous withholding of water, M+ treatment of both cultivars (C0, 11.2%; C8, 9.63%) maintained higher ($P < 0.001$; Table 5.3) SMCs than M- treatment (C0, 8.12%; C8, 7.75%).

Figure 5.1. Soil moisture content ($n = 4$) of mycorrhizal (solid square) and non-mycorrhizal (empty square) treatments of maize cultivars C0 (top) and C8 (bottom) during three weeks of drought (45-65 DAS). Statistical analysis was done at 7, 14 and 21 d after the drought treatment began. The data for well-watered treatment is presented in Table 5.1. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

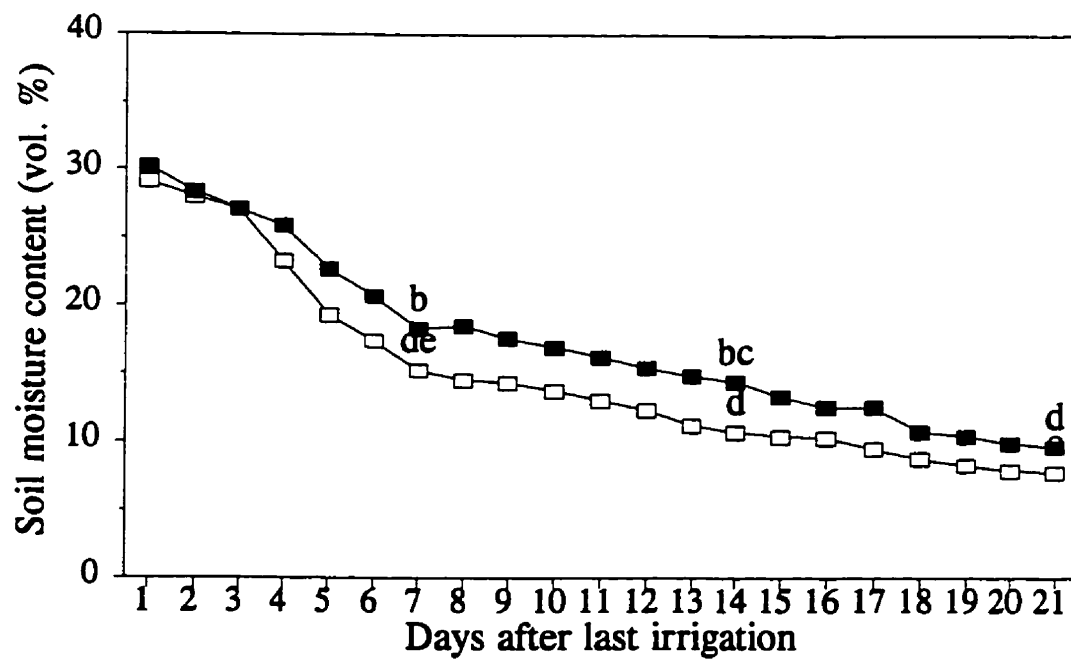
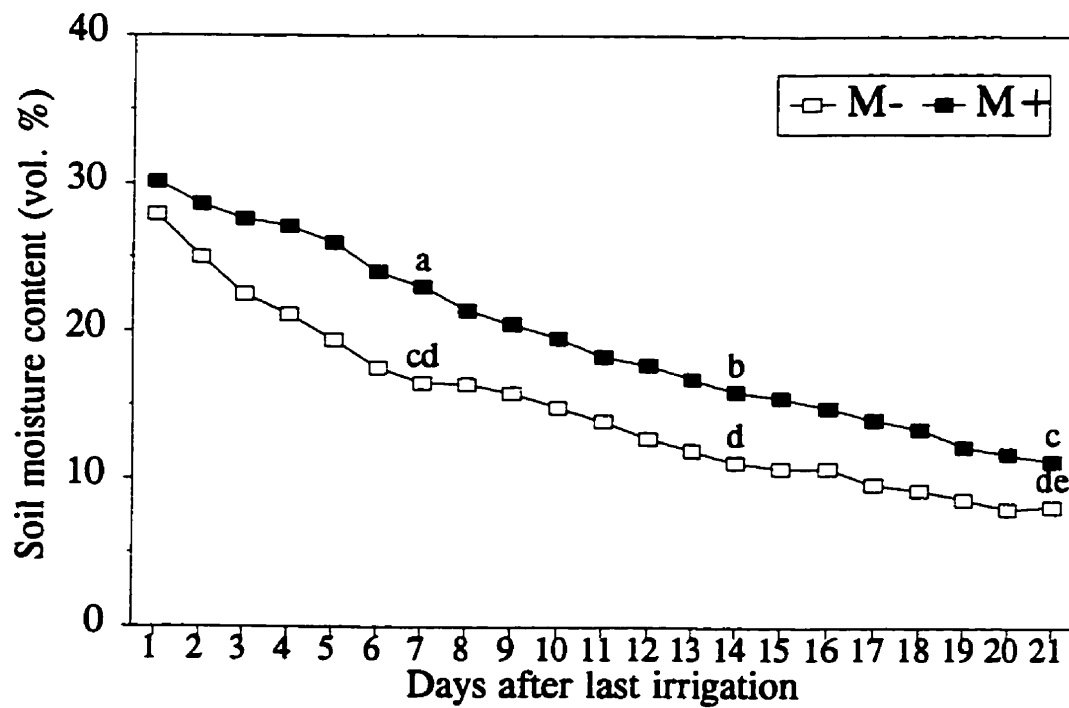


Table 5.1. Means ($n = 4$) and standard error (in parentheses) for daily soil moisture content (SMC) and weekly leaf relative water content (RWC) during the first three weeks of the experiment (45-65 DAS) in the drought-sensitive (C0) and -resistant (C8) cultivars under well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within column indicate significant differences ($P < 0.05$) using Tukey's Studentized Range (HSD) test

DAS	SMC (%)			RWC (%)		
	51	58	65	51	58	65
<i>Cultivar (C0)</i>						
M+	22.9 ^a (0.42)	19.2 ^a (1.22)	16.0 ^a (0.95)	88.5 ^a (3.71)	85.0 ^a (1.76)	85.5 ^{ab} (2.35)
M-	17.1 ^{bc} (0.69)	14.2 ^c (0.38)	16.2 ^a (0.83)	84.2 ^a (2.61)	84.0 ^a (3.36)	82.4 ^{ac} (1.69)
<i>Cultivar (C8)</i>						
M+	18.7 ^b (0.93)	17.8 ^a (0.92)	17.7 ^a (1.54)	86.5 ^a (4.21)	86.2 ^a (2.27)	81.9 ^{ac} (1.57)
M-	14.8 ^c (0.64)	14.9 ^{bc} (0.58)	13.6 ^b (0.88)	87.9 ^a (2.56)	87.3 ^a (1.74)	83.6 ^{ac} (1.94)

Note: These data were statistically analyzed altogether and respectively with the data that appear on the figures 5.1 and 5.4.

5.3.2. Leaf water potential

The LWPs of well-watered M+ and M- plants of the C8 cultivar were similar throughout the course of the experiment while C0 well-watered M+ plants had higher (less negative) LWPs than M- plants except the first and fourth weeks of the experiment (Table 5.2). The LWPs decreased significantly ($P < 0.001$) with the progression of drought stress in M- and M+ plants of both cultivars (Table 5.3; Figures 5.2 & 5.3). After three weeks of drought stress, LWP of C0 M- plants declined to near wilting (-2.32 MPa) while C0 M+ plants maintained LWP well above the wilting point (-1.34 MPa). A similar trend was observed in C8 but the mycorrhizal effect on LWP was much less pronounced than in C0. During rewatering, LWPs of previously drought-stressed M+ and M- plants of C0 and C8 increased progressively with time. As M+ plants maintained higher LWP during the drought period, on rewatering they took less time (C0, 7 d; C8, 4 d) than M- plants (C0, 15 d; C8, 8 d) to attain LWP comparable with well-watered plants.

5.3.3. Relative water content

After three weeks of drought stress, the leaf RWC (Table 5.3; Fig. 5.4) was significantly higher ($P < 0.01$) in M+ (81%) than M- (69%) plants of C0 and was comparable with well-watered plants (Table 5.1). In C8, the RWC values were relatively constant regardless of the treatments, with the exception of a decrease in drought-stressed M- plants (Fig. 5.4).

5.3.4. Sugars

Sugar contents increased significantly ($P < 0.001$) in the M+ roots of C8 under well-watered and drought-stressed conditions (Table 5.3; Fig. 5.5), but decreased in the

Figure 5.2. Leaf water potential ($n = 4$) of mycorrhizal (solid square) and non-mycorrhizal (empty square) plants of maize cultivar C0 during three weeks of drought (left) followed by three weeks of recovery (right). Statistical analysis was done at 7, 14 and 21 d after the drought and recovery treatments began. The data for well-watered plants are presented in Table 5.2. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

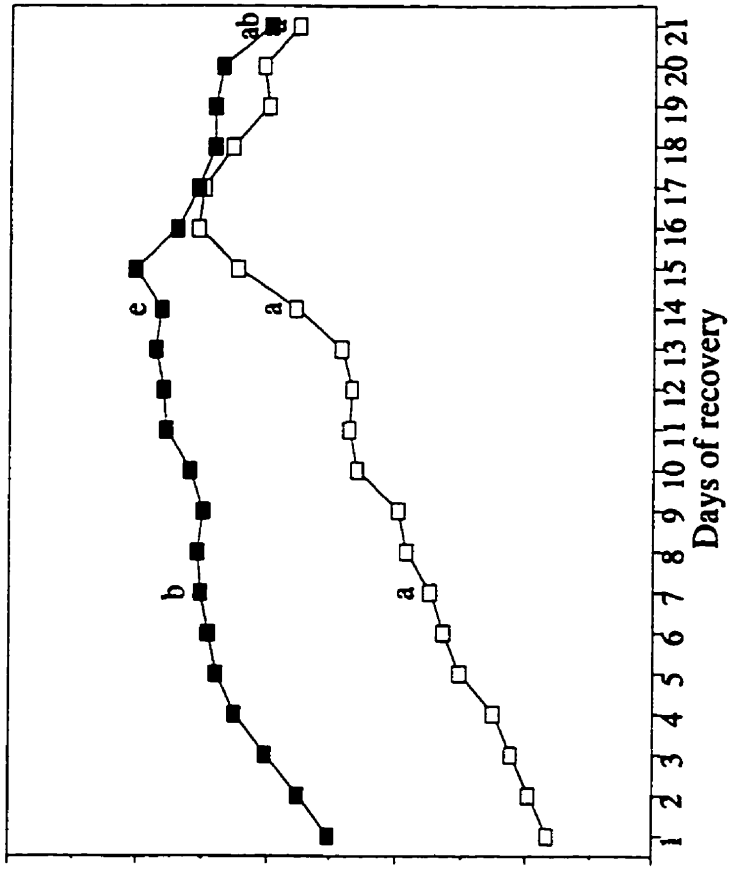
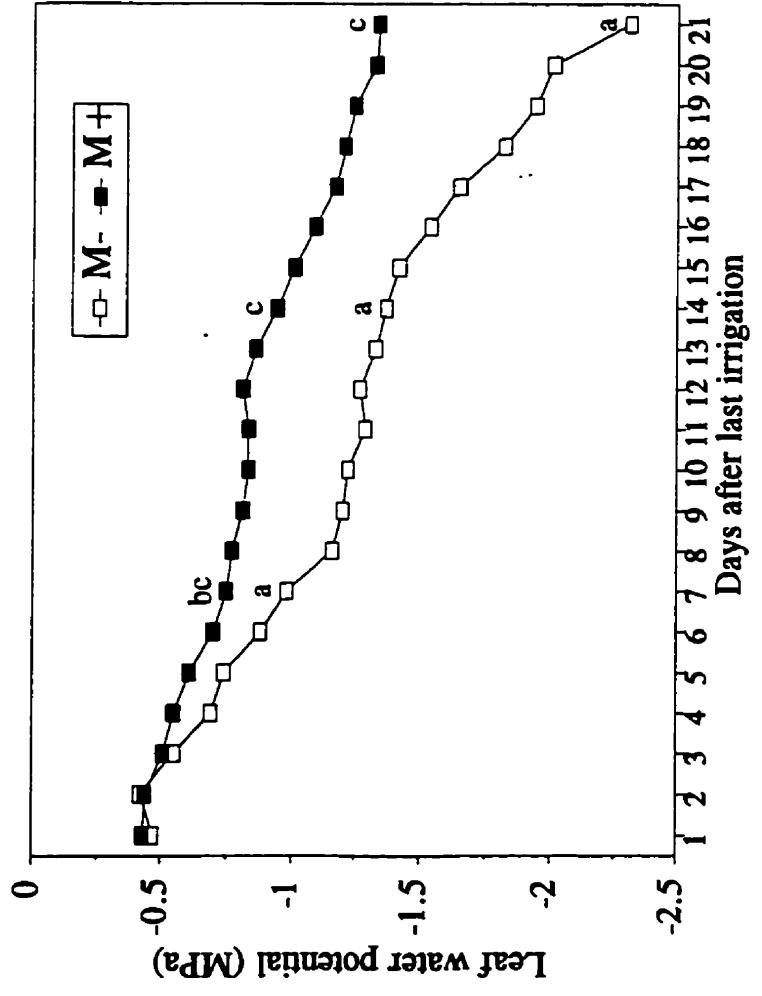


Figure 5.3. Leaf water potential ($n = 4$) of mycorrhizal (solid square) and non-mycorrhizal (empty square) plants of maize cultivar C8 during three weeks of drought (left) followed by three weeks of recovery (right). Statistical analysis was done at 7, 14 and 21 d after the drought and recovery treatments began. The data for well-watered plants are presented in Table 5.2. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

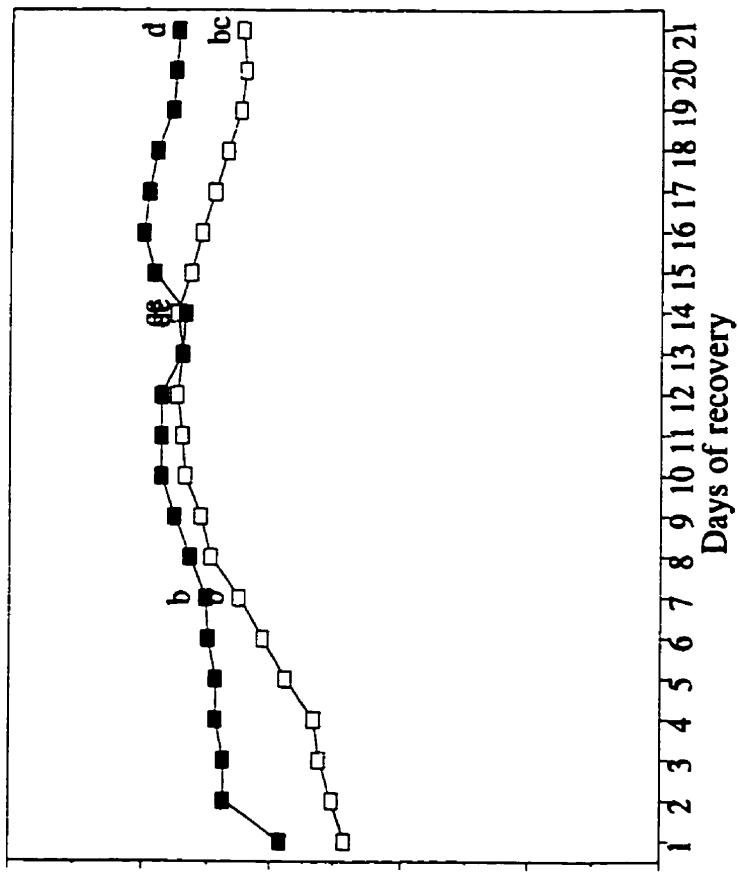
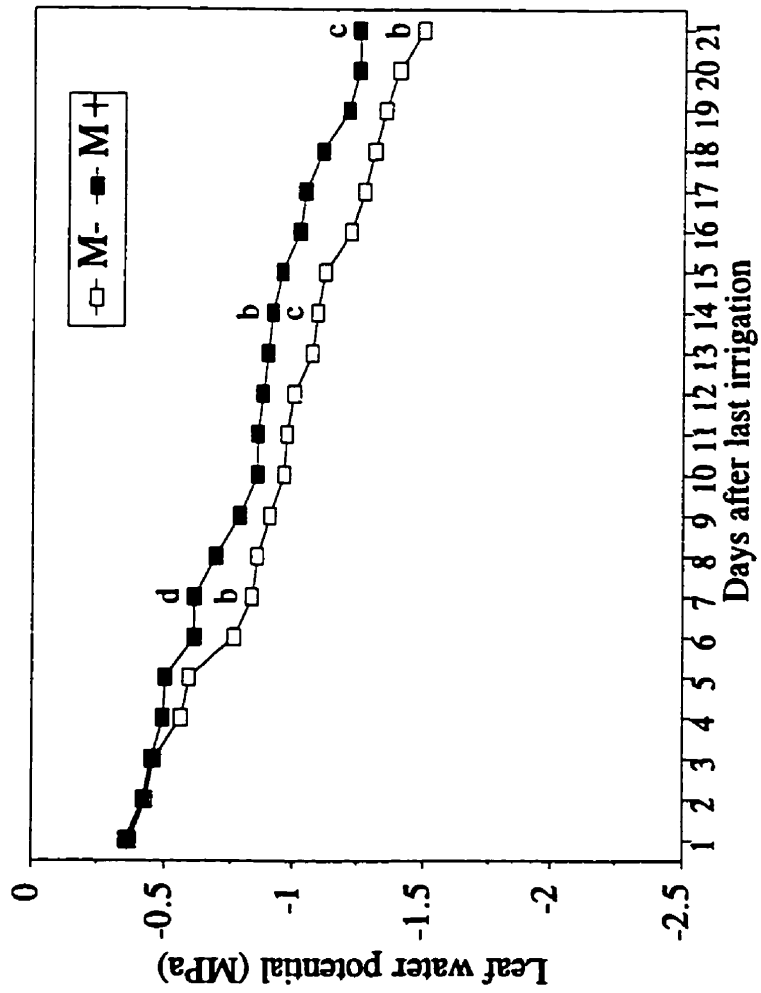


Figure 5.4. Relative water content ($n = 4$) of mycorrhizal (solid bar) and non-mycorrhizal (empty bar) plants of maize cultivars C0 (top) and C8 (bottom) during three weeks of drought. Statistical analysis was done at 7, 14 and 21 d after the drought treatment began. The data for well-watered plants are presented in Table 5.1. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

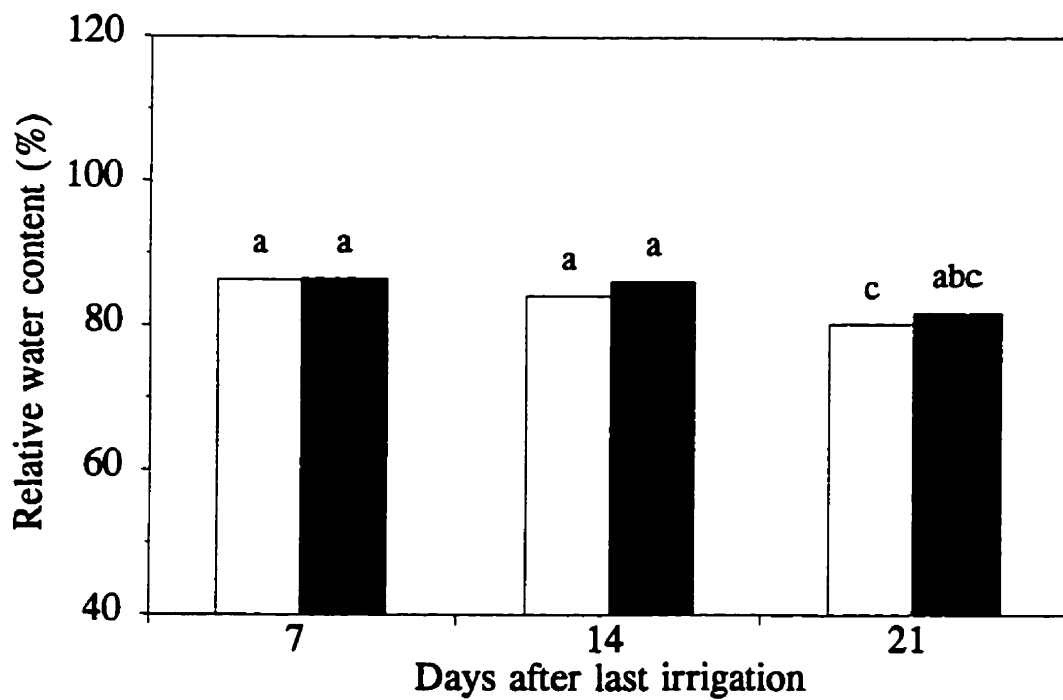
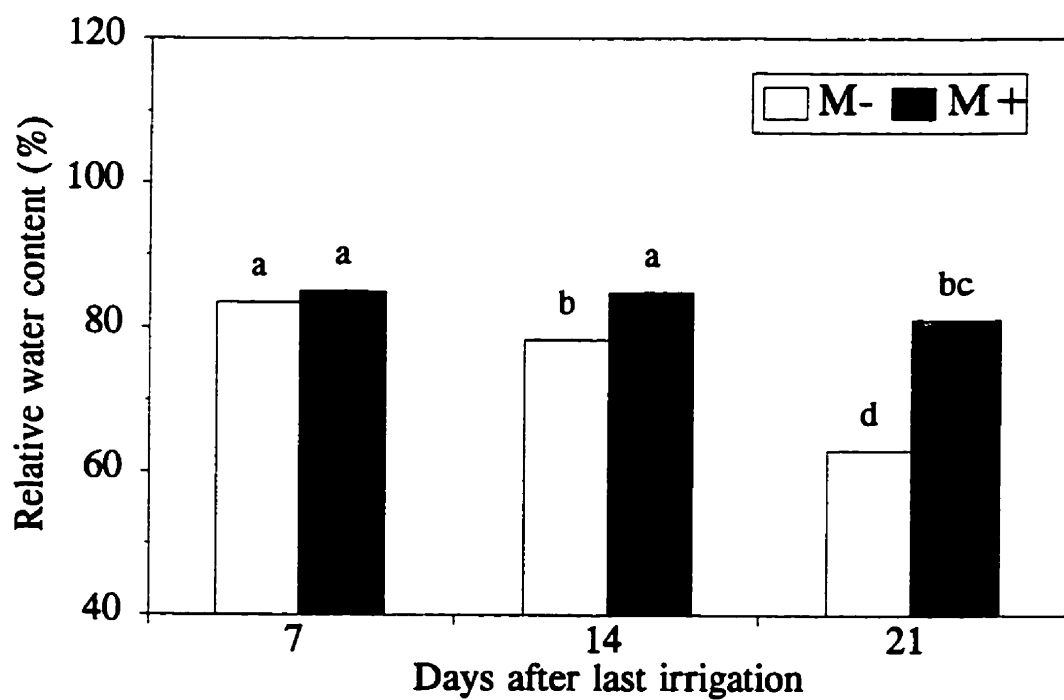


Table 5.2. Means (n = 4) and standard error (in parentheses) for daily leaf water potential (LWP) measured during six weeks of the experiment (45-86 DAS) in the drought-sensitive (C0) and -resistant (C8) cultivars under well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within column indicate significant differences (P < 0.05) using Tukey's Studentized Range (HSD) test

		LWP (MPa)					
DAS		51	58	65	72	78	86
<i>Cultivar (C0)</i>							
M+		-0.70 ^{cd} (0.01)	-0.71 ^d (0.04)	-0.72 ^f (0.02)	-0.73 ^b (0.03)	-0.77 ^c (0.04)	-0.58 ^d (0.02)
M-		-0.78 ^{bc} (0.03)	-0.87 ^c (0.03)	-0.97 ^d (0.04)	-0.78 ^b (0.04)	-0.91 ^b (0.02)	-0.82 ^c (0.04)
<i>Cultivar (C8)</i>							
M+		-0.70 ^{cd} (0.01)	-0.67 ^d (0.03)	-0.81 ^{ef} (0.04)	-0.74 ^b (0.03)	-0.62 ^{dc} (0.01)	-0.57 ^d (0.03)
M-		-0.72 ^c (0.02)	-0.68 ^d (0.03)	-0.84 ^c (0.02)	-0.84 ^b (0.05)	-0.72 ^{cd} (0.01)	-0.78 ^c (0.04)

Note: These data were statistically analyzed altogether and respectively with the data that appear on the figures 5.2 and 5.3.

