

Kizhaeral S. Subramanian · Christiane Charest

## Influence of arbuscular mycorrhizae on the metabolism of maize under drought stress

**Abstract** A greenhouse experiment was carried out to investigate the influence of the arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck & Smith) on metabolic changes in tropical maize (*Zea mays* L.) under drought. Two cultivars, Tuxpeno sequia C0 (drought sensitive) and C8 (drought resistant), were subjected for 3 weeks to water stress following tasselling (75–95 days after sowing). Fully expanded 7th or 8th leaves were sampled and assessed for levels of chlorophyll, sugars, proteins, and amino acids. Chlorophyll content was not altered either by water stress or the presence of mycorrhizae. Mycorrhizal plants (M+) had higher total and reducing sugars than nonmycorrhizal plants (M–) at the end of 3 weeks of the drought cycle. An increase in protein content was observed with drought stress in M+ plants of the cultivar C0. Most of the amino acids showed a linear increase during the period of water stress in M+ and M– plants for both cultivars. Total amino acids increased by 40.6% and 43.7% in M– plants of C0 and C8, respectively. With the presence of AM fungus, amino acid levels increased by only 10.7% and 19.2% of leaf dry mass in C0 and C8, respectively. Alanine, asparagine, glutamine, and glycine accounted for 70% of the amino acid pool. Under drought, AM inoculation enabled the plants to retain considerable amounts of sugars and proteins, especially in the drought-sensitive cultivar C0. This may be of physiological importance in helping the plant to withstand moderate drought.

**Key words** Drought stress · Tropical maize cultivars *Glomus intraradices* · Metabolic changes

### Introduction

Water 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 sugars (Iljin 1957; Kameli and Lösel 1993), amino acids (Brunk et al. 1989; Good and Zaplachinski 1994), and organic acids (Timpa et al. 1986). The metabolic changes are believed to promote drought tolerance in plants by maintaining turgor through osmotic adjustment, i.e. a net increase in solutes leading to a lower osmotic potential (Morgan 1984). Kameli and Lösel (1993) reported that glucose accumulated in wheat under drought more rapidly and to a higher concentration in drought-resistant than in drought-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. 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 for sugars during water 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). Leaf proteins play a vital role in catalytic reactions and as a source of reduced N for vegetative and reproductive growth (Hanson and Hitz 1982).

Arbuscular mycorrhizal (AM) fungi are known to stimulate growth (McArthur and Knowles 1993) and to increase tolerance to extreme conditions such as drought (Nelsen 1987; Kothari et al. 1990) and chilling (Charest et al. 1993). The tolerance is mainly attributed to changes in the host's rate of photosynthesis (Harris et al. 1985), or levels of carbohydrates (Nemec and Guy 1982) and proteins (Nemec and Meredith 1981; Dumas et al. 1990). Higher chlorophyll and leaf starch levels were observed in mycorrhizal rose plants under water

K. S. Subramanian (✉) · C. Charest  
Ottawa-Carleton Institute of Biology, University of Ottawa,  
P. O. Box 450 STN A, Ottawa, Ontario K1N 6N5, Canada  
Tel.: (613) 562-5800; Fax: (613) 562-5178

stress (Augé et al. 1987). Recently, Davis 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 hypothesize 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, we have examined metabolic changes (sugars, proteins and amino acids) in AM and non-AM plants of drought-sensitive and drought-resistant cultivars when water stress was imposed for 3 weeks following tasselling.