Figure 5.5. Soluble sugar contents ($n = 4$) in roots (top) and shoots (bottom) of mycorrhizal (solid bar) and non-mycorrhizal (empty bar) plants of maize cultivars C0 and C8 under well-watered (S-) and drought-stressed (S+) conditions after three weeks of drought. Statistical analysis was done for roots and shoots separately. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

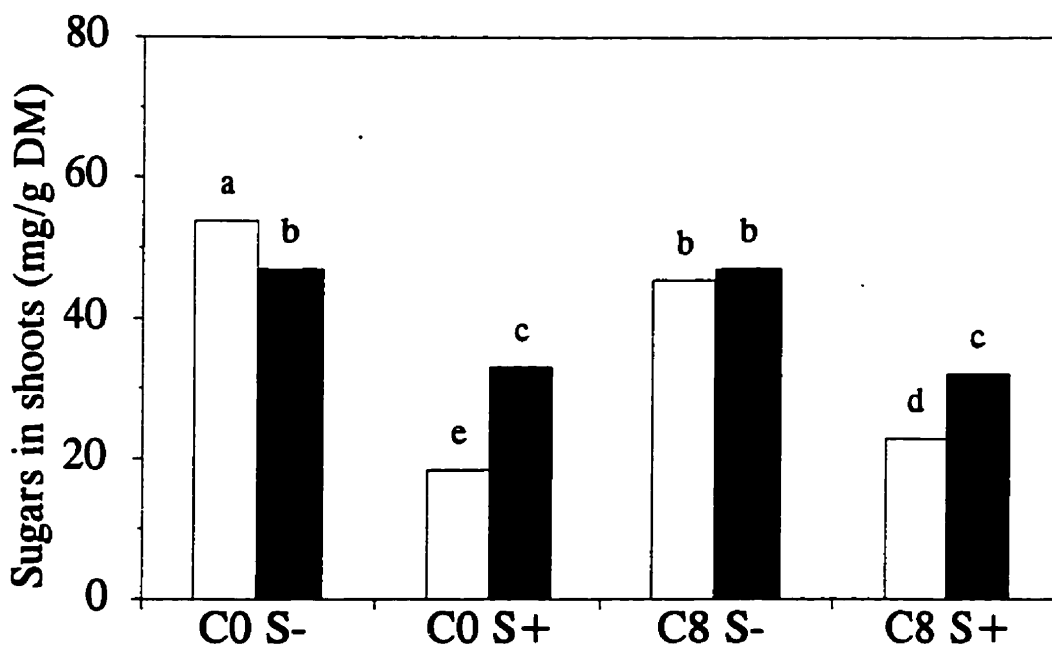
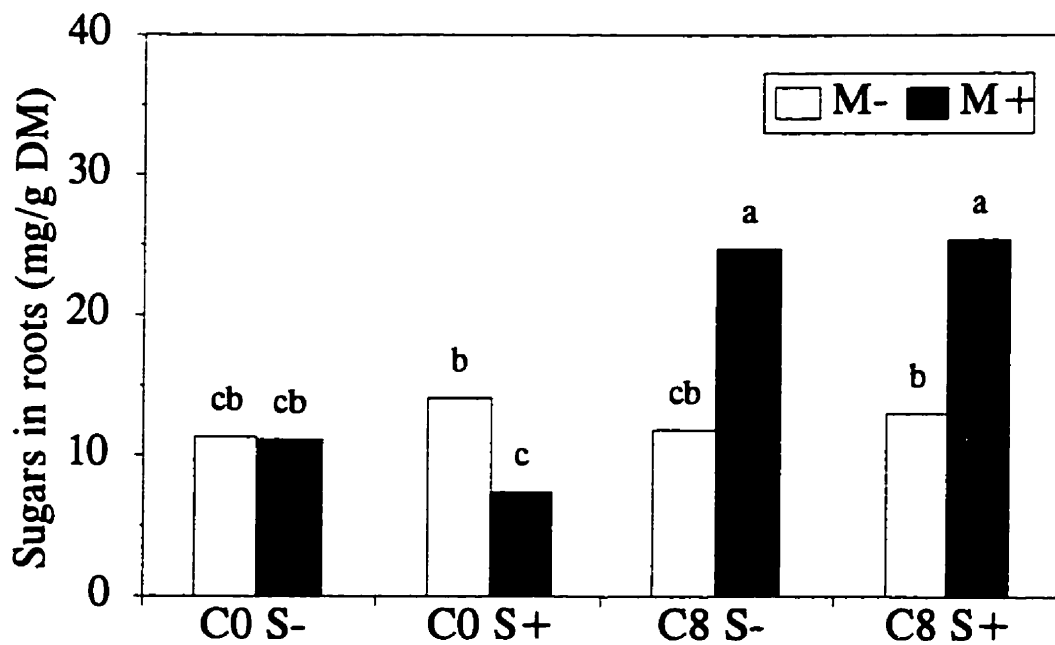


Figure 5.6. Soluble sugar contents ($n = 4$) in roots (top) and shoots (bottom) of mycorrhizal (solid bar) and non-mycorrhizal (empty bar) plants of maize cultivars C0 and C8 under well-watered (S-) and drought-stressed (S+) conditions after three weeks of recovery. Statistical analysis was done for roots and shoots separately. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

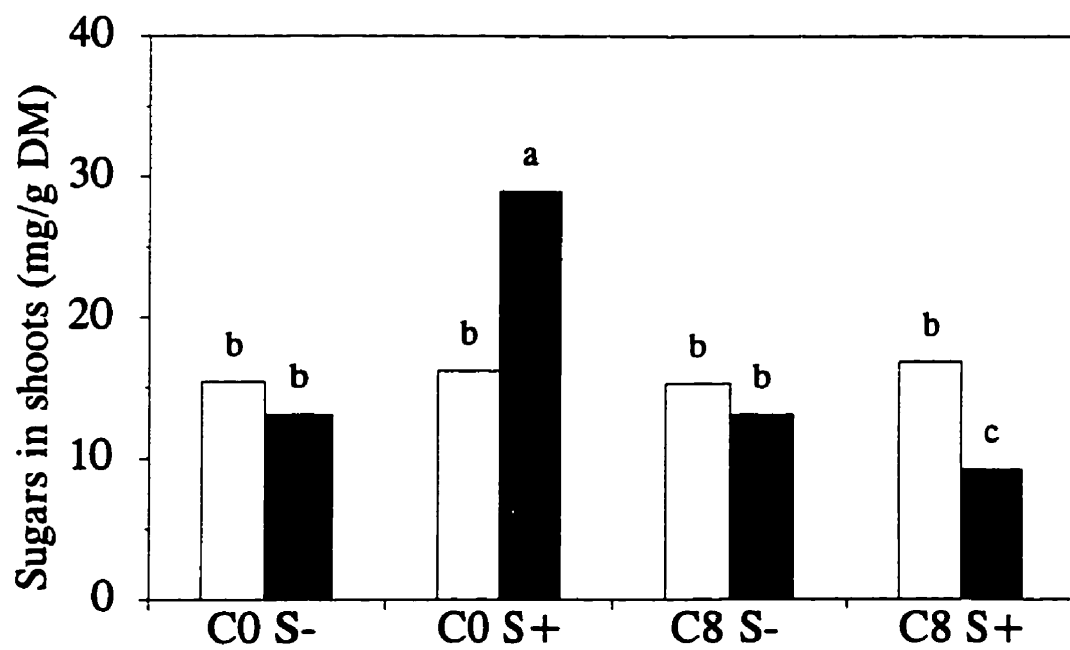
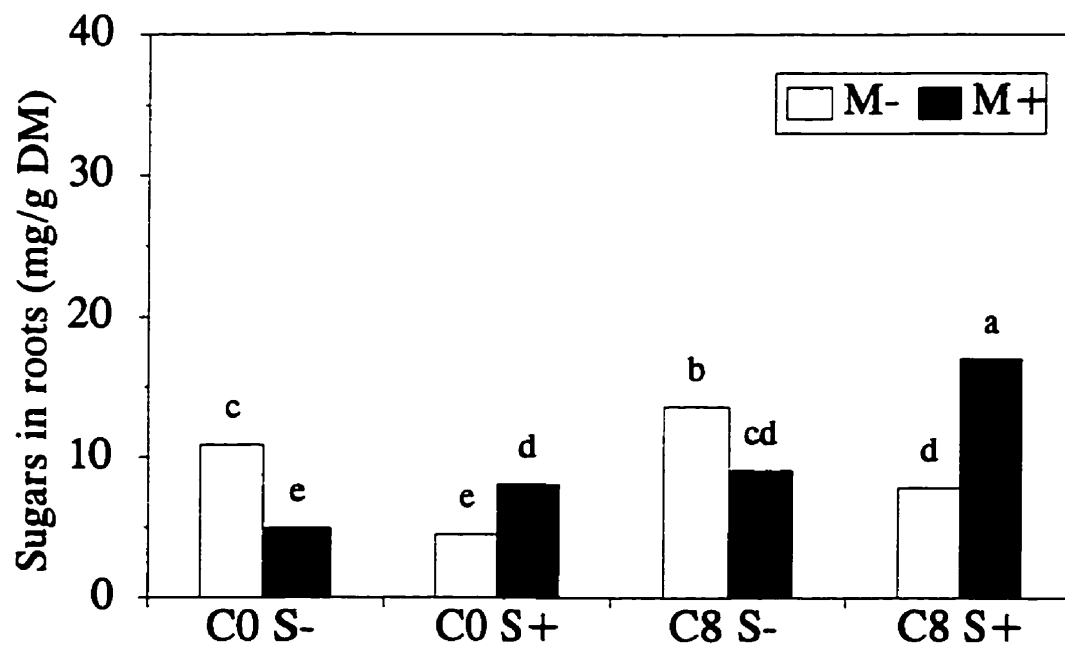


Table 5.3. Levels of significance for ANOVA for soil moisture content (SMC), leaf water potential (LWP), leaf relative water content (RWC), phosphorus status (P), total soluble sugars (TSS) and dry mass at 7, 14, 21 d of drought or recovery treatments (C cultivar; S drought treatment; M mycorrhizal treatment)

	C	S	M	C X S	C X M	S X M	C X S X M
<i>During 3 wk drought</i>							
SMC							
7 d	***	NS	***	NS	***	NS	NS
14 d	**	***	***	NS	**	NS	NS
21 d	**	***	***	NS	**	NS	***
LWP							
7 d	***	***	***	**	***	***	NS
14 d	***	***	***	NS	***	***	NS
21 d	***	***	***	***	***	***	***
RWC							
7 d	NS	NS	NS	NS	NS	NS	NS
14 d	***	**	**	NS	*	**	NS
21 d	***	***	***	**	**	NS	**
P							
Roots	***	***	**	NS	*	NS	*
Shoots	***	***	***	*	NS	*	NS
TSS							
Roots	***	NS	***	NS	***	*	NS
Shoots	NS	***	***	**	NS	***	***
Dry mass							
Roots	***	***	***	NS	NS	**	NS
Shoots	***	***	***	NS	*	**	NS
<i>During 3 wk recovery</i>							
LWP							
7 d	***	***	***	***	***	***	NS
14 d	***	NS	***	NS	***	**	NS
21 d	***	***	***	***	NS	NS	NS
P							
Roots	***	*	***	NS	*	NS	*
Shoots	***	***	***	NS	NS	NS	NS
TSS							
Roots	***	NS	NS	***	***	***	***
Shoots	***	***	**	***	***	***	***
Dry mass							
Roots	***	**	***	NS	NS	**	NS
Shoots	***	***	***	NS	NS	NS	NS

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS not significant

M+ roots of C0 under drought. In the shoots, drought significantly ($P < 0.001$) reduced the soluble sugar contents by 66% and 50% in M- plants of C0 and C8 cultivars, respectively. However, with the presence of mycorrhizae, the sugar contents decreased only by 30% and 32% in C0 and C8, respectively. Soluble sugar contents were significantly higher in M+ shoots of C0 and C8 than M- shoots under drought conditions. After rewatering (Table 5.3; Fig. 5.6), the previously drought-stressed M+ plants had significantly ($P < 0.001$) higher sugar contents in the roots and shoots but not the shoots of C8, compared to M- plants.

5.3.5. Phosphorus content

In general, drought-stressed M+ plants of both cultivars had higher P contents in shoots (C0, 74.5%; C8, 50.8%) and roots (C0, 159%; C8, 49.6%) than M- plants. The M+ C0 plants had significantly higher P contents (Table 5.3; Fig. 5.7) in roots and shoots under both well-watered and drought-stressed conditions than the M- plants. The drought-stressed M+ roots and shoots of C0 and C8 cultivars had P contents comparable with well-watered M- plants. Even after three weeks of recovery (Fig. 5.8), previously drought-stressed M+ plants, in comparison with M- plants, had significantly higher P contents in the roots and shoots of C0 and the roots of C8. In C8, P contents were significantly higher in M+ than M- shoots under well-watered conditions.

5.3.6. Root and shoot dry masses

The dry masses measured after three weeks of drought or recovery periods were significantly ($P < 0.01$) higher in M+ roots and shoots of C0 and roots of C8 than M-

Figure 5.7. Phosphorus contents ($n = 4$) in roots (top) and shoots (bottom) of mycorrhizal (solid bar) and non-mycorrhizal (empty bar) plants of maize cultivars C0 and C8 under well-watered (S-) and drought-stressed (S+) conditions after three weeks of drought. Statistical analysis was done for roots and shoots separately. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

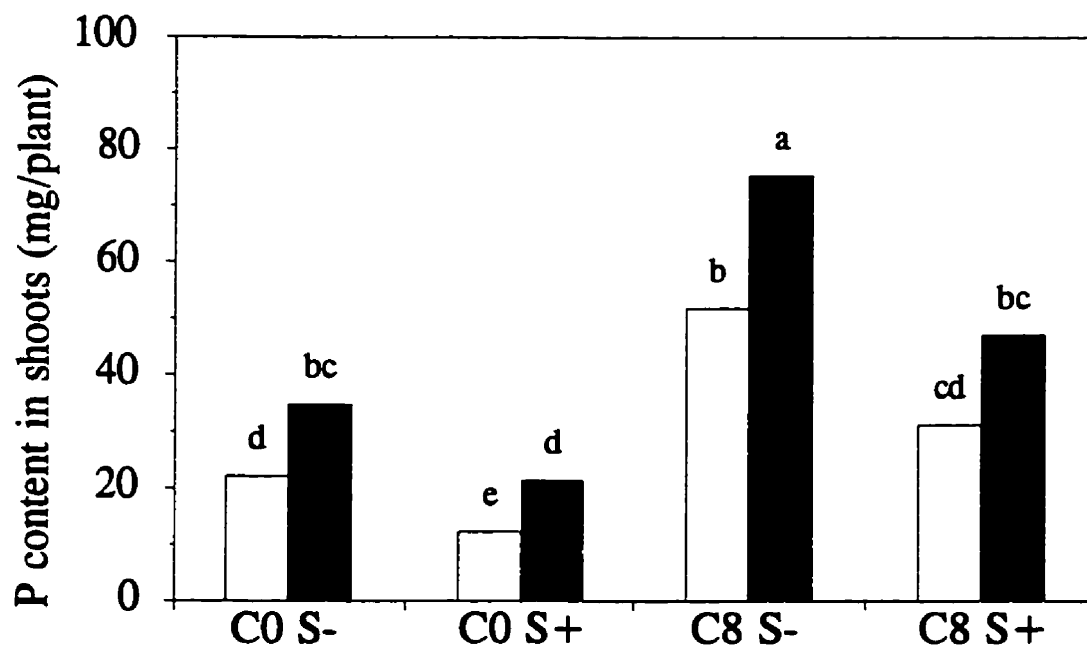
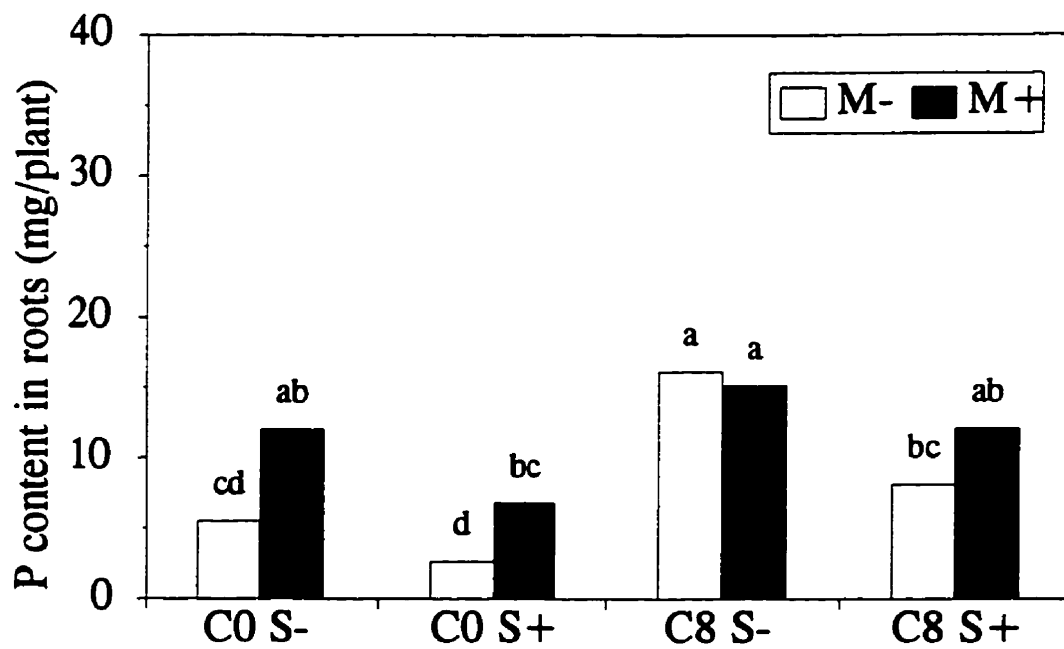


Figure 5.8. Phosphorus contents ($n = 4$) in roots (top) and shoots (bottom) of mycorrhizal (solid bar) and non-mycorrhizal (empty bar) plants of maize cultivars C0 and C8 under well-watered (S-) and drought-stressed (S+) conditions after three weeks of recovery. Statistical analysis was done for roots and shoots separately. Means with different letters are significantly different according to Tukey's test ($P < 0.05$).

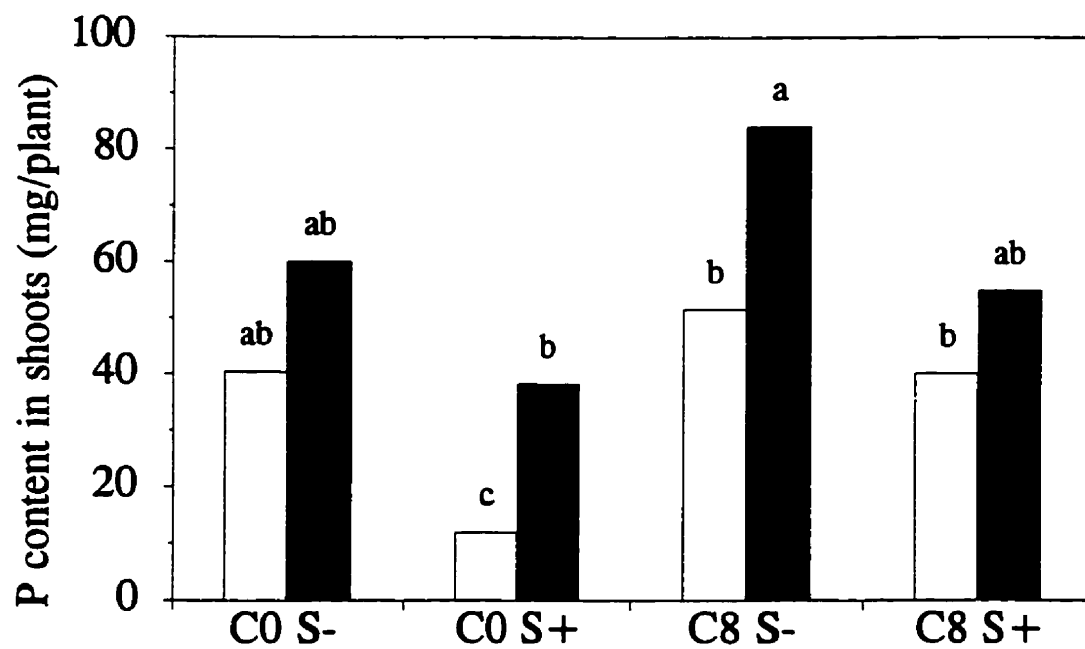
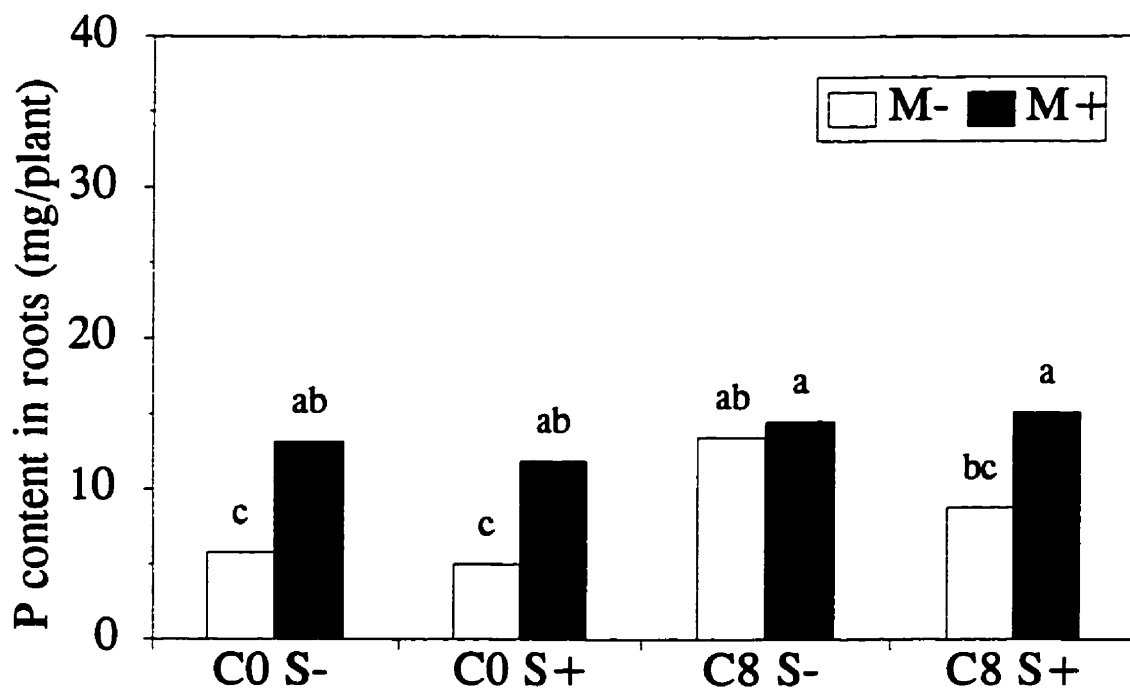
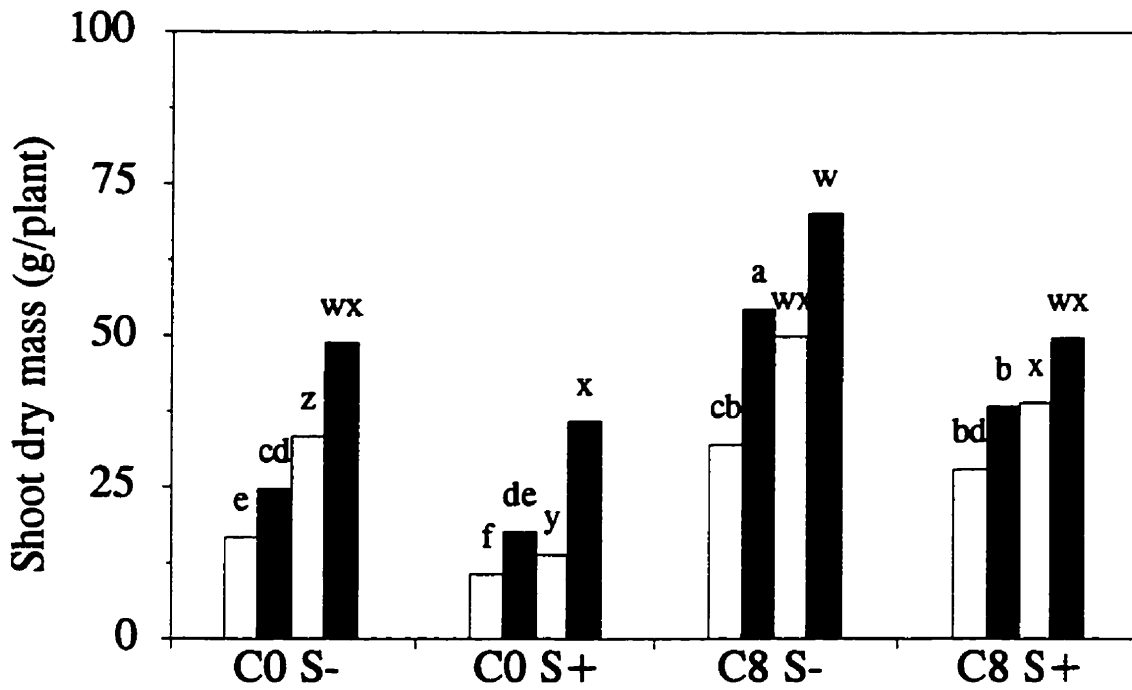
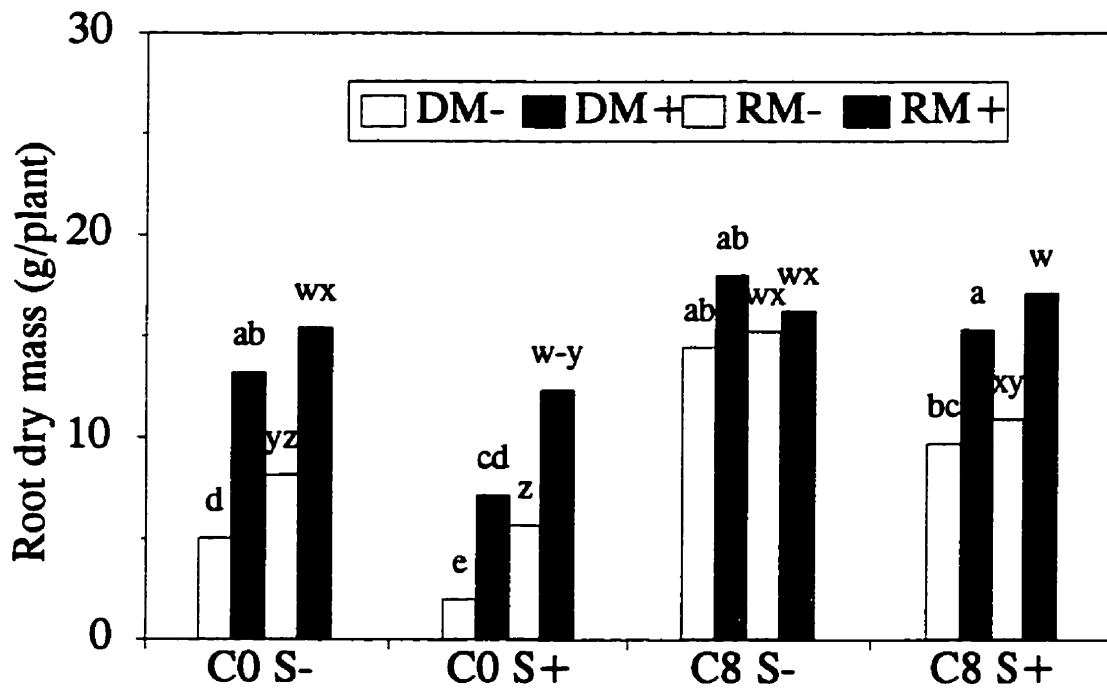


Figure 5.9. Dry masses ($n = 4$) of roots (top) and shoots (bottom) of mycorrhizal and non-mycorrhizal plants of maize cultivars of C0 and C8 at the end of drought (DM+, DM-) or recovery periods (RM+, RM-) under well-watered (S-) and drought-stressed (S+) conditions. Statistical analyses for roots and shoots were done separately. Means with different letters are significantly different according to Tukey's test ($P < 0.05$). Two sets of data collected after drought (a-f) and recovery (w-z) periods were analyzed separately.



plants (Table 5.3; Fig. 5.9). Drought decreased the root and shoot masses of M- C0 plants by 61 % and 36 % but only by 46 % and 28 % in M+ plants, respectively. A similar trend was also observed after three weeks of rewatering. Drought did not significantly decrease dry masses of C8 except for M+ shoots of C8. Under well-watered conditions, AM colonization significantly increased the dry masses in shoot and root of C0 and shoot of C8 plants.

5.4. Discussion

Root colonization with *Glomus intraradices* in the two tropical maize cultivars had a beneficial effect on host plant drought tolerance by maintaining higher (less negative) LWP, higher RWC and higher P and sugar contents and higher dry masses during the drought and recovery periods. This study agrees with the findings of other studies (Nelsen and Safir, 1982; Fitter, 1988; Sylvia et al., 1993; Subramanian and Charest, 1997; Chapter 4) which showed that colonization by AM fungi confers a greater P status of the host plant under drought conditions. In the present study, the higher root and shoot masses of AM plants under drought conditions may be related to increased nutrient content of slowly diffusing elements, especially P. These findings were consistent with other experiments where the same set of treatments were imposed at the tasselling stage (Subramanian and Charest, 1997; Chapter 4). Fitter (1988) stated that the mycorrhizal red clover plants have access to forms of P which were unavailable to non-AM plants under drought conditions. McArthur and Knowles (1993) reported that the external hyphae of AM fungi were able to more rapidly exploit a given volume of soil

for available P than roots of non-AM plants and thus speed the acquisition of soil P by the colonized roots. Higher P status assists the host plant to utilize the available water more efficiently under drought conditions (Bethlenfalvay et al., 1988) and to recover from drought when irrigation is restored (Nelsen and Safir, 1982). Conversely, Augé et al. (1987a) and Davies et al. (1993) have reported that the improvement of water relations by mycorrhizae during drought was independent of the host plant P status. It is also likely that the extraradical mycelium facilitated direct water uptake and transport of water by mycorrhizal roots (Hardie et al., 1985; Faber et al., 1991).

In my study, the increased SMC in the mycorrhizal-drought-treatment appeared to explain the ability of the colonized soil to retain moisture despite greater depletion by the larger dry mass of the host plant. This may be attributed to the formation of water stable soil aggregates (Schreiner and Bethlenfalvay, 1995). This is in contrast with the findings of Augé et al. (1994) who observed no mycorrhizal effects on rates of soil moisture depletion. Reichenbach and Schönbeck (1995) suggested that the intensive hyphal growth in the root zone of AM flax (*Linum usitatissimum*) plants enhances the substrate pore volume which in turn increases the water holding capacity of the soil.

Results of the present study carried out at the preflowering stage are similar to earlier findings (Chapter 2) that LWP of AM-colonized maize plants remained higher even after three weeks of drought following tasselling (Subramanian et al. 1995). However, the LWP values measured in the study at the tasselling stage were lower than in the present study. This may have been due to the higher evapotranspiration rate of maize at the flowering phase (Edmeades et al., 1993). Improved nutrient status of AM plants

may enable the host plant to absorb water more efficiently under drought conditions (Sylvia et al., 1993; Pai et al., 1994; Tobar et al., 1994a,b; Subramanian and Charest, 1997). Maintenance of higher LWP has often been suggested as an indicator of drought resistance in plants (Turner, 1986). The mycorrhizal effect on LWP was more pronounced in the drought-sensitive (C0) than -resistant (C8) cultivars as the latter had been selected for drought tolerance (Edmeades et al., 1993). This supports the view that mycorrhizal responsiveness is under genetic control of the host plant (Hetrick et al., 1996; Khalil et al., 1994). Our study indicated that AM maize plants recovered from drought twice as rapidly as non-AM plants. Since the drought-stressed M+ plants already maintained higher LWP than M- plants, a few days of rewatering resulted in LWPs comparable to those of well-watered plants. This is consistent with previous findings that AM-colonized geranium plants recovered more quickly from drought (Sweatt and Davies, 1984). Ruiz-Lozano et al. (1995) also reported that lettuce plants colonized with *Glomus deserticola* possessed a greater ability to recover from drought than non-mycorrhizal plants as indicated by stomatal conductance and photosynthetic rate.

The sugar accumulation in drought-stressed AM maize leaves may be related to reduced chlorophyll degradation (Subramanian and Charest, 1995; Chapter 3), a higher photosynthetic rate (Ruiz-Lozano et al., 1995), and higher LWP (Subramanian et al., 1995; Chapter 2), or it may result from increased starch hydrolysis (Jones et al., 1980; Augé et al., 1987a). Kameli and Lösel (1993) reported that glucose accumulates in proportion to decreasing LWP more rapidly in drought-resistant than -sensitive wheat cultivars. The sugar accumulation in AM maize plants may result in osmotic adjustment

(Munns, 1988). In contrast, Turner (1979) reported that the massive accumulation of starch in field-grown soybean did not result in osmotic adjustment. This author also suggested that osmoregulation in the form of solute accumulation may occur in plants in which the turgor threshold for cessation of growth is higher than that required for inhibition of photosynthesis.

In conclusion, mycorrhizal association had a significant effect on maintaining higher (less negative) LWP and RWC especially in the drought-sensitive (C0) cultivar, and on retaining more soluble sugars and P status during and after drought. The AM-colonized C0 and C8 plants recovered more rapidly than the non-colonized maize plants. These findings suggest that AM colonization improves drought tolerance of the host plant by maintaining higher leaf water status.

CHAPTER 6

ARBUSCULAR MYCORRHIZAE AND NITROGEN ASSIMILATION IN MAIZE AFTER DROUGHT AND RECOVERY

This chapter is the reproduction of a paper published in *Physiologia Plantarum* (102: 000-000, 1998) by K.S. Subramanian and C. Charest. This chapter fulfills the fifth objective of this thesis: To examine the effects of AM colonization on the levels of major enzymes involved in N assimilation after drought and recovery of maize and to assess these enzyme activities as potential factors in host plant drought tolerance.

6.1. Introduction

In many soils, nitrate is the main form of N available to plants, and hence its assimilation represents a major metabolic function (Oaks, 1994a). Nitrogen assimilation includes uptake of NO_3^- , its reduction to NO_2^- , the conversion of NO_2^- to NH_4^+ , and the incorporation of NH_4^+ into amino acids (Chapter 1.13). All these steps take place primarily in shoots (Campbell, 1988; Merlo et al., 1994; Sivasankar and Oaks, 1995) and to a lesser extent in roots (Oaks and Hirel, 1985; Oaks, 1994a). There is a vast body of literature concerning the regulation of N-assimilating enzymes by nitrate, light and drought (Sivaramakrishnan et al., 1988; Hoff et al., 1992; Kenis et al., 1994; Oaks, 1994b). Among these factors, NO_3^- uptake by the plant is the major determinant of the extent of N assimilation. Nitrate ion mobility in soils is severely restricted under drought conditions due to its low concentration and diffusion rate (Azcón et al., 1996). In

addition, drought-stressed plants suffer from a reduction in photosynthesis which appears to limit the supply of reductants and energy for NO_3^- reduction (Warner and Huffaker, 1989, Oaks, 1994b).

Under drought conditions, arbuscular mycorrhizal (AM) colonization plays a key role in mobilizing slowly diffusing ions and water which are not accessible to the host-plant roots (Bethlenfalvay et al., 1988, Faber et al., 1991). The AM colonization appears to improve plant N nutrition (Read, 1991, Turnbull et al., 1996, Subramanian and Charest, 1997) in addition to its well-established role in P uptake (George et al., 1995). Several ^{15}N studies revealed that AM fungi transport slowly diffusing NH_4^+ and NO_3^- through the extraradical mycelium especially under water deficit environments (Frey and Schüepp, 1993; Tobar et al., 1994a,b).

A number of studies have shown that the formation of ectomycorrhizae alters the characteristics of N acquisition and assimilation depending on the fungus and host plant species (Vézina et al., 1989; Botton and Chalot, 1995). Relatively few studies have been reported on N assimilation in plants colonized by AM fungi. Ho and Trappe (1975) detected NR activities in AM fungal spores and Kaldorf et al. (1994) provided genetic evidence for the presence of this enzyme in a *Glomus* isolate. Cliquet and Stewart (1993) reported that NR and GS activities in roots and shoots of maize plants increase when colonized with *G. fasciculatum*, indicating that NO_3^- mobilized from the soils by an AM fungus could be transferred directly to the root cells for further reduction and assimilation. Recently, Ruiz-Lozano and Azcón (1996) reported that NR activities

increase in drought-stressed lettuce plants when colonized with *G. deserticola*. These data suggested that mycorrhizal association assists the host plant to assimilate greater amounts of soil N under drought conditions.

We hypothesized that AM colonization in maize enhances acquisition and assimilation of N which may be factors related to host plant drought tolerance. To test this, we examined key enzymes involved in N assimilation (nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase), amino acid and protein concentrations and total N contents in roots and shoots of AM and non-AM maize plants which had been subjected to three weeks of drought (45-65 days after sowing, DAS) followed by three weeks of recovery (66-86 DAS) or to well-watered conditions at the preflowering stage.

6.2. Materials and Methods

6.2.1. Plant growth conditions

The details of growth conditions, treatments and sampling procedure are presented in Chapter 5.2.1. Maize plants of tropical maize cultivars (C0 and C8) inoculated with or without arbuscular mycorrhizal (AM) fungus were subjected to three weeks of drought (45-65 DAS) followed by three weeks of recovery (66-86 DAS) or well-watered conditions. Roots and shoots of each treatment sampled after the drought and the recovery periods were analyzed for N assimilating enzymes, amino acid and protein concentrations and total N contents.

6.2.2. Enzyme extraction and assays

6.2.2.1. Nitrate reductase (NR; EC 1.6.6.1)

NR was determined by a method adapted from Sivasankar and Oaks (1995). Freeze-dried root and shoot tissues (100 mg) were ground on ice in a mortar and pestle with 10 ml extraction buffer containing 25 mM Tris-HCl (pH 8.5), 1 mM EDTA, 1mM DTT, 20 μ M FAD, 1% (w/v) BSA (bovine serum albumin) and 10 mM cysteine. To stabilize NR activity, chymostatin (10 μ M dissolved in DMSO, dimethyl sulfoxide) and leupeptin (10 μ M) were added to the extraction buffer for roots and shoots, respectively. The extracts for all enzymes were centrifuged at 10 000 rpm for 25 min, filtered through Miracloth (Calbiochem, Biodesign Inc. of New York, Carmel, NY, USA) and assayed for enzyme activity. The assay mixture consisted of 0.2 ml of 0.65 M N-2-hydroxypiperazine-N-2-ethanesulfonic acid (HEPES) buffer (pH 7.0), 0.2 ml KNO₃ (0.1 M) and 0.1 ml extract. The volume of the assay mixture was brought to 1.4 ml with deionized H₂O, the reaction started by the addition of 0.1 ml NADH (3.6 mg ml⁻¹ in 0.04 M KH₂PO₄ pH 7.0) and incubated at 25°C for 15 min. After this period, the reaction was terminated by adding 1 ml of 1% (w/v) sulfanilamide in 1N HCl and 1 ml of 0.01% (w/v) N-1 naphthylethylene-diamine-dihydrochloride (NED) in d H₂O. The resultant chromophore was measured at 540 nm, 30 min after the termination of reaction. The NR activity was expressed as μ mol NO₂⁻¹ produced h⁻¹ g⁻¹ dry mass.