## Materials and methods

### Plant material

Greenhouse experiments were conducted at the Central Experimental Farm, Ottawa, using two tropical maize cultivars Tuxpeno sequia C0 and C8 obtained from Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico. The 2 × 2 × 2 factorial experimental design included two cultivars C0 (drought-sensitive) and C8 (drought-resistant), two moisture levels (irrigation once each week throughout the crop period (S-) and irrigation withheld for 3 weeks (S+) following tasselling (75–95 days after sowing), and with (M+) and without (M-) mycorrhizal inoculation. Thus, there were eight treatment combinations replicated four times. Six maize plants were grown in each plastic container (65 cm × 40.6 cm × 42 cm) in vermiculite at 30°C:20°C (day:night) with a 14-h photoperiod, 70% RH, and a light intensity of 800 μmol m<sup>-2</sup> s<sup>-1</sup> provided by high-pressure sodium lamps. Peatmoss (1 l, 300 g) (provided by Les Tourbières Premier Peatmoss, Rivière-du-Loup, Québec) carrying an AM inoculum (*Glomus intraradices* Schenck & Smith) was applied uniformly as a 1-cm layer 5 cm below seeds in each container of M+ plants. The same was performed for M- plants but with inoculum-free peatmoss. All the plants were fertilized with Hoagland's solution (500 ml/pot per week) in the irrigation water. The quantity of water required was predetermined before the start of the experiment (data not shown). Four well-watered plants were removed at 15-day intervals beginning 45 days after sowing until 90 days for the assessment of root colonization. Two plants per container remained at the time of the imposition of the water stress treatment. Irrigation was carried out each week based on the cumulative evapotranspiration rate. Irrigation was withheld from half the M- and M+ plants for 3 weeks at 75 days after sowing. Thereafter, all plants were watered until harvest to compensate for weekly cumulative evapotranspiration.

### Mycorrhizal colonization

Plant roots were stained with aniline blue (Dalpé 1993) before mounting on slides in polyvinyl-alcohol-lactic acid-glycerol. A total of 200 1-cm root segments per cultivar were examined for the presence of arbuscules, vesicles or both, and the percentages of colonization and arbuscules were determined. These measurements were repeated at 6, 8, 10, and 12 weeks after sowing.

**Table 1** Mycorrhizal colonization (%) and arbuscules (%) in the maize cultivars C0 and C8 (*n* = 200)

	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

### 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 contents. Chlorophyll was extracted in 95% ethanol, and assayed by the method of Bruinsma (1963). Sugars were analyzed by the classical method of Nelson as adapted by Potvin and Charest (1991). Leaf protein was extracted by the method of Charest and Phan (1990), and determined according to the method of Bradford (1976). Data collected on chlorophyll, sugars, and proteins at the beginning, middle and end of the experiment were statistically analyzed by a three-way analysis of variance. 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 g 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.

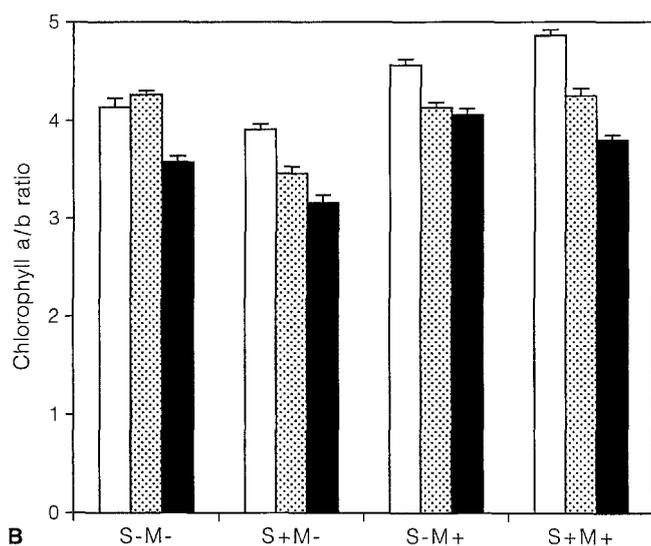
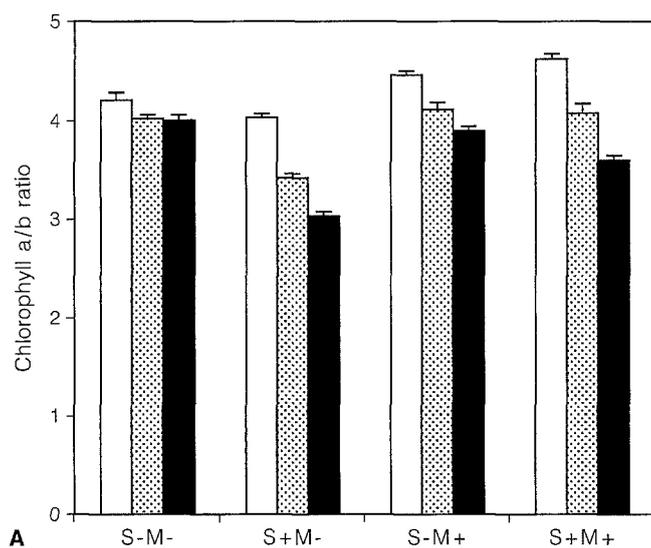
## Results