6.2.2.2. Nitrite reductase (NiR; EC 1.7.7.1)

Tissue samples (200 mg) were homogenized on ice in a mortar with an extraction buffer (10 ml) containing 50 mM KH₂PO₄ (pH 7.5), 100 mM KCl, 5 mM EDTA, 12 mM

DTT, 1mM PMSF (phenyl methylsulfonyl fluoride), 2 mM oxoglutarate and 0.05 % (v/v) Triton X 100. The reaction mixture consisted of 0.1 ml 50 mM KH_2PO_4 (pH 7.0), 0.1 ml 1mM KNO_2 , 0.1 ml 0.05 mM FAD, 0.2 ml enzyme extract of root or shoot tissues and 0.4 ml d H_2O . The reaction was started by the addition of 0.05 ml 12 mM methyl viologen and 0.05 ml 12 mM sodium dithionite. After 15 min of incubation at 25°C, the reaction was stopped as described in NR. NiR activity ($\mu\text{mol h}^{-1} \text{g}^{-1}$ dry mass) was determined by measuring the consumption of the substrate NO_2^- in the presence of reduced methyl viologen at 540 nm (Merlo et al., 1994).

6.2.2.3. *Glutamine synthetase (GS; EC 6.3.1.2)*

The GS activity was measured by the transferase assay (Shapiro and Stadtman, 1970). The extracting medium contained 25 mM Tris-HCl (pH 7.8), 1 mM EDTA, 1 mM DTT, 1 mM GSH (glutathione reduced), 10 mM MgSO_4 and 5 mM glutamate. Two hundred mg of freeze-dried root or shoot tissues were ground up on ice with 10 ml buffer, inert sand and 0.2 g PVP (insoluble polyvinylpyrrolidone) in a mortar and pestle on ice. The reaction mixture contained 80 μmol MES (2-[N-morpholino] ethanesulfonic acid), 60 μmol L-Gln, 25 μmol Na_2HAsO_4 , 2.5 μmol hydroxylamine, 2 μmol of MnCl_2 and 15 μmol ADP (final pH 7.6). The reaction was initiated by the addition of 0.35 ml of enzyme extract and terminated after 15 min by the addition of 0.70 ml ferric chloride reagent (4 ml FeCl_3 10%; 1 ml TCA 24%; 0.5 ml 6N HCl; 6.5 ml d H_2O). Control tubes contained enzyme extract and all the reagents except substrates (glutamine and hydroxylamine). The microfuge tubes were centrifuged at 10 000 rpm for 2 min to precipitate the proteins. The GS activity was detected at 540 nm using a standard curve

prepared with different concentrations of gamma-glutamylhydroxamate (Appendix 5).

The GS activity was expressed in $\mu\text{mol h}^{-1} \text{g}^{-1}$ dry mass.

6.2.2.4. Glutamate synthase (GOGAT; EC 1.4.1.14)

GOGAT extract was prepared together with NiR and assayed according to Lea et al. (1990). Assay mixture contained 0.2 ml 50 mM KH_2PO_4 buffer (pH 7.5), 0.1 ml 10 mM glutamine, 0.1 ml 10 mM 2-oxoglutarate, 0.1 ml d H_2O and 0.4 ml enzyme extract. Reaction was started by the addition of 0.1 ml freshly prepared 0.5 mM NADH. Control tubes were incubated with all the reagents except the substrates (glutamine and 2-oxoglutarate). The oxidation of NADH was followed at 340 nm for 5 min at 25°C and the activity expressed as $\mu\text{mol NADH oxidized h}^{-1} \text{g}^{-1}$ dry mass.

6.2.3. Amino acid, protein and nitrogen analyses

Soluble proteins from roots or shoots (100 mg freeze-dried tissue) were extracted using 10 ml Tris-HCl buffer (pH 7.8), centrifuged at 10 000 rpm for 25 min and determined according to Bradford (1976). Amino acids from roots or shoots (0.1 - 0.2 g) were extracted with 10 ml of 95 % ethanol. The homogenates were centrifuged at 5000 rpm for 10 min. Amino acids were screened by automated precolumn phenylthiocarbamyl amino acid analysis using the applied Biosystems Inc. model 420A-Boa-92a free amino acid analyzer, Foster City, CA, USA (Subramanian and Charest 1995; Chapter 3). The N percentages in roots and shoots were estimated using an Elemental Analyzer (Perkin Elmer Series II 2400, Foster City, CA, USA) and multiplied by the dry mass to calculate the total N contents (Subramanian and Charest 1997; Chapter 4).

6.2.4. Statistical analysis

A three-way analysis of variance (ANOVA) was applied (SAS Institute Inc, 1989) to all data, and they were also examined using Tukey's Studentized Range (HSD) test.

6.3. Results

6.3.1. Enzymes involved in N assimilation

The N assimilating enzymes, except NiR in the roots and shoots of the C0 and C8 cultivars, were affected by drought or mycorrhizal treatments. Drought stress significantly ($P < 0.01$ or $P < 0.001$) decreased NR and GS activities of C0 and GS activity of C8 in non-AM roots compared to well-watered roots (Table. 6.1). However, in the presence of AM colonization, NR, GS and GOGAT activities in C0 roots were significantly ($P < 0.01$ or $P < 0.001$) higher than in non-AM roots under drought conditions. Such mycorrhizal response was pronounced only for the GOGAT activity in C8. Drought-stressed AM roots of both cultivars had higher activities of NR (C0, 45%; C8, 26%), GS (C0, 76%; C8, 33%) and GOGAT (C0, 41%; C8, 53%) than non-AM roots and were comparable to well-watered AM or non-AM plants. Under well-watered conditions, enzyme activities in AM and non-AM roots were similar except GS which was higher in the AM roots of C0.

In shoots, the NR activity was 3 to 5 times higher than in roots of C0 and C8 regardless of mycorrhizal or drought treatments. In the absence of AM association, drought significantly ($P < 0.05$ or $P < 0.01$) decreased NR and GS activities in C0, and GS as well as GOGAT activities in C8 in comparison to well-watered non-AM shoots of

both cultivars (Table 6.1). With AM association, shoots of C0 and C8 had higher NR (C0, 46%; C8, 28%), GS (C0, 44%; C8, 50%) and GOGAT (C0, 67%; C8, 72%) activities than non-AM shoots under drought conditions. Enzyme activities in drought-stressed AM shoots were comparable to well-watered AM or non-AM shoots. Under well-watered conditions, all the enzymes in AM and non-AM plants were similar in both cultivars except in AM shoots of C8 which had significantly higher GS activity.

Even after three weeks of rewatering (Table 6.2), previously drought-stressed non-AM roots of C0 had significantly ($P < 0.01$ or $P < 0.001$) lower NR, GS and GOGAT activities than well-watered non-AM roots but the enzyme activities were constant in C8. In comparison to non-AM roots, colonization increased NR & GS activities in the roots of C0 in recovered and well-watered plants by 2.2 & 1.7 and 2.9 & 1.9 times, respectively. In contrast, NiR in recovered AM roots of C0 was lower than non-AM roots. In C8, GS activities in AM roots were significantly ($P < 0.001$) higher than non-AM roots under well-watered and recovery treatments.

In shoots, NR activity in recovered AM and non-AM plants of C0 and C8 declined by nearly 50% compared to the enzyme activity detected after drought (Table 6.2). The rewatered non-AM plants had significantly ($P < 0.01$ or $P < 0.001$) lower activities of GS and GOGAT in C0 and NR in C8, respectively, than well-watered non-AM plants. Recovered AM plants had significantly higher activities of NR and GS in C0 and GOGAT in C8 than non-AM plants. On the other hand, NiR significantly declined in recovered AM plants of C0 and C8. Mycorrhizal colonization enhanced NR, GS and GOGAT activities of previously drought-stressed C0 plants by 48%, 60% and 45%

Table 6.2. Means (n = 4) for nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS) and glutamate synthase (GOGAT) activities in roots and shoots of C0 and C8 cultivars after 3 weeks of drought recovery (66-86 DAS) or under well-watered conditions with (M+) or without (M-) arbuscular mycorrhizal colonization. Different letters within a column indicate significant differences at 5% level using Tukey's Test; and the levels of significance for ANOVA * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS not significant

Treatment	NR		NiR ($\mu\text{mol g}^{-1} \text{DM h}^{-1}$)		GS		GOGAT	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
<i>Drought-sensitive (C0) cultivar</i>								
Drought-stressed								
M+	27 ^d	83 ^{ab}	34 ^b	27 ^c	74 ^{ab}	95 ^{ab}	23 ^{bc}	24 ^{cd}
M-	12 ^e	43 ^d	41 ^a	35 ^{ab}	26 ^d	38 ^d	17 ^c	13 ^d
Well-watered								
M+	49 ^{ab}	104 ^a	38 ^{ab}	32 ^{bc}	89 ^a	127 ^a	19 ^c	20 ^{cd}
M-	29 ^d	91 ^b	42 ^a	39 ^a	48 ^{bc}	62 ^{bc}	34 ^{ab}	46 ^b
<i>Drought-resistant (C8) cultivar</i>								
Drought-stressed								
M+	40 ^c	67 ^c	39 ^{ab}	28 ^c	87 ^a	69 ^{bc}	35 ^a	42 ^{bc}
M-	39 ^c	52 ^{cd}	40 ^a	39 ^a	39 ^{cd}	60 ^{bc}	37 ^a	14 ^d
Well-watered								
M+	65 ^a	103 ^a	40 ^a	37 ^{abc}	95 ^a	87 ^{ab}	39 ^a	116 ^a
M-	55 ^{ab}	77 ^{ab}	39 ^{ab}	34 ^{ab}	50 ^{bc}	50 ^{bc}	42 ^a	27 ^{bcd}
ANOVA : C (cultivar), S (drought treatment), M (mycorrhizal treatment)								
C	***	NS	NS	NS	NS	NS	***	***
S	***	***	NS	**	**	**	NS	***
M	***	***	**	***	***	***	NS	***
C X S	NS	NS	NS	NS	NS	NS	NS	*
C X M	NS	NS	**	*	NS	**	NS	***
S X M	**	*	NS	***	NS	NS	*	**
C X S X M	NS	*	NS	***	NS	NS	**	**

compared to non-AM plants. Such mycorrhizal response was observed only for GOGAT in C8 under similar conditions. Even under an optimal irrigation regime, AM plants had higher NR and GS in C0 and GOGAT in C8 but there was a reduction in GOGAT in AM plants of C0.

6.3.2. Amino acids

Total amino acid concentrations in drought-stressed AM roots of C0 were significantly ($P < 0.001$) increased by almost 5 times compared to other treatments (Table 6.3; Fig. 6.1). In contrast, there was a lower concentration of amino acids in well-watered AM roots of C0. After three weeks of recovery, total amino acid concentrations in AM and non-AM roots of both cultivars were similar except for well-watered AM C0 and non-AM C8 which were higher than other treatments. In shoots, amino acids were generally higher in AM than non-AM plants of C0 and C8 at the end of drought and recovery periods. The most abundant amino acids detected in AM or non-AM plants were Ala, Arg, Asn, Asp, Gln and Glu which constituted about 56% and 75% of the total pool in roots and shoots, respectively (data not shown). Among the predominant amino acids, AM colonization significantly ($P < 0.01$ or $P < 0.001$) increased glutamine concentrations in roots and shoots of C8 under drought conditions but such an increase was not significant for C0 (Fig. 6.2). Glutamate concentrations were significantly ($P < 0.001$) increased in drought-stressed AM roots of both cultivars. After irrigation was restored, mycorrhizal treatment had no effect on Gln and Glu in roots or shoots except in the well-watered roots of AM C0 and non-AM C8 which showed an increase in Glu concentrations (Fig. 6.3)

Figure 6.1. Total amino acid (AA) concentrations ($n = 2$) of roots (top) and shoots (bottom) of mycorrhizal (M+) and non-mycorrhizal (M-) plants of maize cultivars C0 and C8 at the end of drought (DM+, DM-) or recovery (RM+, RM-) periods under well-watered (S-) or drought-stressed (S+) conditions. Means with different letters are significantly different according to Tukey's test ($P < 0.05$). The two data sets after drought (a-c) and recovery (x, y) periods were analyzed separately.

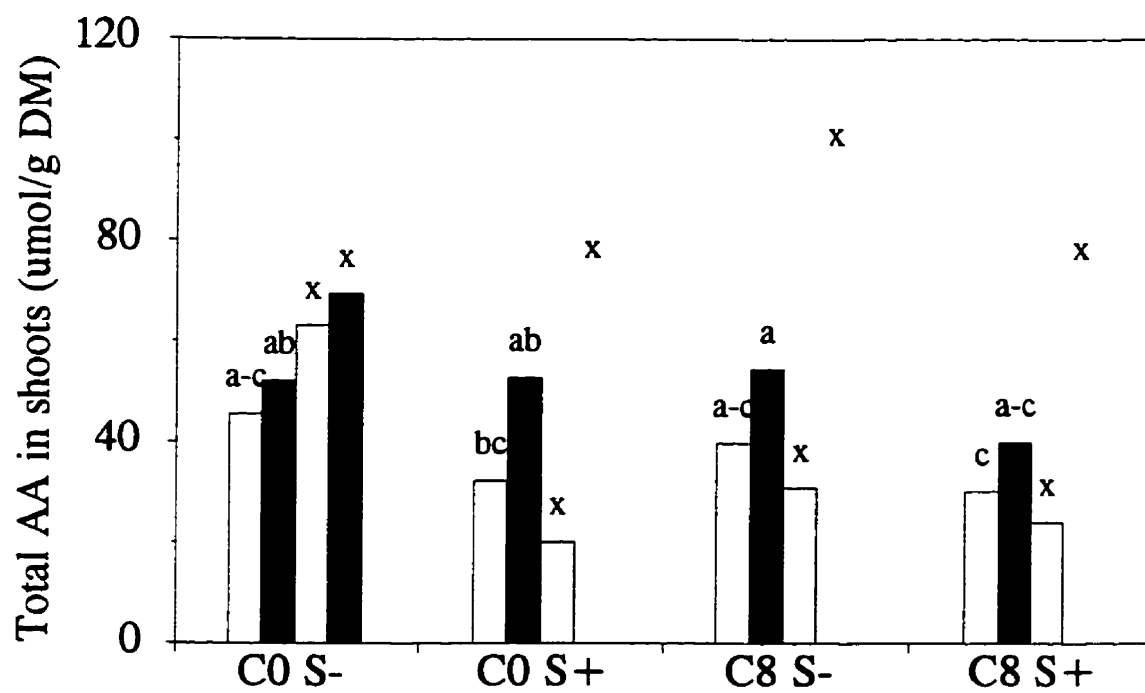
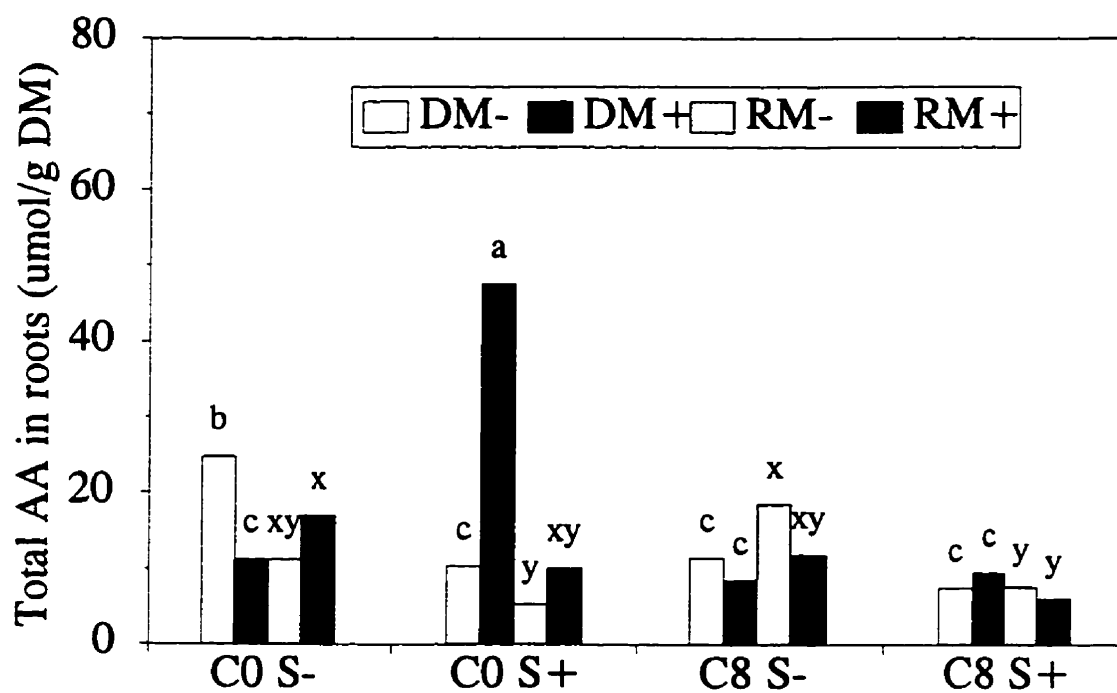


Figure 6.2. Glutamine (Gln) concentrations ($n = 2$) of roots (top) and shoots (bottom) of mycorrhizal (M+) and non-mycorrhizal (M-) plants of maize cultivars C0 and C8 at the end of drought (DM+, DM-) or recovery (RM+, RM-) periods under well-watered (S-) or drought-stressed (S+) conditions. Means with different letters are significantly different according to Tukey's test ($P < 0.05$). The two data sets after drought (a-d) and recovery (x) periods were analyzed separately.

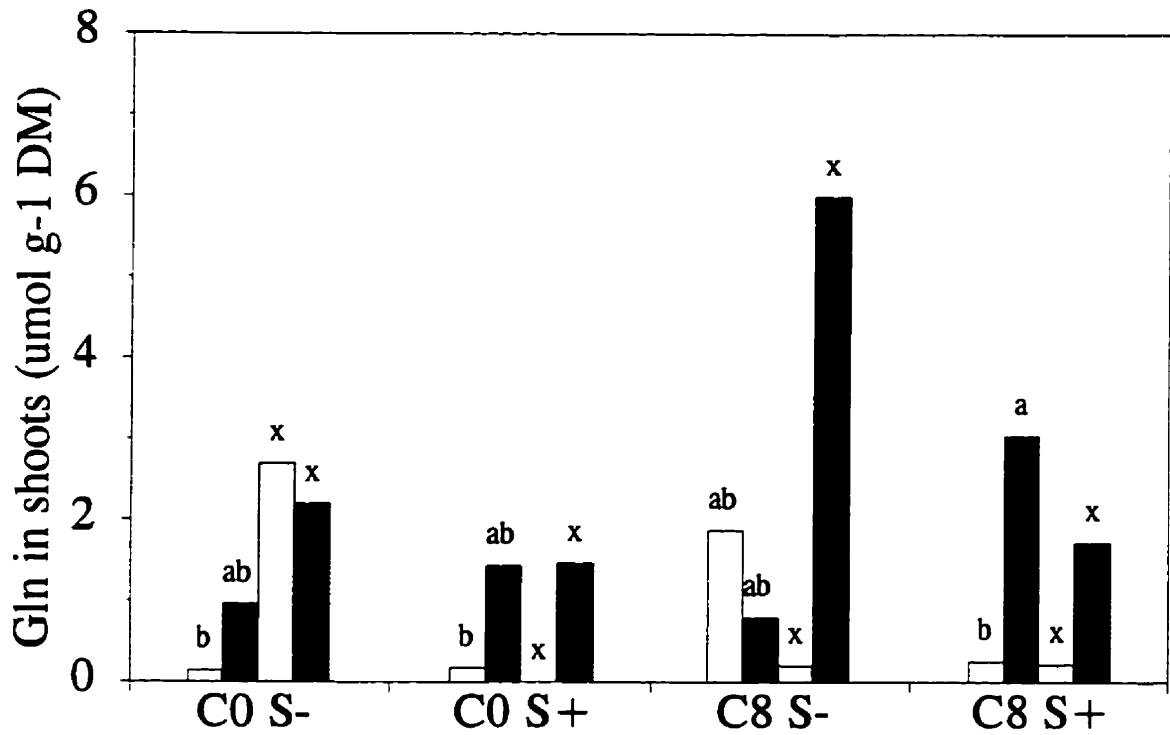
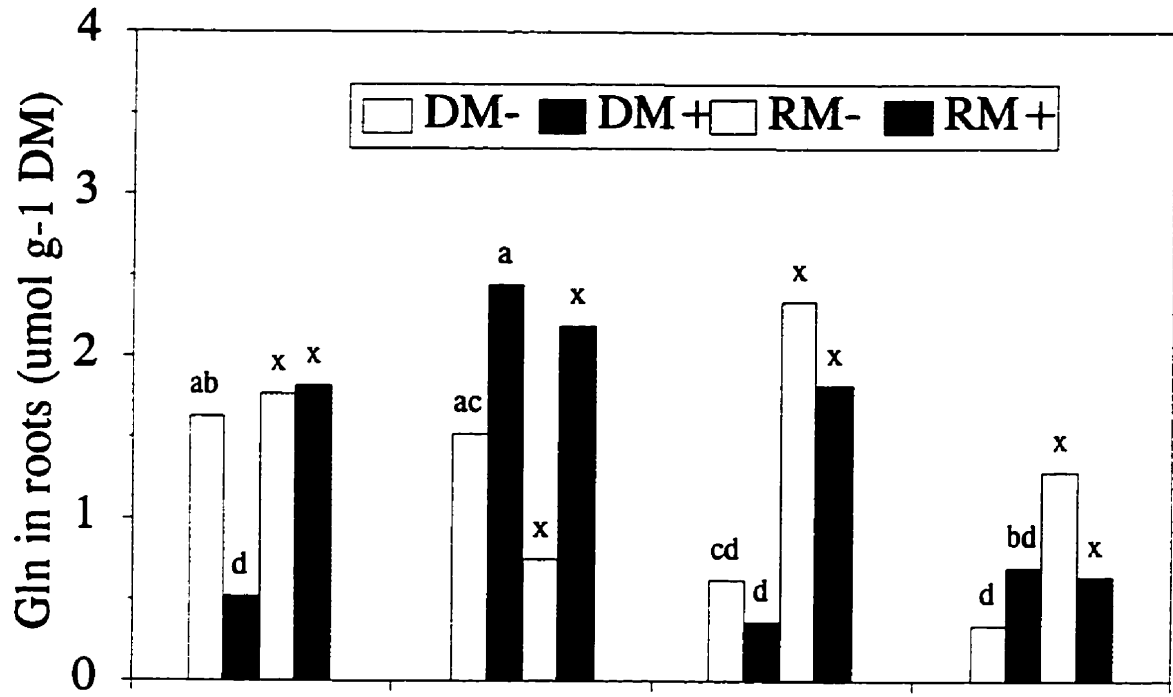
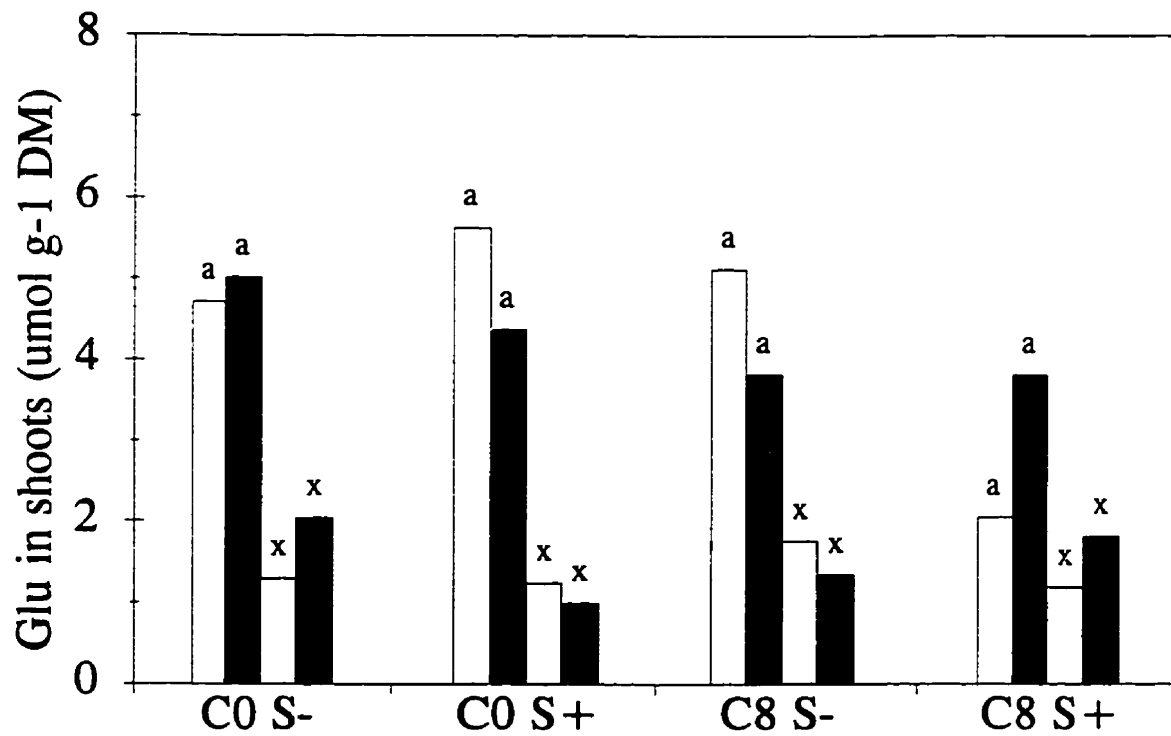
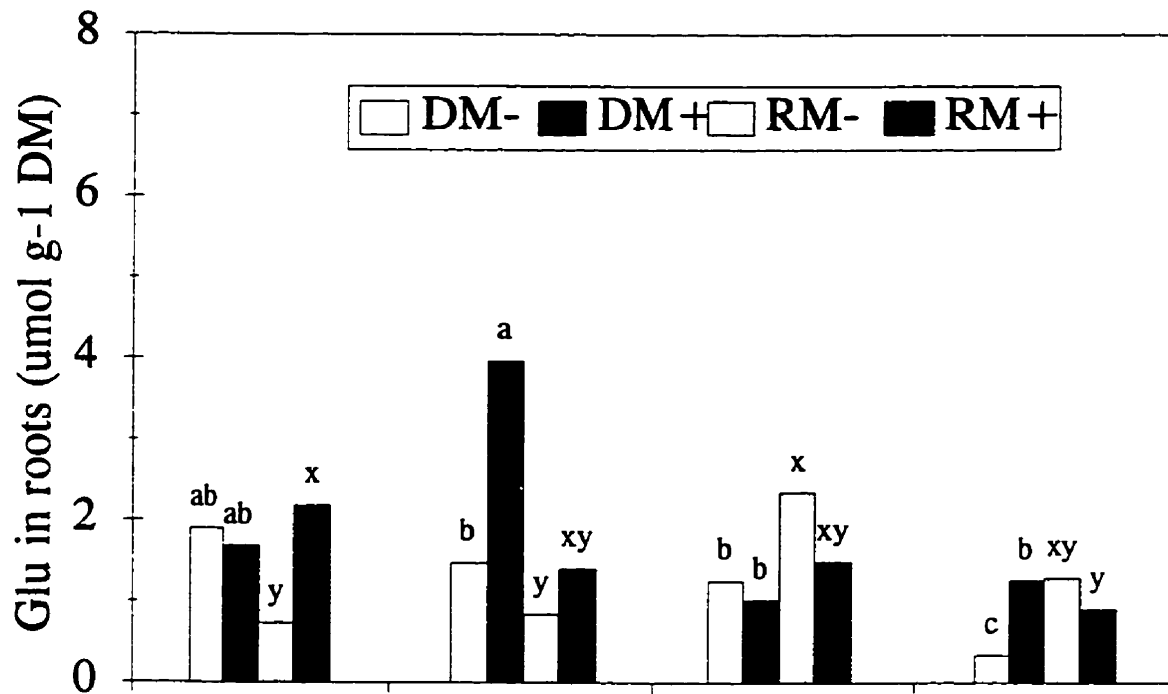


Figure 6.3. Glutamate (Glu) concentrations ($n = 2$) of roots (top) and shoots (bottom) of mycorrhizal (M+) and non-mycorrhizal (M-) plants of maize cultivars C0 and C8 at the end of drought (DM+, DM-) or recovery (RM+, RM-) periods under well-watered (S-) or drought-stressed (S+) conditions. Means with different letters are significantly different according to Tukey's test ($P < 0.05$). The two data sets after drought (a-d) and recovery (x, y) periods were analyzed separately.



6.3.3. Proteins

Drought significantly ($P < 0.01$ or $P < 0.001$) decreased soluble protein concentrations in non-AM roots and shoots of C0 and in the shoots of C8 (Table 6.3; Fig. 6.4). With AM association, plants had significantly ($P < 0.001$) higher protein concentrations in drought-stressed roots of both cultivars than non-AM roots under drought conditions. Mycorrhizal plants of C0 and C8 had higher protein concentrations in shoots under well-watered and drought-stressed conditions. However, after rewatering, proteins in AM and non-AM roots of both cultivars were similar except in well-watered C8 roots where they were higher. In shoots, after the recovery period, the protein increases due to AM association were significant only in C0.

6.3.4. Nitrogen content

Drought significantly ($P < 0.01$ or $P < 0.001$) reduced the total N contents in non-AM C0 roots and shoots (Table 6.3; Fig. 6.5). In the presence of AM association, total N contents in roots and shoots of C0 plants were significantly increased under well-watered or drought-stressed conditions. Such increase was observed in shoots of C8 only under well-watered conditions. Even after three weeks of rewatering, the recovered AM roots of C0 and C8 and shoots of C0 had significantly ($P < 0.01$ or $P < 0.001$) higher N contents than non-AM plants.

Figure 6.4. Protein concentrations ($n = 4$) of roots (top) and shoots (bottom) of mycorrhizal (M+) and non-mycorrhizal (M-) plants of maize cultivars C0 and C8 at the end of drought (DM+, DM-) or recovery (RM+, RM-) periods under well-watered (S-) or drought-stressed (S+) conditions. Means with different letters are significantly different according to Tukey's test ($P < 0.05$). The two data sets after drought (a-d) and recovery (w-z) periods were analyzed separately.

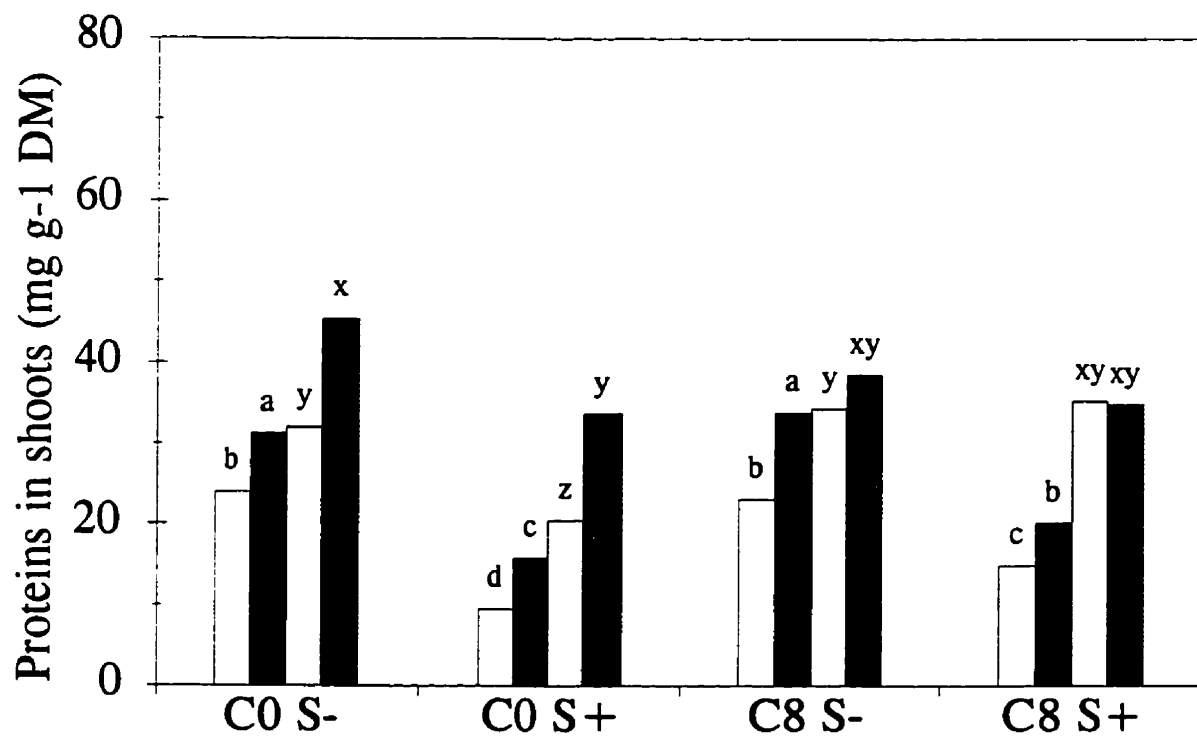
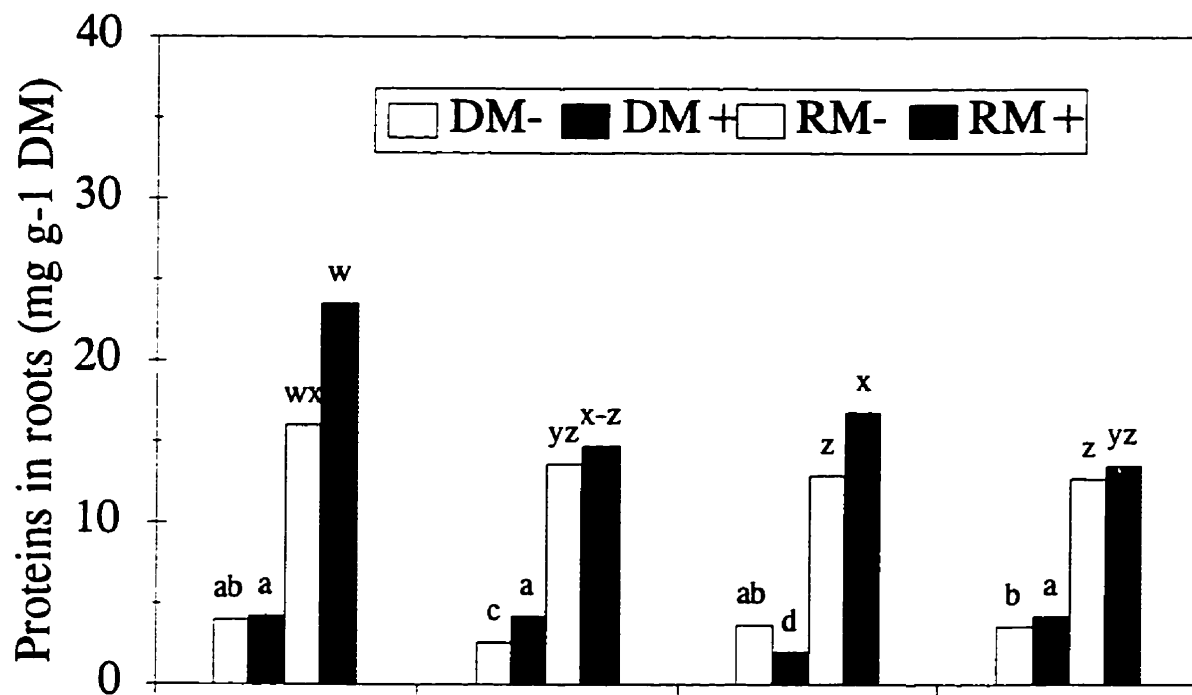


Figure 6.5. Nitrogen content ($n = 4$) of roots (top) and shoots (bottom) of mycorrhizal (M+) and non-mycorrhizal (M-) plants of maize cultivars C0 and C8 at the end of drought (DM+, DM-) or recovery (RM+, RM-) periods under well-watered (S-) or drought-stressed (S+) conditions. Means with different letters are significantly different according to Tukey's test ($P < 0.05$). The two data sets after drought (a-d) and recovery (w-z) periods were analyzed separately.

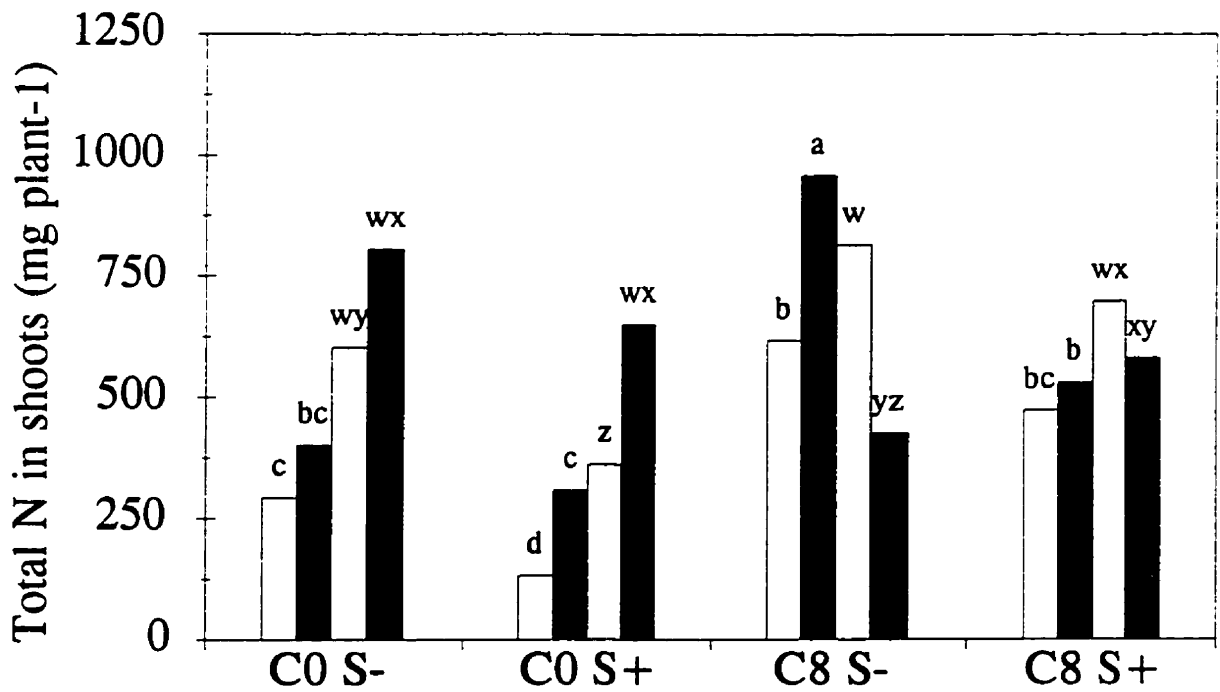
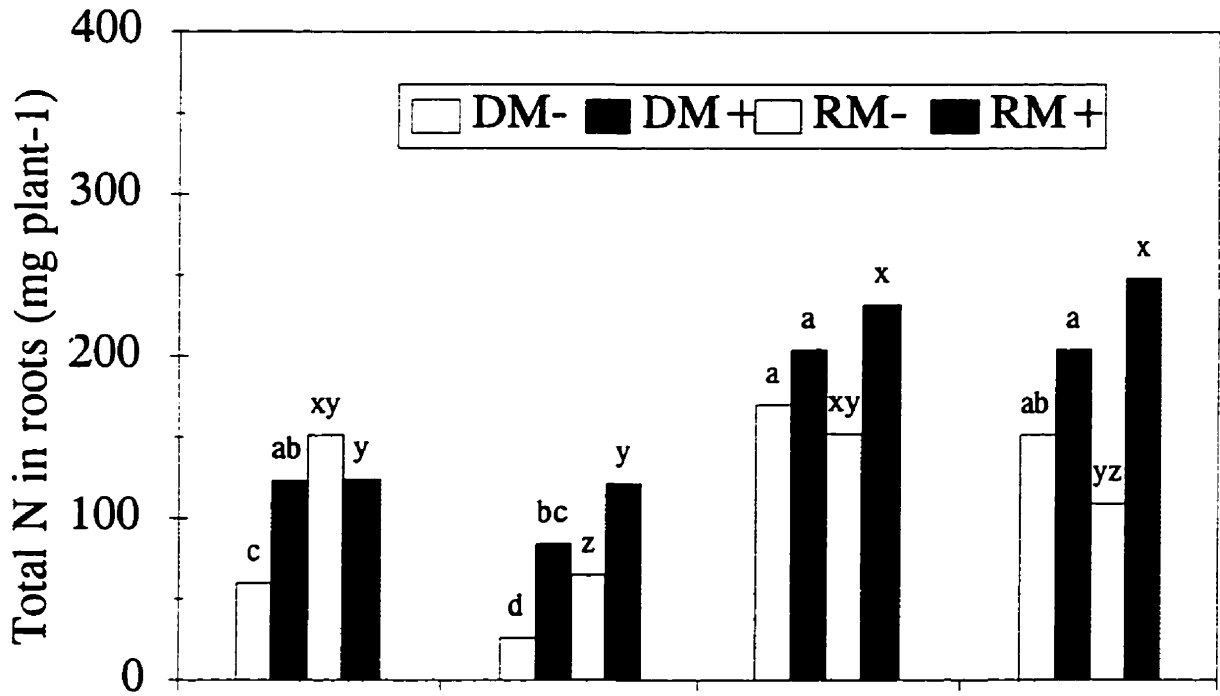


Table 6.3. Levels of significance for ANOVA for total amino acids, glutamine, glutamate, protein and nitrogen in roots and shoots at the end of three weeks of drought (45-65 DAS) and recovery (66-86 DAS) periods (C cultivar; S drought treatment; M mycorrhizal treatment).