### AM colonization

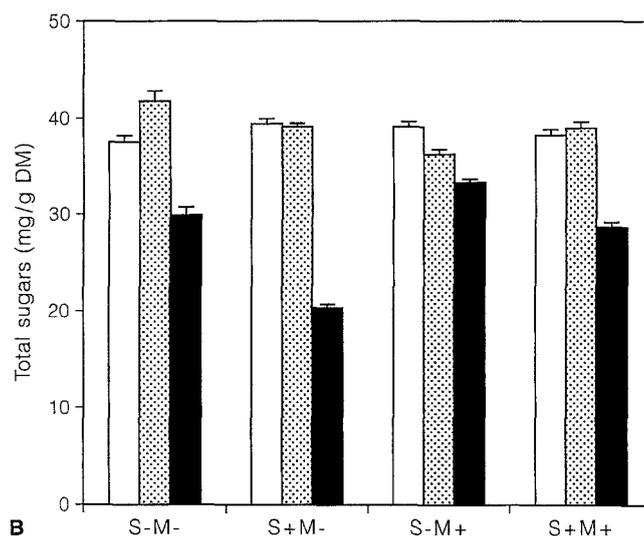
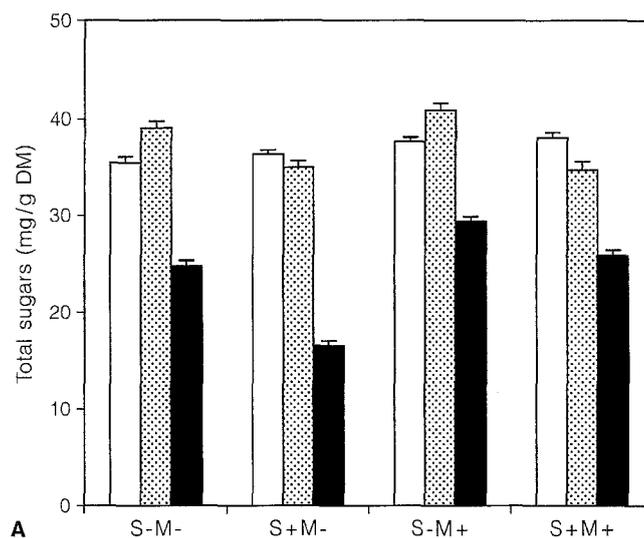
Mycorrhizal fungus colonization in cultivars C0 and C8 increased through 8 weeks, and C8 had higher values than C0 throughout the experiment (Table 1). At week 12, C8 had the highest colonization of 96.5%, which was 25% higher than C0. Arbuscules as a percentage of total colonization declined progressively from 53.2 and 65.3% (6th week) to 13.1 and 13.8% (12th week) in C0 or C8, respectively. Regardless of growth stage, the numbers of arbuscules and vesicles were higher in C8 than C0, but the differences between the cultivars were less pronounced for arbuscules than vesicles (data not shown).

### Chlorophyll

Chlorophyll content in maize leaves was not altered either by moisture level or mycorrhizal colonization in the two cultivars. The average total chlorophyll contents for AM and non-AM plants were 7.85 and 8.09 mg/g for C0 and 7.87 and 8.18 mg/g for C8, respectively. Moisture stress significantly ( $P \leq 0.001$ ) reduced the chlorophyll a/b ratio in C0 and C8 during the drought



**Fig. 1** Chlorophyll a/b ratios in maize cultivars C0 (**A**) and C8 (**B**) in well-watered non-AM (S-M-) and AM (S-M+) and water-stressed non-AM (S+M-) and AM (S+M+) plants at the beginning (empty bars), middle (dotted bars), and end (solid bars) of the experiment. SEMs ( $n=4$ ) are indicated



**Fig. 2** Total sugars in maize cultivars C0 (**A**) and C8 (**B**) in well-watered non-AM (S-M-) and AM (S-M+) and water-stressed non-AM (S+M-) and AM (S+M+) plants at the beginning (empty bars), middle (dotted bars), and end (solid bars) of the experiment. SEMs ( $n=4$ ) are indicated (DM dry mass)

spell and non-AM plants had significantly ( $P \leq 0.001$ ) lower ratios than AM plants (Fig. 1).

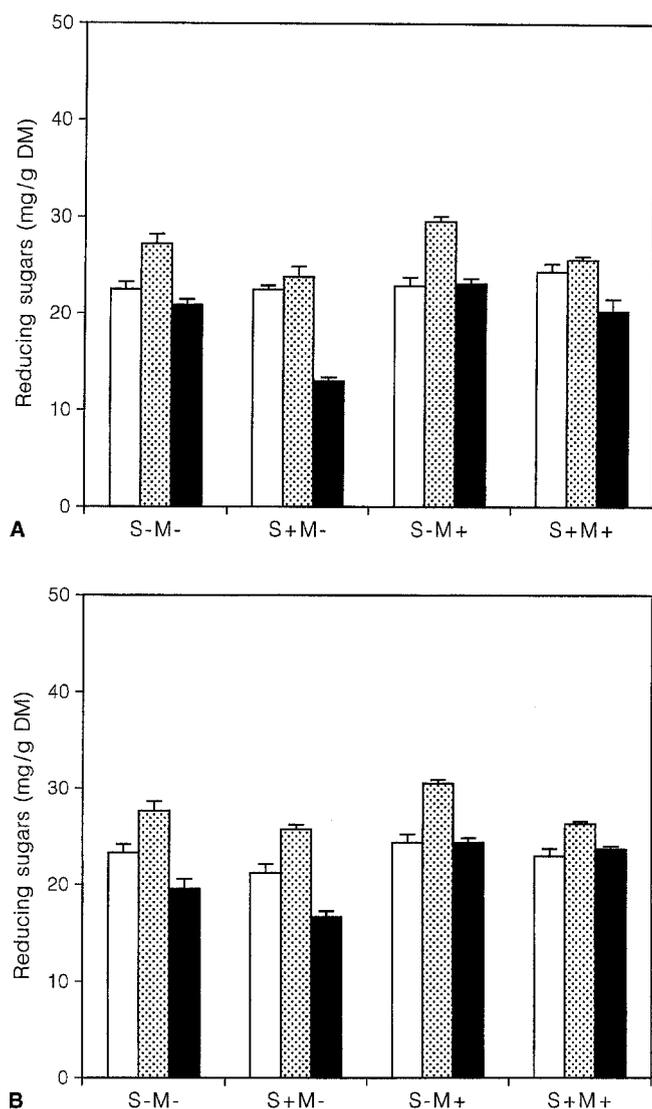
### Sugars

Total sugars in maize plants showed an increase at the middle of the experiment and then declined. Response to AM fungus was lower in unstressed plants (Fig. 2). Total sugar content was significantly ( $P \leq 0.05$ ) reduced in moisture-stressed M+ and M- plants of C0 and C8 compared with unstressed plants. Continuously withheld irrigation of 3 weeks led to a reduction in total sugars by 32.2% in M- plants but only 13.7% in M+ plants. Reducing sugars were significantly altered by

moisture stress ( $P \leq 0.01$ ) and AM colonization ( $P \leq 0.05$  and  $P \leq 0.001$ ) in the two cultivars at the middle and the end of the experiment (Fig. 3). Under water-stressed conditions, M+ plants had higher levels of reducing sugars (C0 20 and C8 23.4 mg/g dry mass) than M- plants (C0 12.8 and C8 16.6).