	C	S	M	C X S	CX M	S X M	C X S X M
Total amino acids							
<i>After drought</i>							
Roots	***	***	**	**	***	***	***
Shoots	*	**	***	NS	NS	NS	*
<i>After recovery</i>							
Roots	NS	***	NS	NS	**	NS	NS
Shoots	NS	NS	NS	*	NS	NS	NS
Glutamine							
<i>After drought</i>							
Roots	***	***	NS	***	NS	***	*
Shoots	**	NS	**	NS	NS	**	**
<i>After recovery</i>							
Roots	NS	NS	NS	NS	NS	NS	NS
Shoots	NS	NS	NS	NS	NS	NS	NS
Glutamate							
<i>After drought</i>							
Roots	**	NS	*	*	*	**	*
Shoots	NS	NS	NS	NS	NS	NS	NS
<i>After recovery</i>							
Roots	NS	**	NS	NS	***	NS	*
Shoots	NS	NS	NS	NS	NS	NS	NS
Protein							
<i>After drought</i>							
Roots	***	**	*	***	***	***	**
Shoots	***	***	***	**	NS	*	NS
<i>After recovery</i>							
Roots	***	***	***	***	**	***	*
Shoots	*	***	***	***	***	NS	NS
Nitrogen							
<i>After drought</i>							
Roots	***	**	***	NS	NS	NS	NS
Shoots	***	***	***	*	NS	**	*
<i>After recovery</i>							
Roots	***	*	***	NS	***	***	NS
Shoots	NS	**	NS	**	***	**	NS

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS not significant

6.4. Discussion

Mycorrhizal colonization with *Glomus intraradices* in the two tropical maize cultivars improved the plant N nutritional status possibly by the hyphal transport of N from the growth medium. This may have led to increases in activities of key N assimilating enzymes (NR, GS and GOGAT) and protein and amino acid concentrations. This increased capacity of N acquisition and assimilation may enable the host plant to withstand drought conditions. The present study agrees with other reports (Tobar et al., 1994a,b; Ruiz-Lozano and Azcón, 1996; Subramanian and Charest, 1997) which indicated that mycorrhizae actively assist the host plants to enhance NO_3^- uptake and assimilation under limited water environments. The enhancement of N assimilating enzymes in host plants has also been reported under normal conditions (Smith et al., 1985; Cliquet and Stewart, 1993).

The increased N-assimilating enzymes may be attributed to the contribution of hyphal transport of N in the form of NO_3^- (Johansen et al., 1993, 1994; Tobar et al., 1994a,b). Johansen et al., (1996) indicated that NO_3^- or NH_4^+ assimilated into free amino acid pool of the AM mycelium. Recently, in an elegant petri plate system using carrot transformed roots, Shachar-Hill et al. (1997) have shown that the extraradical mycelium assimilated most of the added inorganic N or urea into free amino acids (Arg, Asp, Asn, Gln) and those amino acids were subsequently transferred to the host plant. These forms of N or increased N uptake may stimulate the N metabolism enzymes in the host plant. As drought stress impedes the mobility of NO_3^- ions (Azcón et al., 1996), mycorrhizal plants may have access, through the extraradical mycelium, to the forms of N which are

usually unavailable to the non-AM plants (Azcón-Aguilar et al., 1993). Mycorrhizal fungi constitute a major part of microbial biomass of the rhizospheric soil and they may assist in the turnover of N by competing with other microorganisms for this nutrient (Johansen et al., 1996).

Recently, we have shown improved nutritional status of N and P and higher dry masses in mycorrhizal plants under drought conditions (Subramanian et al., 1997). The enhanced P status in mycorrhizal plants might have altered the activities of N-assimilating enzymes. Our data correspond with the findings of Oliver et al. (1983) who reported that mycorrhizal subclover (*Trifolium subterraneum* L.) had a greater capacity to synthesize NR which was attributed to an indirect effect associated with improved host plant P nutrition. The improved P status due to AM association may facilitate enzyme reactions, especially GS that requires ATP (Lea et al., 1990).

In the present study, NR and GS activities were increased by 2 to 4 times in AM roots, especially under drought conditions. Such increases were previously reported by Cliquet and Stewart (1993) for maize roots colonized with *G. fasciculatum*. The enhanced N assimilation in roots suggests two scenarios: either AM fungi induce an increase of these enzymes in the host plant or AM fungal structures may have such enzyme activities. Kaldorf et al. (1994) provided molecular evidence for the presence of genes coding NR in AM fungi and this may account for the increased enzyme activities in maize roots. We also detected higher GS and GOGAT activities in AM colonized roots and this may be due to the contribution of a functional GS-GOGAT system in the mycorrhizal fungi (Johansen et al., 1996). During rewatering, the enhanced NR and GS activities in AM

roots of the sensitive C0 cultivar suggests that AM association is an important factor in sustaining N assimilation until the full recovery of the host plant.

In this study, NO_3^- reduction in maize was higher in the shoots than roots. This agrees with the findings of Campbell (1988) who detected higher NR in leaves of several plant species. In the absence of AM colonization, drought decreased the NR, GS and GOGAT activities by 25-75% depending on the sensitivity of the cultivar. This drought inhibitory effect may be attributed to the lower flux of NO_3^- from the roots to the shoots (Ruiz-Lozano and Azcón, 1996). Our previous study (Subramanian et al., 1995, Chapter 2) had shown that by maintaining higher (less negative) leaf water potential, the mycorrhizal roots may assist in exporting NO_3^- to the shoots for further reduction and assimilation under drought conditions. In addition, mycorrhizal plants were shown to be photosynthetically more efficient (Augé et al., 1987a; Ruiz-Lozano et al., 1995) and to supply carbon for nitrate reduction and assimilation under stress conditions (Merlo et al., 1994). Even under well-watered conditions or after rewatering, mycorrhizal plants of both cultivars had higher N enzyme activities suggesting that mycorrhizae may be a crucial factor under normal and limited water environments. In recovered maize shoots, NR activity dropped by nearly 50% which may be due to the progression of developmental stages. Ta (1991) also observed a decline in NR activities in maize leaves at post-anthesis stage.

Amino acids were 2 to 3 fold higher in the mycorrhizal than non-mycorrhizal plants during drought and recovery periods. This may indicate an altered N assimilation pathway in the presence of AM colonization (Attiwill and Adams, 1993). The most

abundant free amino acids detected in the mycorrhizal shoots were Ala, Arg, Asn, Asp, Gln and Glu which constituted 56 to 75% of the pool. These data agree with the findings of Cliquet and Stewart (1993) who observed an increase in the same set of amino acids in AM plants of another maize cultivar colonized with another *Glomus* sp.. Johansen et al. (1996) also found Asn, Asp, Gln and Glu contents to account for over 90% of the free amino acid pool of AM extraradical mycelium. The presence of the GS-GOGAT system had been found in ectomycorrhizal fungi (Vézina et al., 1989; Chalot et al., 1994). Other studies indicated that the external hyphae of AM fungi were able to transport N as inorganic (Frey and Schüepp, 1993; Johansen et al., 1993; Tobar et al., 1994 a,b) or organic forms (Johansen et al., 1996) and translocate them to the host plant. The N contribution by the hyphae may alter N metabolism in the host plant under drought conditions.

The increase in protein concentrations by mycorrhizal association agrees with our earlier findings at the tasselling stage of maize (Subramanian and Charest, 1995, Chapter 3). Arines et al. (1993) also detected a 2 to 6 fold increase in soluble proteins in mycorrhizal clover roots. Other studies had identified endomycorrhizins (AM-inducible proteins) in host-plant species (Dumas et al., 1990; Simoneau et al., 1994). These new proteins may play an adaptive role under drought conditions. The AM colonization also increased the total N contents, especially in the sensitive C0 cultivar under drought or non-drought conditions, likely due to an enhanced biomass (Subramanian et al., 1997; Chapter 5). These results agree with the study of Ruiz-Lozano and Azcón (1996) who

reported that lettuce colonized with *G. deserticola* had total N contents twice that of control plants under drought conditions.

In summary, the present study shows that AM colonized plants have enhanced NR, GS and GOGAT activities and higher nitrogenous compounds during drought or recovery periods. These overall results suggest that AM association plays a major role in improving host plant N assimilation and nutritional status. This may be a key factor that enables the plants to withstand drought and recover after stress is relieved.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1. General discussion

The main purpose of this study was to determine potential factors involved in mycorrhizae-assisted drought tolerance in tropical maize cultivars under controlled conditions. The general hypothesis was that arbuscular mycorrhizal (AM) colonization promotes drought tolerance of the host plant. This may be as a consequence of altered water relations, metabolism and nutritional status of the host plant. These changes enable the host plant to withstand water deficit conditions and recover rapidly when irrigation is restored. In addition, the host plant response to AM colonization varies with the drought sensitivity of the cultivar and the stage of development that coincided with drought. To test these hypotheses, five objectives were set up. (i) To measure the physiological responses of two tropical maize cultivars (C0 and C8) having differential sensitivity to drought during three weeks of withheld irrigation at the tasselling stage in order to determine the ability of these plants to sustain water deficit conditions in the presence or absence of AM inoculation. (ii) To examine the effects of AM association on metabolic changes in these cultivars under the different treatments. (iii) To determine the nutritional status of maize plants to assess whether AM colonization enables the host plant to supply enough minerals to support kernel growth. (iv) To evaluate the drought recovery of tropical maize cultivars in the presence or absence of AM colonization. (v) To examine the effects of AM colonization on the levels of enzymes involved in nitrogen assimilation

in maize and to assess the N enzyme modifications as a potential factor in host plant drought tolerance.

To accomplish the first objective, the physiological responses of the two tropical maize cultivars (C0, drought-sensitive; C8, drought-resistant) to arbuscular mycorrhizal (AM) colonization were measured (Chapter 2). The AM association in maize had a beneficial effect on plant water relations and leaf enlargement under water deficit conditions. The AM colonized plants maintained higher (less negative) leaf water potential than non-AM plants even after three weeks of continuously withholding irrigation at tasselling (Chapter 2) or preflowering stages (Chapter 5). This study supports the hypothesis that the AM association assists the host plant to maintain higher water status under moderate drought conditions regardless of developmental stage of the crop.

Under drought conditions, AM colonization promotes water relations of the host plant as a consequence of stimulated plant nutrition (indirect effect) and possibly through enhanced water uptake (direct effect). I have shown that AM colonization confers a greater P status of the host plant under water deficit conditions (Chapters 4 & 5). It is widely believed that AM association alters host plant water status as a secondary consequence of enhanced P nutrition (Nelsen and Safir, 1982; Fitter, 1988; Smith and Read, 1997). I have performed a simple linear regression analysis in order to ascertain the relationship between P content and the host plant water status. Our data have shown a significant positive correlation between root (**non-mycorrhizal** $r^2 = 0.66$ ***; **mycorrhizal** $r^2 = 0.52$ *) or shoot P contents (**non-mycorrhizal** $r^2 = 0.63$ ***;

mycorrhizal $r^2 = 0.49^*$) and leaf water potential (Appendices 6 & 7). This agrees with the observation of Fitter (1988) who reported that P status of the host plant has an effect on the maintenance of leaf water potential. In our study, the increased P uptake in drought-stressed AM plants may be due to the extraradical mycelium which can absorb P from the soil solution and translocate it to the roots (Jakobsen et al., 1992). The improved P status of the host plant provides an ability to exploit the available soil moisture more efficiently and thus AM plants appeared to be more drought tolerant than non-AM plants. In addition, the AM colonized plants recovered from drought more rapidly possibly due to the increased root growth and efficient extraction of water from the soil as a result of increased P uptake (Chapter 5). In contrast, Davies et al. (1993) and Augé et al. (1994) found that mycorrhizal symbiosis in helping host plant drought tolerance acted independently of P nutrition. Bethlenfalvay et al. (1988) showed an ability of the soybean AM roots to take up soil water that was not available to the non-AM roots. The advantage of this increased ability of AM plants may be expected to increase with decreasing availability of water.

The improved water status of AM plants may be attributed to the transport of water through extraradical hyphae and this may directly affect water relations in plants (Allen, 1982; Hardie, 1985; Faber et al., 1991). This evidence clearly indicated that the rate of water transport through the extraradical mycelium to the root was sufficient enough to maintain normal plant water relations. The hyphal transport of water may depend on the status of drought stress (severity and duration) or the functional compatibility between the plant species and the AM fungal species (Ruiz-Lozano et al.,

1995). However, others have found no direct water transport by AM hyphae to the host plants (Kothari et al., 1990; George et al., 1992).

In contrast to our findings, Levy et al. (1983) reported that the drought-stressed AM citrus plants had significantly lower leaf water potential than non-AM plants. They suggested that the higher transpiration and slightly (not significantly) larger size of the AM plants may have more quickly depleted the available soil moisture and resulted in the AM plants being exposed to severe drought stress than the non-AM plants. These authors also argued that small pot size may have aggravated the difference in treatments and the results may be different in either field or larger pots. In our experiments, we have used relatively larger containers (65 litres) to circumvent such pot size effects and the results were reproducible.

In addition, I have shown that the mycorrhizae-treated soil maintained higher moisture contents under drought conditions (Subramanian et al., 1997, Chapter 5). This appeared to indicate the ability of AM colonized soil to retain more moisture despite greater depletion by the larger dry mass of the host plant. We observed a significant positive correlation (**non-mycorrhizal** $r^2 = 0.77^{***}$; **mycorrhizal** $r^2 = 0.90^{***}$) between soil moisture content and leaf water potential (Appendix 8). Augé et al. (1995) have shown that sorghum plants colonized with *Glomus intraradices* depleted soil moisture more slowly than *G. etunicatum* in spite of the fact that shoot and root sizes were similar. The higher moisture content detected in AM colonized soil may be related to the formation of water stable aggregates (Schreiner and Bethlenfalvay, 1995). The AM fungus-mediated soil aggregation is mainly attributed to the binding of soil particles by

extraradical hyphae in microaggregates and entanglement of microaggregates into macroaggregates (Schreiner and Bethlenfalvay, 1995).

In this study, AM colonization modified the stomatal behaviour of maize plants under drought conditions as indicated by lower stomatal resistance and higher transpiration rates than non-AM plants (Chapter 2). This tended to indicate that AM plants were able to keep the stomata partially open for longer periods of time than non-AM plants. The symbiosis between maize and *G. intraradices* allowed the plants to maintain water balance and keep stomata open suggesting that AM plants may fix more carbon under drought conditions. As AM colonized soil retained higher soil moisture content, these plants could have been exposed to less strain in comparison to non-AM plants. It is also possible that the lower stomatal resistance values in AM plants are as a result of improved P nutritional status (Chapter 4). Recently, there have been suggestions that under drought conditions, AM association influenced the host plant stomatal behaviour by lowering the production and loading of abscisic acid (ABA) into xylem (Ebel et al., 1997). Since ABA appears to be a hormonal signal for stomatal closure in droughted plants (Davies et al., 1994), its lower concentration in AM plants may allow these plants to keep the stomata open longer than non-AM plants under drought conditions.

Mycorrhizal plants of the tropical maize cultivars retained higher green leaf area (GLA) throughout the three weeks of drought. The retention of higher GLA in AM plants may be attributed to the improved N and P nutritional status (Chapter 4). This suggests that AM association reduces senescence of leaf area caused by drought. Some reports

indicated that mycorrhizal symbiosis eliminated the inhibitory effects of the drought-induced non-hydraulic root-to-shoot signaling process on leaf growth in sorghum (Augé et al., 1995). Leaf area production is playing an important factor in determining water use and carbon uptake by plants and therefore potential productivity.

Our data on water relations clearly showed that AM colonization is advantageous to plants under moderate drought conditions. However, it must be pointed out from another study (Levy et al., 1983) that during prolonged periods of drought stress, AM plants may suffer more due to their lower stomatal resistance, higher transpiration rate and larger size. In order to assess the potential benefits of AM colonization, I also measured metabolic and nutritional changes in the host plant at the same time. In the first phase of this research work, the potential benefits of mycorrhizal association on the physiological aspect of host plant drought tolerance have been accomplished. When discussing drought resistance in the host plant, it is important to integrate other metabolic and nutritional plant responses to the limitation of water and to ascertain the realistic benefits of mycorrhizal association.

The second objective was to determine the effect of mycorrhizal association on metabolic changes in maize cultivars. I have shown that the AM maize plants retained more soluble sugars and proteins than non-AM plants under drought conditions (Chapters 3 & 5). Higher sugar concentrations accompanying decreasing LWP with the progression of drought stress appeared to be physiologically important in helping the plants to withstand water deficit conditions and recover after irrigation was restored. A direct relationship between soluble sugars and degree of adaptation to drought has been

observed in cotton (Ackerson, 1981). In another study, sugars accumulated more rapidly in drought-resistant than -sensitive wheat cultivars (Kameli and Lösel, 1993). Our data suggested that the increased sugar concentration in AM maize plants may have assisted in osmotic adjustment and enabled the host plant to maintain higher LWP under water deficit conditions. This observation is further supported by a significant positive correlation (**non-mycorrhizal** $r^2 = 0.87^{***}$; **mycorrhizal** $r^2 = 0.89^{***}$) between sugar concentration in shoots and LWP (Appendix 9).

The enhanced soluble protein concentration in AM plants may be linked to the greater acquisition and assimilation of N by the mycorrhizal roots (Chapters 3 & 6). The increase in protein concentration in AM plants appears to be an indicator of stress tolerance (Charest et al., 1993; Subramanian and Charest, 1995). The higher proteins in AM maize plants may also be attributed to the reduced extent of protein degradation as indicated by lower amino acid concentrations (Chapter 3) or enhanced N assimilation (Chapter 6). Mycorrhizal symbiosis had been shown to induce host plants to produce new proteins called mycorrhizins (Dumas et al., 1990; Arines et al., 1993; Simoneau et al., 1994). These may have contributed to the increase in protein concentrations of AM plants in our experiment. The functional role of mycorrhizins is however yet to be determined. The metabolic indicators of stress (sugars and proteins) seemed to indicate that the AM plants had relatively less degradation of these metabolites compared to non-AM plants under water deficit conditions.

The third objective was to examine nutritional and reproductive responses of maize to AM colonization during and after drought stress at tasselling. The AM

association improved the nutritional status of maize through the enhanced uptake of slowly diffusing mineral ions such as PO_4^- , Cu^{2+} and Zn^{2+} (Chapter 4). This indirectly helps the plants to utilize the soil available water more effectively. Numerous studies have demonstrated conclusively that AM colonized plants are much more efficient at taking up soil P than non-AM plants (Smith and Gianinazzi-Pearson, 1988; Subramanian and Charest, 1997; Subramanian et al., 1997). Extraradical mycelium of AM fungi increases host plant P uptake by growing beyond the rhizospheric zone around the roots and by providing access to the P which is otherwise transported by the slow diffusion processes (Jakobsen et al., 1992).

In the past, it was generally believed that AM association was less important for N than P nutrition of the host plant, especially in tropical soils. This was due to the fact that NO_3^- ions are the predominant form of N in tropical soils and they are highly mobile in moist conditions. Drought impedes the mobility of NO_3^- ions due to their low concentration and diffusion rate. Under such conditions, AM fungi may be crucial for host plant N nutrition. Our data indicated that the total N content in drought-stressed maize plants were nearly doubled in the presence of AM association (Chapters 4 & 6). This strongly suggests that AM colonization may be an important factor in the host plant N acquisition under drought conditions. The ^{15}N studies have revealed that extraradical mycelium of AM fungi plays a vital role in transporting N from the soil to the host plants (Frey and Schüepp, 1993; Johansen et al., 1994; Tobar et al., 1994a). In these studies, the hyphal contribution of N was estimated at 30-40% of the total N uptake. The AM hyphal uptake and translocation of N may alter the host plant N assimilation (Chapter 6) which

may be a potential factor involved in drought tolerance. Our data in conjunction with other experimental evidence of ^{15}N studies indicate the significance of AM fungi to host plant N nutrition under water deficit conditions. This may contradict the conventional view that in AM association N transfer is only of secondary importance (Read, 1989).

A part of the third objective was to study whether the improved nutritional status in relation to AM association altered the reproductive behaviour in tropical maize cultivars. The enhanced uptake of N, P and other micronutrients assisted AM plants to grow faster, and resulted in significant reduction in days to silking and anthesis-silking interval (ASI) in the drought-sensitive cultivar C0 under well-watered and drought-stressed conditions (Chapter 4). Such a modification in flowering is very important in terms of agronomic advantage because ASI alone constitutes 70% of the yield variation in maize exposed to water deficit. Shortening of ASI by 2-3 days in C0 could have contributed to its higher grain yield. This is the first report that shows a key contribution of AM association to changes in flowering behaviour of maize.

In this study, the beneficial effect of AM colonization was more pronounced in the drought-sensitive cultivar (C0) as indicated by grain yield and mycorrhizal dependency data. In the drought-sensitive cultivar C0, drought stress reduced the grain yield by 55% when roots were not colonized, while the reduction was only 31% with mycorrhizal association. Drought-stressed C0 AM plants produced grain yield comparable to that of the drought-resistant cultivar (C8) with or without AM colonization under drought conditions. This appears to imply that the drought tolerance attained by the

C0 cultivar due to AM association can be comparable to the drought resistance in the C8 cultivar acquired through the recurrent selection procedure.

Mycorrhizal association also appeared to modify the remobilization of nitrogen and carbon in order to assist kernel development. Westgate and Boyer (1985) have shown that under normal growth conditions, the carbohydrate and nitrogen contents in the leaves and stalks are remobilized to support kernel growth. It has been estimated that about 60-80% of the N was remobilized from the leaves to the kernels at the flowering stage in maize (Ta and Weiland, 1992). Thus the majority of carbon and nitrogen delivered to the developing kernels is derived from current photosynthates and NO_3^- reduction. Photosynthesis and nitrate reduction in leaves are usually inhibited by low water status. The kernel growth depends mainly on the remobilization of carbon and nitrogen reserves and this process is generally suppressed by drought. The mycorrhizal association modifies the remobilization process of nutrients and assimilates. Higher N and P contents were measured in the grains of the drought-stressed maize plants in the presence of AM association. In addition, in the same experiment, I measured higher sugar concentrations in the ear leaves of AM plants indicating that these plants were able to supply sufficient amounts of carbon to the developing kernels. These findings suggest that mycorrhizal colonization assists the plants in mobilizing considerable amounts of carbohydrates and minerals from the source (leaf) to the kernels (sink) thus alleviating the loss of grain yield under water deficit conditions (Chapter 4).