### Proteins

Protein content in maize leaves of C0 and C8, with or without mycorrhizae, in most cases showed a decrease with the age of the plant (Fig. 4). At the beginning of the experiment, protein content in C8 was significantly ( $P \leq 0.001$ ) higher than C0 and the difference was re-

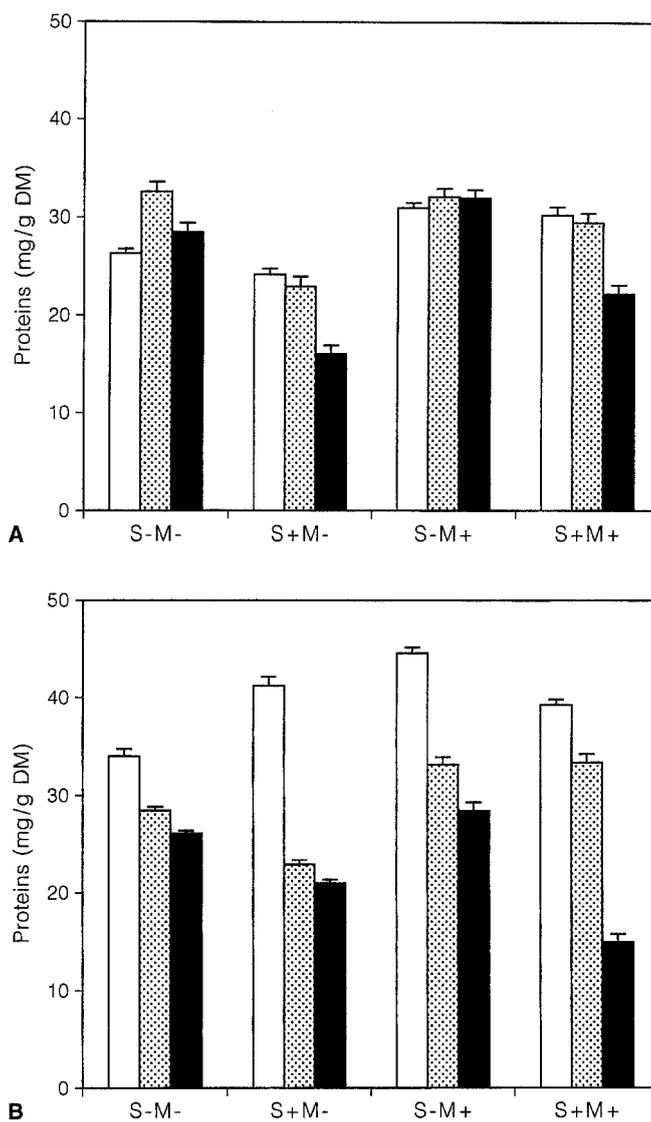


**Fig. 3** Reducing sugars in maize cultivars C0 (**A**) and C8 (**B**) in well-watered non-AM (S-M-) and AM (S-M+) and water-stressed non-AM (S+M-) and AM (S+M+) plants at the beginning (empty bars), middle (dotted bars), and end (solid bars) of the experiment. SEMs ( $n=4$ ) are indicated

duced during the later stages. Moisture stress lowered the protein content regardless of cultivar. The protein contents in C0 or C8 dropped by 44% and 20%, respectively, as the drought spell progressed, 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 marked response was not seen in C8.

#### Amino acids

Cultivars C0 and C8 showed pronounced increases in the amino acid levels during the drought stress period (Table 2), namely by 40.6% and 43.7% in nonAM C0 and C8 plants, respectively, compared with unstressed plants. The amino acid levels increased only by 10.7%



**Fig. 4** Proteins in maize cultivars C0 (**A**) and C8 (**B**) in well-watered non-AM (S-M-) and AM (S-M+) and water-stressed non-AM (S+M-) and AM (S+M+) plants at the beginning (empty bars), middle (dotted bars), and end (solid bars) of the experiment. SEMs ( $n=4$ ) are indicated

and 19.2% in AM plants of C0 and C8 plants, respectively. The drought-resistant cultivar C8 had a higher amino acid content than C0. The predominant amino acids detected in both cultivars were asparagine, alanine, serine, glutamate, glutamine, and glycine, which together accounted for over 70% of the amino acid 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.

#### Discussion

The colonization levels of cultivars C0 and C8 by *G. intraradices* were higher than those previously reported

**Table 2** Amino acid content (mmoles/g dry mass) of leaves from well-watered non-AM (S-M-) and AM (S-M+) and water-stressed non-AM (S+M-) and AM (S+M+) maize cultivars C0 and C8. Each value is the mean of 6 replicates two each at the beginning, middle, and end of the experiment)  $\pm$  SE

	S-M-	S-M+	S+M-	S+M+
<b>C0</b>				
Ala	19.0 $\pm$ 1.2	23.0 $\pm$ 3.3	25.0 $\pm$ 4.6	19.4 $\pm$ 2.3
Asn	13.0 $\pm$ 2.3	13.0 $\pm$ 0.2	29.0 $\pm$ 9.8	17.0 $\pm$ 12.0
Asp	2.4 $\pm$ 0.2	3.7 $\pm$ 0.5	2.5 $\pm$ 0.7	3.1 $\pm$ 1.8
Gln	5.1 $\pm$ 2.5	1.4 $\pm$ 0.4	14.0 $\pm$ 6.3	16.0 $\pm$ 7.6
Glu	3.5 $\pm$ 0.3	3.9 $\pm$ 0.7	4.7 $\pm$ 1.0	4.6 $\pm$ 0.4
Gly	2.9 $\pm$ 0.8	5.0 $\pm$ 1.0	5.2 $\pm$ 1.6	4.8 $\pm$ 0.8
Ser	6.5 $\pm$ 1.0	9.3 $\pm$ 0.2	9.8 $\pm$ 2.4	7.3 $\pm$ 1.3
Thr	1.9 $\pm$ 0.3	3.1 $\pm$ 0.6	2.2 $\pm$ 0.7	1.5 $\pm$ 0.3
Tyr	1.1 $\pm$ 0.5	1.6 $\pm$ 0.2	1.6 $\pm$ 0.4	0.7 $\pm$ 0.3
Val	1.1 $\pm$ 0.2	1.3 $\pm$ 0.2	1.6 $\pm$ 0.3	1.2 $\pm$ 0.3
Other <sup>a</sup>	3.0 $\pm$ 0.6	4.9 $\pm$ 1.2	4.6 $\pm$ 1.1	3.0 $\pm$ 0.9
Total	59.5 $\pm$ 9.9	70.2 $\pm$ 8.5	100.2 $\pm$ 28.9	78.6 $\pm$ 28.0
<b>C8</b>				
Ala	19.0 $\pm$ 2.7	19.0 $\pm$ 4.0	32.0 $\pm$ 5.6	31.0 $\pm$ 2.5
Asn	17.0 $\pm$ 9.7	45.0 $\pm$ 10	45.0 $\pm$ 10	51.0 $\pm$ 20
Asp	3.6 $\pm$ 0.8	3.5 $\pm$ 0.9	4.5 $\pm$ 1.2	3.0 $\pm$ 0.6
Gln	5.2 $\pm$ 1.9	7.4 $\pm$ 2.3	7.7 $\pm$ 2.2	5.1 $\pm$ 3.1
Glu	5.3 $\pm$ 0.8	4.6 $\pm$ 0.6	5.6 $\pm$ 0.1	5.4 $\pm$ 1.5
Gly	3.5 $\pm$ 0.7	4.0 $\pm$ 0.9	5.4 $\pm$ 1.4	6.8 $\pm$ 3.5
Ser	7.7 $\pm$ 1.3	7.7 $\pm$ 1.5	13.0 $\pm$ 4.1	12.0 $\pm$ 3.6
Thr	2.4 $\pm$ 0.5	2.4 $\pm$ 0.7	2.1 $\pm$ 0.4	2.4 $\pm$ 1.0
Tyr	1.0 $\pm$ 0.3	0.7 $\pm$ 0.1	1.3 $\pm$ 0.4	1.0 $\pm$ 0.6
Val	1.4 $\pm$ 0.3	1.5 $\pm$ 0.3	0.1 $\pm$ 0.0	0.6 $\pm$ 0.1
Other <sup>a</sup>	2.8 $\pm$ 0.7	4.0 $\pm$ 1.1	5.6 $\pm$ 1.1	5.2 $\pm$ 2.2
Total	68.9 $\pm$ 19.7	99.8 $\pm$ 22.4	122.3 $\pm$ 26.5	123.5 $\pm$ 38.7