The fourth objective was to assess the progression of drought recovery of the maize cultivars in the presence or absence of AM inoculation. In this study, AM maize

plants recovered twice as rapidly as non-AM plants as determined by the time required to return to a LWP comparable to the values of well-watered plants (Subramanian et al., 1997, Chapter 5). This suggests that AM plants recovered more quickly from drought, and this rapid recovery may be due in part to their higher sugar concentration which might have helped the plants to osmotically adjust and regain leaf water status. The recovery of AM plants may also be related to the improved P status which assists these plants to regain LWP in a shorter period of time through the enhanced root growth.

The fifth objective was to examine the effects of AM colonization on the levels of major enzymes involved in N assimilation and to relate these activities as a potential factor in host plant drought tolerance. Our data indicated that mycorrhizae benefit the host plants by enhancing NO_3^- assimilation in water limited environment. The enhancement of N assimilation in AM plants may be attributed to the hyphal transport of N either in the form of NO_3^- (Johansen et al., 1994) or as amino acids (Johansen et al., 1996). These forms of N may serve as substrates for N assimilating enzymes in the host plant. As drought stress restricts the mobility of NO_3^- ions in soil, mycorrhizal plants have access, through the extraradical mycelium, to the forms of N which are usually unavailable to the non-AM plants (Azcón-Aguilar et al., 1993).

We have shown that nitrate reductase (NR), glutamine synthetase (GS) and glutamate synthase (GOGAT) activities in AM roots increased by 45-75% in non-AM plants under drought conditions. Such an increase in AM roots suggests two scenarios: either AM fungi induce an increase of these enzyme activities in roots or AM fungal colonized structures have such enzyme activities. Recently, Johansen et al. (1996)

indicated the presence of a functional GS-GOGAT system in the extraradical mycelium of the AM fungi. This may correspond to our concurring observation of increased GS and GOGAT activities in AM colonized roots. In addition, we detected 25-75 % higher N assimilating enzyme activities in AM shoots, suggesting that mycorrhizal association assisted the plants to transport considerable amounts of NO_3^- from the roots to the shoots for further reduction and assimilation under drought conditions (Subramanian and Charest, 1998). The AM plants were shown to maintain higher water status (Chapters 2 & 5) and to be photosynthetically more efficient (Ruiz-Lozano et al., 1995), and they appeared to supply carbon for NO_3^- reduction and assimilation under water deficit conditions. We have shown a highly significant correlation (**non-mycorrhizal** $r^2 = 0.80^{***}$; **mycorrhizal** $r^2 = 0.56^{***}$) between leaf water potential and GS activity in shoots (Appendix 10). This suggests that water status of the host plant has a direct impact on N assimilation. Even after three weeks of recovery, the AM plants continued to maintain higher N assimilation enzyme activities implying that mycorrhizae may be a critical factor for the drought recovery of host plants. Interestingly, the most abundant amino acids detected in AM roots of our experiments were consistent with the free amino acid pool in the extraradical mycelium of the same *Glomus* sp. in another study (Johansen et al., 1996). In an elegant petri plate system using carrot transformed roots, Shachar-Hill et al. (1997) have shown that the extraradical mycelium assimilated most of the added inorganic ^{15}N or urea into free amino acids (Arg, Asp, Asn, Gln) and that these amino acids were subsequently transferred to the host plant. This clearly indicates that the amount of N being assimilated in the extraradical mycelium has a greater impact on N

assimilation pathways in the host plant. Our data support the active participation of AM fungi on host plant N assimilation pathways under drought conditions. This appears to be one of the potential mechanisms related to the host plant drought tolerance.

The overall altered host plant physiological processes induced by AM association may increase tolerance to a number of other stresses including mineral deficiencies, heavy metal toxicities and high salt concentrations (Sylvia and Williams, 1992). The AM fungi alleviate deficiencies of immobile nutrients (P, Zn, Cu) in the host plant through the rapid transport of these minerals from the soil by the extraradical mycelium (Jakobsen et al., 1992; Evans and Miller, 1988; Li et al., 1991). Mycorrhizal association protects the plants from heavy metal toxicities by sequestering them in polyphosphates of the AM fungus (Turnau et al., 1993). The AM fungi are also known to reduce the incidence of root diseases and nematodes as a result of mycorrhizae-mediated changes in host physiology (Linderman, 1994). Benhamou et al. (1994) observed a direct inhibitory effects of AM hyphae on wilt causing pathogen (*Fusarium oxysporum* f.sp.*chrysanthemi*) in a root-organ culture system. The use of mycorrhizal fungi as biocontrol agents has not yet been widely explored. External to the roots, mycorrhizal fungi can alter the chemical and physical properties of soil due to the effects of the extraradical hyphae (Smith and Read, 1997). As the AM plants are nutritionally rich, these plants can modify the quality and quantity of root exudates, resulting in a new microbial equilibrium in the rhizospheric soil, called the mycorrhizosphere. The chemical and physical effects of the fungal symbiont extending out into the soil creates a whole dimension, both spatially and biologically (Linderman, 1992). The extraradical mycelium of AM fungi exude organic

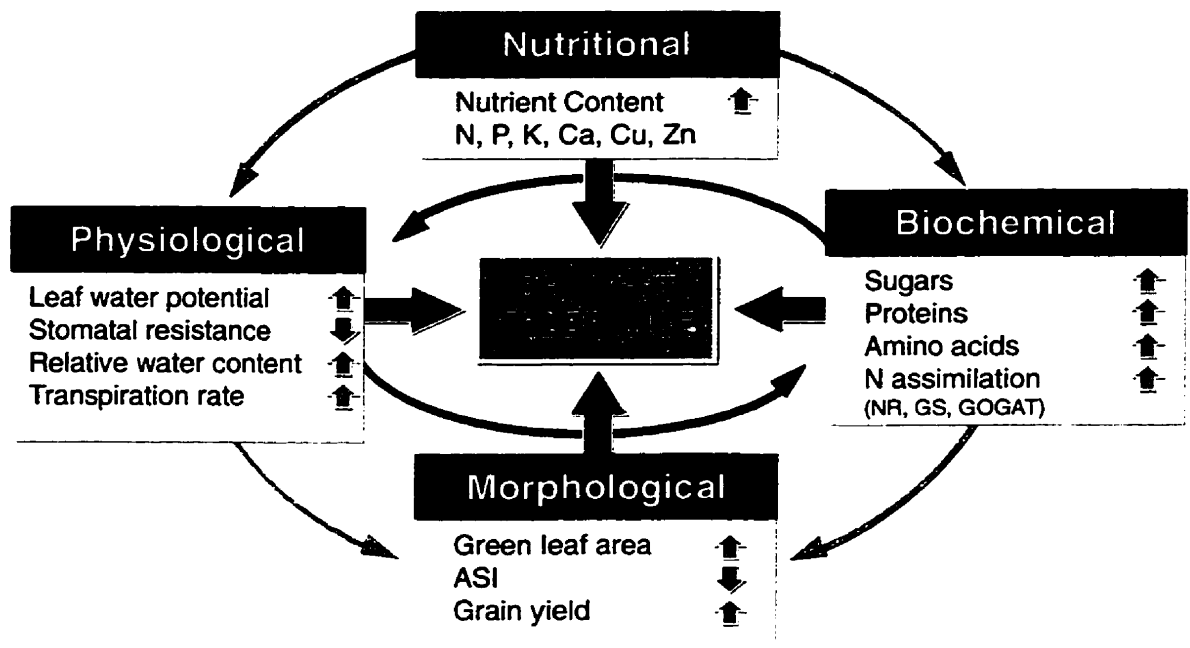
materials that are substrates for other soil microbes. Secilia and Bagyaraj (1987) detected greater bacterial population in mycorrhizae-treated soil than non-inoculated soil. These hyphal associates frequently produce sticky material that cause soil particles to adhere and form aggregates (Oades, 1993). Thus mycorrhizae can provide all aspects of a protective environment to plant growth, and play a major role in the quest for sustained plant productivity in agriculture.

7.2. Conclusions

This thesis has clearly shown that arbuscular mycorrhizal (AM) colonization promotes the tolerance of the two tropical maize cultivars under moderate controlled drought stress conditions. The potential factors involved in mycorrhizae-assisted drought tolerance are summarized in Figure. 7.1. The host plant water relations were modified in some ways by the mycorrhizal interactions. The drought tolerance was achieved due to the nutritional, physiological, biochemical and morphological modifications in the host plants. The mechanisms involved in mycorrhizae-assisted drought tolerance are quite complex, but most of the effects can be related to the changes in nutritional status, especially N and P. This provided an ability for the AM maize plants to maintain higher water status under moderate drought conditions and recover rapidly when irrigation was restored. Since the AM colonized soil retained relatively higher moisture content, these plants could have been exposed to less strain and appeared to keep the stomata partially open longer and carry out photosynthetic functions. As a result of higher water status and possibly by better photosynthetic efficiency, the AM colonized plants retained larger

Figure 7.1. Potential factors involved in mycorrhizae-assisted drought tolerance in maize. Nutritional, physiological, biochemical and morphological changes in AM colonized maize plants under drought conditions are summarized. The arrows within a box represent changes in the mycorrhizal plants i.e. increase (up) or decrease (down) in comparison to the nonmycorrhizal plants under drought conditions.

Mycorrhizal effects on drought tolerance in maize



green leaf area under moderate drought conditions. Mycorrhizal maize plants also retained significant amounts of sugars and nitrogenous compounds that may have contributed for the drought tolerance.

Mycorrhizal association altered growth and reproductive behaviour of tropical maize cultivars under water deficit conditions. The improved nutritional status assisted the AM maize plants to grow faster which resulted in shortening of anthesis-silking interval (ASI), especially in the drought-sensitive cultivar (C0). The AM plants were able to remobilize sufficient amounts of nutrients and assimilates from the leaves to the developing kernels thus alleviating loss of grain yield. The response to mycorrhizal association was more pronounced for the drought-sensitive than -resistant maize cultivars as indicated by the grain yield and mycorrhizal dependency data, under moderate drought conditions.

Mycorrhizal colonization participates actively in N acquisition and assimilation of the host plant especially under drought conditions. I detected 25-75% higher activities of the major enzymes involved in N assimilation in AM plants, suggesting that mycorrhizal association helped the plants to transport considerable amounts of NO_3^- from the roots to shoots for further reduction and assimilation under drought conditions. The AM plants continued to maintain higher N enzyme activities even after three weeks of rewatering, indicating that mycorrhizae may be a crucial factor for drought recovery of the host plant. Such an increase in AM plants suggests two scenarios: either AM fungi induce an increase of these enzyme activities in roots or AM fungal structures have such enzyme activities.

My thesis has provided potential insights for the research advancement in the sphere of mycorrhizae-assisted drought tolerance. Drought restricts the mobility of mineral ions in general and particularly nitrate ions. Under such circumstances, mycorrhizal association may play a crucial role in N nutrition, therefore contributing to the plant growth and productivity. I presented evidence to support the active participation of mycorrhizal association on N assimilation pathway in the host plants. This provides an ability for the AM plants to sustain especially under water deficit conditions. I have also indicated that mycorrhizal association assisted the plants to remobilize minerals and metabolites from the leaves to the developing kernels and this alleviates the grain yield loss due to drought. In addition, this is the first report that suggests an important role of mycorrhizal symbiosis on alteration in maize flowering behaviour. These findings contribute to understand the role of mycorrhizae in N cycling and developing management strategies for improved N economies of agricultural crop plants. My Ph.D work provided sufficient evidence to consider the “use of mycorrhizal association” as an alternative drought management strategy in sustainable agriculture.

7.2. Implications of the present study in the alternative drought management strategy

Drought is the major constraint for agricultural production in arid and semiarid areas causing considerable yield losses. Modern farming practices have been developed to combat drought effects in agricultural crops. But these practices are rarely adopted by farmers in drought-prone areas due to practical difficulties. For economic and

environmental reasons, the National Research Council of USA (NRC, 1989) recommended sustainable farming options to “reduce costs, protect health and environmental quality and enhance beneficial biological interactions and natural processes”. Mycorrhizal fungi are ubiquitous beneficial organisms that might be considered in the design of sustainable systems. However, current agricultural practices do not yet take into account the mycorrhizal symbioses.

The most important aspect of drought tolerance in an agricultural context is the pattern of the water supply in relation to the crop water requirement. In our experimental model, maize plants were exposed to a short-term drought at a critical stage that coincides with the establishment of a functional mycorrhizal symbiosis from which the host plant is likely to benefit the most. Mycorrhizal colonization alters the host plant responses which include physiological, biochemical and nutritional changes. Such changes may be of adaptive value in the sense that they contribute to productive processes that lessen the impact of drought on yield. Some of these changes may assist the plants for their survival under water deficit environment.

Mycorrhizal fungi are an integral part in natural agricultural soils. From a sustainability perspective, it would be useful to identify how the benefits from these organisms can be maximized in order to endure agricultural productivity. There are several recommendations that can enhance the function of AM fungi in the agricultural system. Soil disturbance has been shown to have a major effect on the mycorrhizal symbiosis, reducing root colonization. No till system is appropriate to realize the maximum benefits of AM fungi (Anderson et al., 1987). The literature on the effects of

fertilizers on colonization is controversial. In most cases, high levels of fertilizer application suppress AM colonization (Johnson and Pflieger, 1992). Because AM fungi are obligate symbionts and thus highly dependent on the plant, proper crop rotation is necessary in order to maintain their population dynamics in the soil (Harinikumar and Bagyaraj, 1988). Most fungicides are shown to be detrimental to either the root colonization or function of the AM fungi (Johnson and Pflieger, 1992). Rationalization of their use by selection of only fungicides that do not interact negatively with mycorrhizal association may bring significant benefits. These data suggest that a coordinated approach is needed to realize the full benefits of AM symbiosis.

7.3. Future work

The increased interest in mycorrhizae and their interaction with host plant drought tolerance are an exciting and promising area of research. With addition of more greenhouse and field experiments, much can be learned about mycorrhizae and plant water use since the available literature for the past two decades has brought up intriguing ideas. This thesis has answered some questions pertaining to mycorrhizae-assisted drought tolerance in the two tropical maize cultivars. During the accomplishment of this thesis more questions and ideas for further avenues of exploration have been arisen. To this end, future work should include the following:

The mechanisms involved in AM-assisted host plant drought tolerance are quite complex, but most of the effects appeared to be related to changes in nutritional status. The question of whether direct or indirect effects of mycorrhizal association induced the

host plant drought tolerance remains questionable. Very recently, MacFall and Johnson (1997) have developed a magnetic resonance imaging (MRI) technique to visualize the transport of water through roots and ectomycorrhizal hyphae associated with pine seedlings. Further advancement in this research extended to endomycorrhizal fungi may resolve an important question as whether hyphal contribution constitutes a significant part in host plant water relations.

We have observed that the modifications in host plant N assimilation may be as a result of the substrate contribution by the extraradical mycelium of AM fungi. The determination of uptake and assimilation of inorganic N by the extraradical mycelium may answer this question. A compartmental box system or a dual *in vitro* culture system may allow to carry out this experiment using ^{15}N tracer.

Mycorrhizal association is usually considered non-specific but this relationship is tightly regulated by both structural and physiological levels. Little is known about physiological specialization and functioning of AM fungi. The knowledge concerning functional compatibility between plants and AM fungi is important for successful utilization of these microorganisms in particular environments.

There is a clear indication that during the establishment of a functional mycorrhizal association, the host plants are induced to produce specific proteins or polypeptides (mycorrhizins). The qualitative and quantitative modifications in mycorrhizins may play a protective role in host plant tolerance against abiotic and biotic stresses.

The beneficial effects of the mycorrhizal fungi on host plant physiology may be as a consequence of molecular interaction between the two symbiotic partners. Identifying the genes involved in the interaction is a prerequisite for a greater understanding of the functional role of this symbiosis. Research on these topics is relatively recent and much work has to be done to gain insight in the molecular-genetic regulation of the symbiosis.

Most mycorrhizal research have been conducted in controlled greenhouse or growth chamber conditions. Relatively little information exists on the function of AM in field environments. The potential uses of mycorrhizae in agriculture may be realistic if more field experiments are done. Although significant advances have been accomplished in the recent past toward understanding the role of mycorrhizae, the inherent complexities of their function within ecosystems should be explored extensively. Application of AM fungi in future agricultural management will depend to a large degree on our ability to identify the specific functions that AM fungi are performing within a particular field system and integrate these findings into management strategies.

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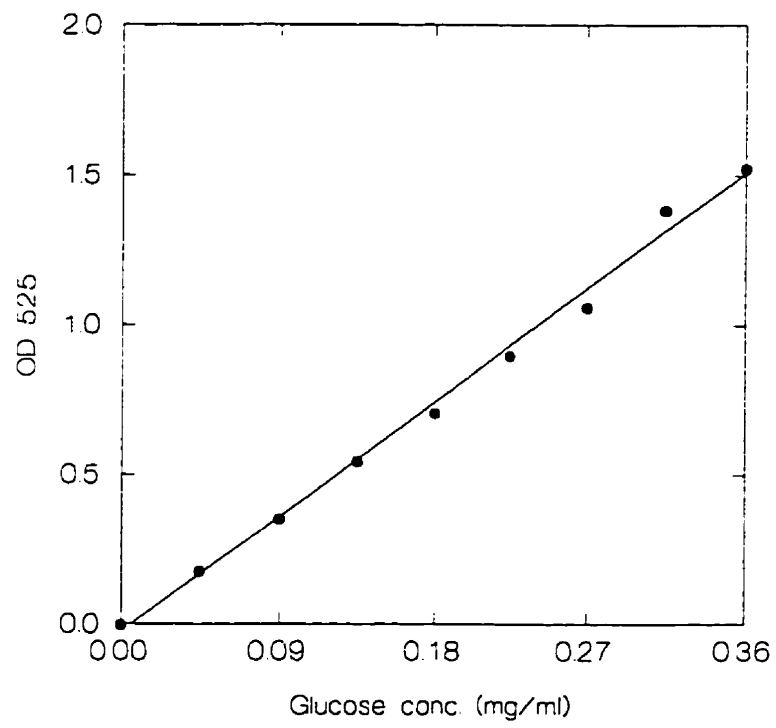
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Appendix 1. A standard curve of glucose for the determination of sugar concentrations in plant tissues.

<i>Glucose conc. (mg/ml)</i>	<i>OD₅₂₅</i>
0.000	0.000
0.045	0.180
0.090	0.355
0.135	0.547
0.180	0.708
0.225	0.900
0.270	1.063
0.315	1.386
0.360	1.526

Regression output

Constant	0
Std Err of Y Est	0.0099628
R squared	0.9934645
No. of observations	9
Degrees of Freedom	8
X Coefficients	0.2401603
Std Err of Coef.	0.0037263

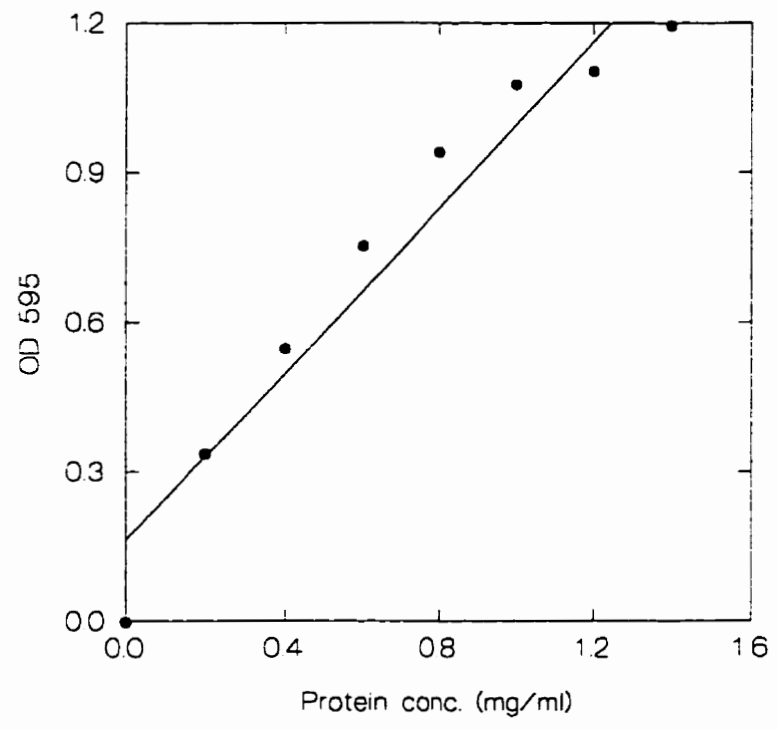


Appendix 2. A standard curve of BSA (bovine serum albumin) for the determination of protein concentrations in plant tissues.