<sup>a</sup> Arg, Cys, His, Ile, Leu, Lys, Met, Phe, and Pro

for different maize cultivars with this *Glomus* species (Augé et al. 1994) or with other *Glomus* species (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).

Under water stress, AM plants alleviated the chlorophyll degradation and maintained the chlorophyll content at levels comparable with unstressed plants. Reduction in chlorophyll content due to drought stress is well-established (Hsiao 1973; Sung 1985). Our results agrees with the findings of Augé et al. (1987) who observed higher chlorophyll content in water-stressed AM than non-AM rose leaves. Mycorrhizae, by improving nutrition (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 a sugar level during drought stress is physiologically important in helping the plant withstand the effects of withheld irrigation and recover after stress is relieved. The enhanced sugar content in water-stressed AM maize

plants observed in our study may be related to these plants having higher leaf water potential (less negative) and lower stomatal resistance than non-AM plants (unpublished data). 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 water 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 water-stressed conditions, especially in the drought-sensitive cultivar. Our results agrees with the findings of Arines et al. (1993) who reported a two- to sixfold increase in soluble protein content in mycorrhizal clover roots. The enhanced soluble proteins appear to be an indicator of stress tolerance (Charest and Phan 1990; Charest et al. 1993). The AM-inducible proteins or polypeptides (endomycorrhizins) have been identified in roots of tobacco and onion (Dumas et al. 1990) and tomato (Simoneau et al. 1994), and they may have an adaptive role in drought resistance. 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. 1994). The relative increase in amino acids also demonstrates that N utilization of maize was altered in the presence of mycorrhizae. Recently, Cliquet and Stewart (1993) have shown that AM fungi increase ammonium assimilation, glutamine production, and xylem nitrogen translocation in maize. This illustrates the role of mycorrhizae in regulating and making modifications in N metabolism of host plants (Attiwill and Adams 1993).

In summary, we have demonstrated that AM inoculation orchestrates metabolic changes that play an adaptive role in drought resistance of maize. The increase of organic solutes such as sugars and nitrogenous compounds contributes to osmotic adjustment, resulting in drought tolerance in the host plant. This may be of agronomical significance, particularly in semi-arid tropics where drought is not uncommon. Further investigations are under way to determine the AM-inducible signalling process of drought tolerance.

**Acknowledgements** K. S. S. thanks the Canadian Commonwealth Scholarship and Fellowship Plan for a scholarship to conduct this research. The authors are grateful to G. O. Edmeades, CIMMYT, Mexico, for providing seeds, R. I. Hamilton for greenhouse facilities, Y. Dalpé, Agriculture Canada, for root colonization studies, and S. Parent, Tourbière Premier Peat moss, for providing the fungal inoculum.