<i>Protein conc. (mg/ml)</i>	<i>OD₅₉₅</i>
0.0	0.000
0.2	0.338
0.4	0.549
0.6	0.755
0.8	0.943
1.0	1.079
1.2	1.105
1.4	1.196

Regression output

Constant	0
Std Err of Y Est	0.1415283
R squared	0.9165406
No. of observations	8
Degrees of Freedom	7
X Coefficients	0.9795099
Std Err of Coef.	0.0593286



Appendix 3. A conversion table for the determination of volumetric soil moisture content (SMC)

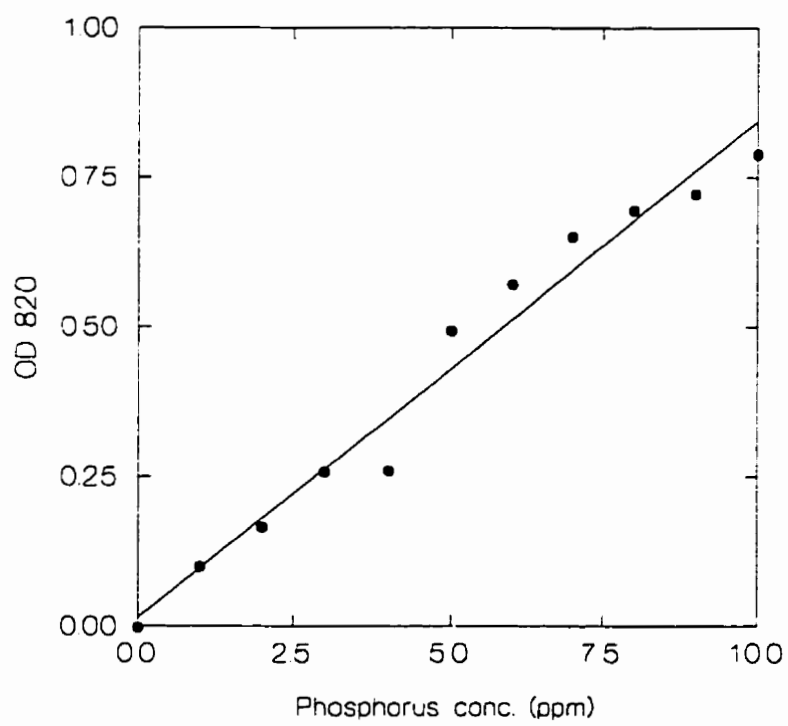
<i>Reading</i>	<i>SMC (%)</i>	<i>Reading</i>	<i>SMC (%)</i>	<i>Reading</i>	<i>SMC (%)</i>
3.0	6.6	5.1	24.0	7.2	40.6
3.1	7.4	5.2	24.9	7.3	41.3
3.2	8.1	5.3	25.7	7.4	41.9
3.3	8.9	5.4	26.6	7.5	42.6
3.4	9.6	5.5	27.5	7.6	43.2
3.5	10.4	5.6	28.3	7.7	43.8
3.6	11.2	5.7	29.1	7.8	44.4
3.7	12.0	5.8	30.0	7.9	45.0
3.8	12.9	5.9	30.8	8.0	45.6
3.9	13.7	6.0	31.6	8.1	46.2
4.0	14.5	6.1	32.4	8.2	46.7
4.1	15.4	6.2	33.2	8.3	47.3
4.2	16.2	6.3	34.0	8.4	47.8
4.3	17.1	6.4	34.8	8.5	48.4
4.4	17.9	6.5	35.6	8.6	48.9
4.5	18.8	6.6	36.3	8.7	49.4
4.6	19.7	6.7	37.1	8.8	50.0
4.7	20.5	6.8	37.8		
4.8	21.4	6.9	38.5		
4.9	22.3	7.0	39.2		
5.0	23.2	7.1	39.9		

Appendix 4. A standard curve for the determination of phosphorus concentration in plant tissues.

<i>P conc. (ppm)</i>	<i>OD₈₂₀</i>
0.0	0.000
1.0	0.102
2.0	0.168
3.0	0.260
4.0	0.262
5.0	0.495
6.0	0.573
7.0	0.653
8.0	0.697
9.0	0.724
10.0	0.790

Regression output

Constant	0
Std Err of Y Est	0.5759993
R squared	0.9698386
No. of observations	11
Degrees of Freedom	10
X Coefficients	11.657267
Std Err of Coef.	0.3436907

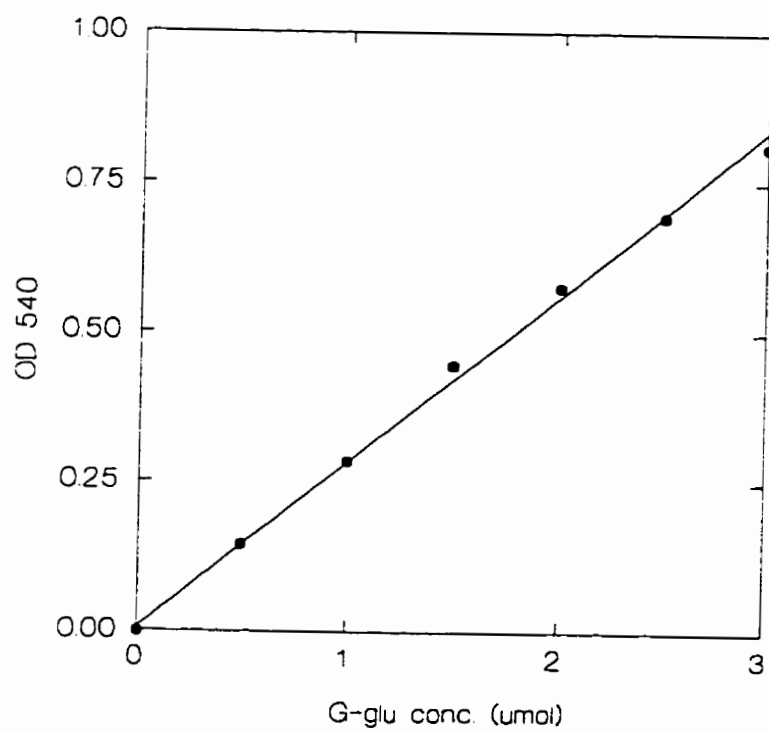


Appendix 5. A standard curve of gamma-glutamylhydroxamate concentration for the determination of GS (glutamine synthetase) activity in plant tissues.

<i>Gamma-glu. conc. (umol)</i>	<i>OD₅₄₀</i>
0.0	0.000
0.5	0.147
1.0	0.286
1.5	0.447
2.0	0.577
2.5	0.696
3.0	0.812

Regression output

Constant	-0.045914
Std Err of Y Est	0.0631232
R squared	0.9971539
No. of observations	7
Degrees of Freedom	6
X Coefficients	3.6497136
Std Err of Coef.	0.0872002



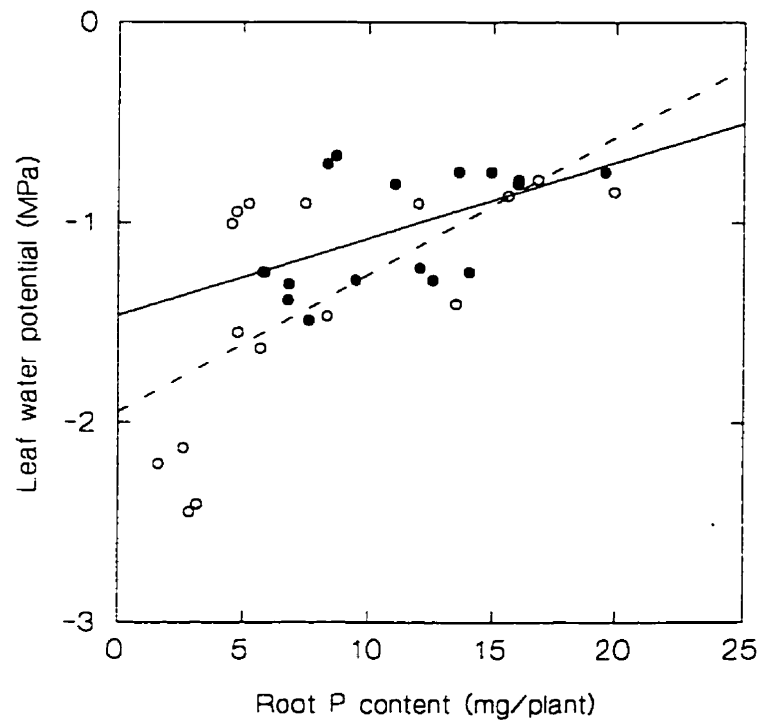
Appendix 6. Linear regression analysis of relationship between leaf water potential (LWP) and root phosphorus content (RP). Different regressions are fitted to each group (non-mycorrhizal in dashed line and mycorrhizal in solid line). Standard errors (SEs) are given in parentheses. (* $P < 0.05$; *** $P < 0.001$)

Non-mycorrhizal (empty circles)

$$\text{LWP} = -1.95 (\pm 0.21) + 0.07 (\pm 0.02) \times \text{RP} \quad n = 16 \quad r^2 = 0.66^{***}$$

Mycorrhizal (filled circles)

$$\text{LWP} = -1.46 (\pm 0.20) + 0.04 (\pm 0.02) \times \text{RP} \quad n = 16 \quad r^2 = 0.52^*$$



Appendix 7. Linear regression analysis of relationship between leaf water potential (LWP) and shoot phosphorus content (SP). Different regressions are fitted to each group (non-mycorrhizal in dashed line and mycorrhizal in solid line). Standard errors (SEs) are given in parentheses. (* $P < 0.05$; *** $P < 0.001$)

Non-mycorrhizal (empty circles)

$$\text{LWP} = -2.05 (\pm 0.25) + 0.02 (\pm 0.00) \times \text{SP} \quad n = 16 \quad r^2 = 0.63***$$

Mycorrhizal (filled circles)

$$\text{LWP} = -1.32 (\pm 0.16) + 0.01 (\pm 0.00) \times \text{SP} \quad n = 16 \quad r^2 = 0.49*$$

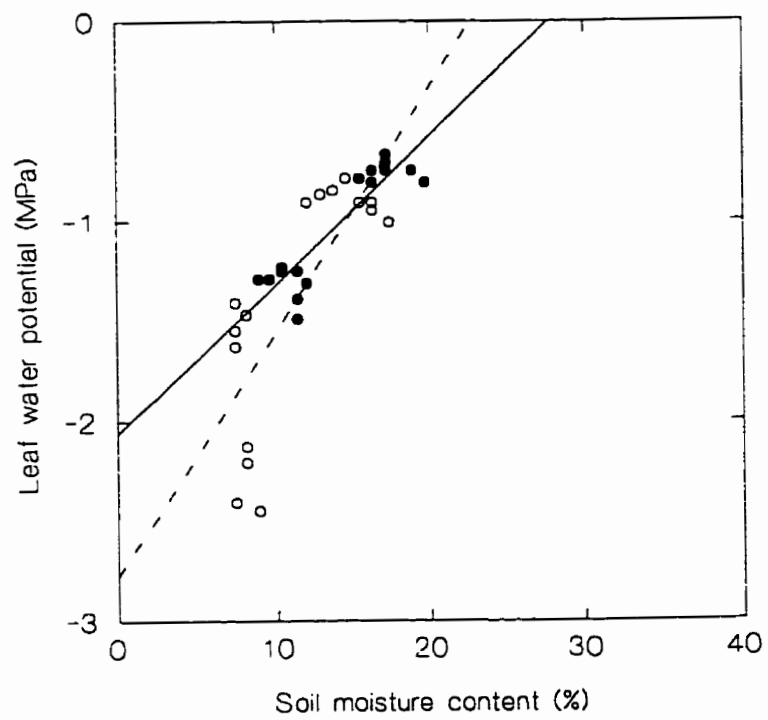
Appendix 8. Linear regression analysis of relationship between leaf water potential (LWP) and soil moisture content (SMC). Different regressions are fitted to each group (non-mycorrhizal in dashed line and mycorrhizal in solid line). Standard errors (SEs) are given in parentheses. (***) $P < 0.001$

Non-mycorrhizal (empty circles)

$$\text{LWP} = -2.78 (\pm 0.32) + 0.12 (\pm 0.03) \times \text{SMC} \quad n = 16 \quad r^2 = 0.77^{***}$$

Mycorrhizal (filled circles)

$$\text{LWP} = -2.06 (\pm 0.14) + 0.07 (\pm 0.01) \times \text{SMC} \quad n = 16 \quad r^2 = 0.90^{***}$$



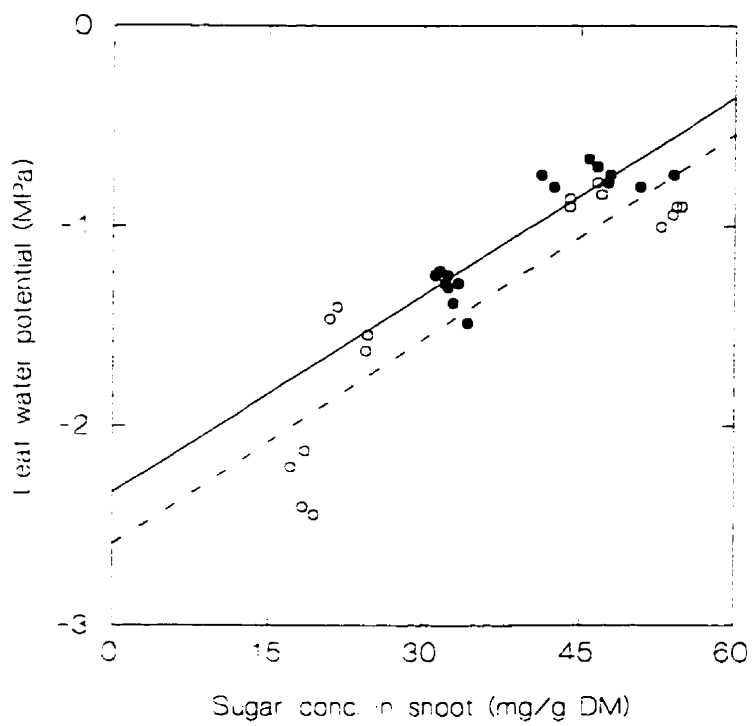
Appendix 9. Linear regression analysis of relationship between leaf water potential (LWP) and sugar concentration in shoot (SS). Different regressions are fitted to each group (non-mycorrhizal in dashed line and mycorrhizal in solid line). Standard errors (SEs) are given in parentheses. (******* $P < 0.001$)

Non-mycorrhizal (empty circles)

$$\text{LWP} = -2.59 (\pm 0.19) + 0.03 (\pm 0.01) \times \text{SS} \quad n = 16 \quad r^2 = 0.87^{***}$$

Mycorrhizal (filled circles)

$$\text{LWP} = -2.33 (\pm 0.18) + 0.03 (0.00) \times \text{SS} \quad n = 16 \quad r^2 = 0.89^{***}$$



Appendix 10. Linear regression analysis of relationship between leaf water potential (LWP) and glutamine synthetase (GS) activity in shoot. Different regressions are fitted to each group (non-mycorrhizal in dashed line and mycorrhizal in solid line). Standard errors (SEs) are given in parentheses.

Non-mycorrhizal (empty circles)

$$\text{GS} = 131.6 (\pm 12.8) + 42.3 (\pm 8.5) \times \text{LWP} \quad n = 16 \quad r^2 = 0.80^{***}$$

Mycorrhizal (filled circles)

$$\text{GS} = 251.0 (\pm 30.4) + 129.0 (\pm 28.6) \times \text{LWP} \quad n = 16 \quad r^2 = 0.77^{***}$$

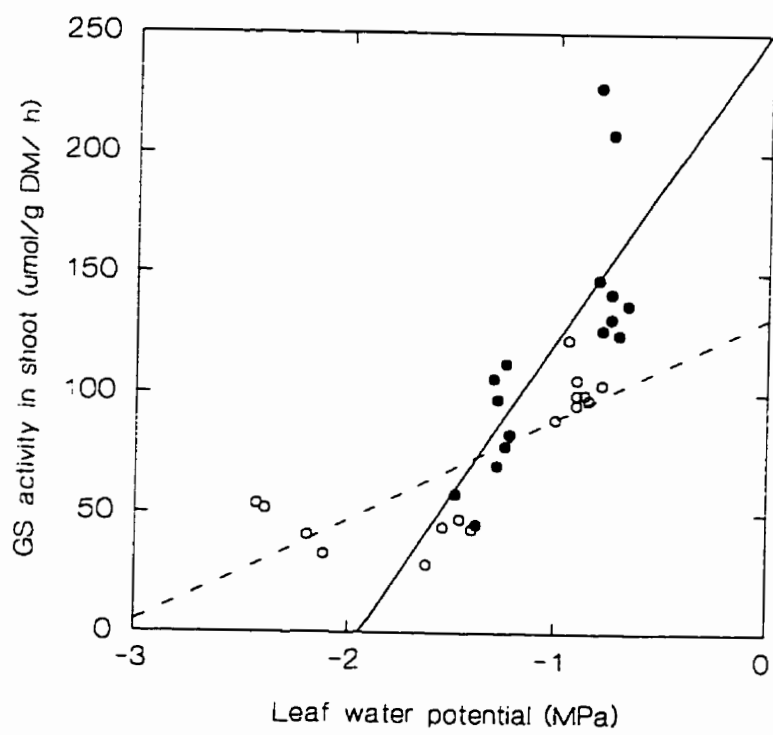
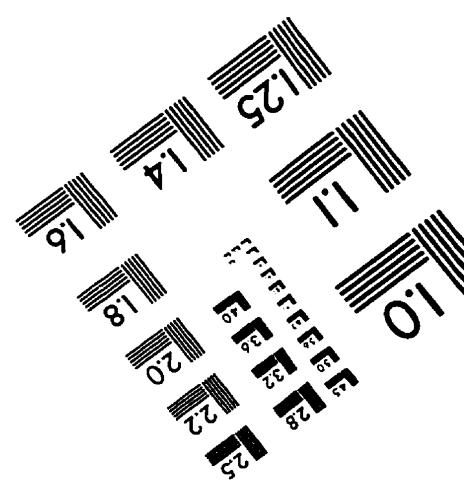
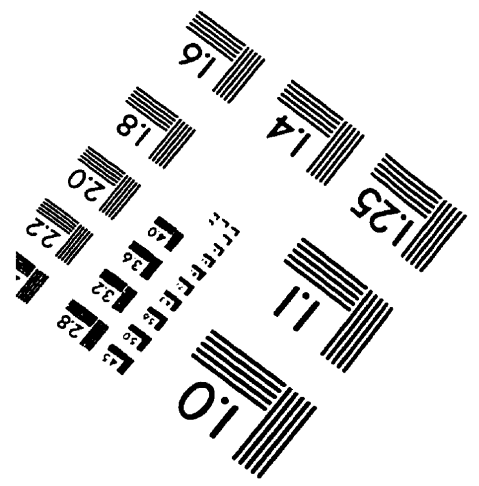
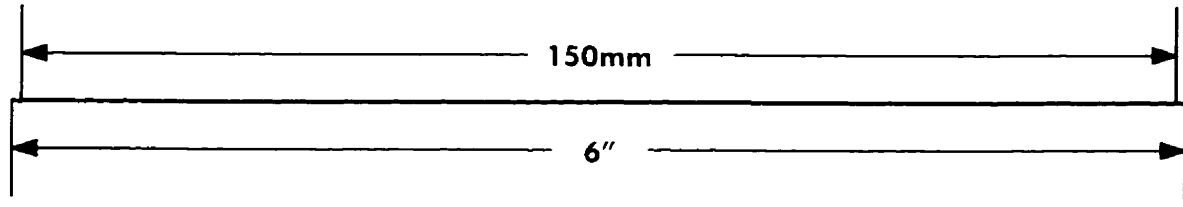
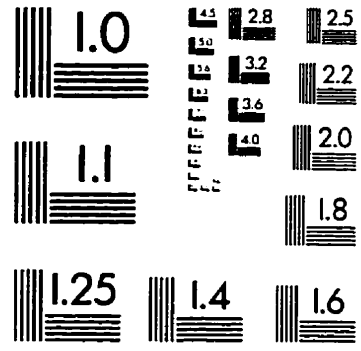
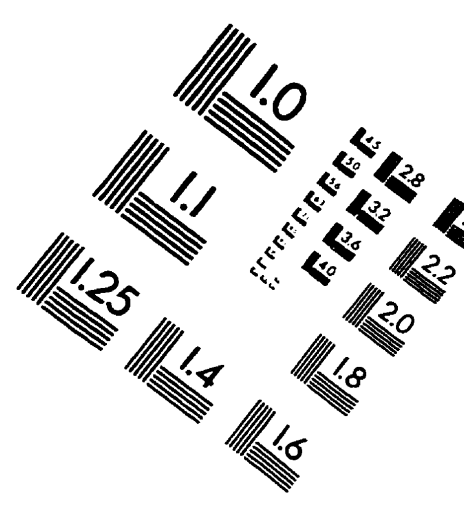
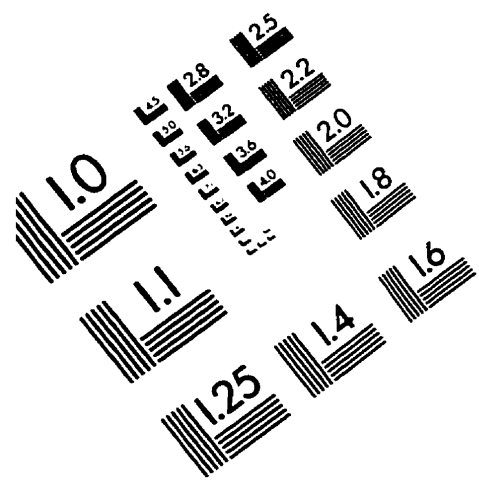


IMAGE EVALUATION TEST TARGET (QA-3)



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