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Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance

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Abstract The effects of an arbuscular mycorrhizal (AM) fungus and drought stress on the growth, phosphorus, and micronutrient uptake of two wheat genotypes exhibiting differences in drought resistance were investigated. Plants were grown on a low P (4 mg kg^{-1} soil) silty clay (Typic Xerochrept) soil-sand mix. Mycorrhizal infection was higher under well-watered than under dry soil conditions and the drought-resistant genotype CR057 had a higher mycorrhizal colonization than the drought-sensitive genotype CR006. Total and root dry matter yields and total root length were higher in mycorrhizal than in nonmycorrhizal plants of both genotypes. CR057 had higher total dry matter but not root dry matter than CR006 plants. The enhancement in total dry matter due to AM inoculation was 42 and 39% under well-watered and 35 and 45% under water-stressed for CR057 and and CR006, respectively. For both genotypes, the contents of P, Zn, Cu, Mn, and Fe were higher in mycorrhizal than in nonmycorrhizal plants and higher under well-watered than under dry soil conditions. The enhancement of P, Zn, Cu, Mn, and Fe uptake due to AM inoculation was more pronounced in CR006 than in CR057, particularly under water-stressed conditions. Thus CR006 benefitted from AM infection more than the CR057 under dry soil conditions, despite the fact that CR057 roots were highly infected. It appears that CR006 is more dependent on AM symbiosis than CR057.

Key words AM · Drought resistance · Genotype · Phosphorus · *Triticum durum*

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Introduction

In many semiarid regions of the world, drought and infertile soils with low a phosphorus concentration combine to limit crop productivity. In these regions, most wheat (*Triticum durum* L.) is grown under rainfed conditions, where drought can occur at any time during the growing season. It has been demonstrated, in the absence of indigenous arbuscular mycorrhizal (AM) fungi, that plant nutrition and growth can be improved by inoculating the soil with these symbiotic microorganisms (Ellis et al. 1985; Kupulnik and Kushnir 1991).

The mycorrhizal enhancement of P, Zn, Cu, Mn, and Fe uptake, and the growth of plants subjected to low soil P and adequate water supply is well documented (Nelsen 1987; Michelsen and Rosendahl 1990; Raju et al. 1990a; Manjunath and Habte 1991). Mycorrhizal associations with plant roots not only enhance growth and mineral element uptake, but may also confer a greater resistance to drought (Hardie and Leyton 1981; Davies et al. 1992; Ruiz-Lozano et al. 1995). This improved drought resistance may be caused by more efficient P uptake in P deficient soils (Huang et al. 1985; Nelsen 1987). However, the drought resistance of AM plants has also been reported to be independent of plant P concentration (Auge et al. 1986; Bethlenfalvay et al. 1988).

Wide variation in plant response to AM inoculation exists for different wheat cultivars (Azcon and Ocampo 1981; Kapulnik and Kushnir 1991) and genotypes differ in their responses to AM infection under drought stress (Kapulnik and Kushnir 1991). It has been suggested that mycorrhizal colonization is a host-dependent and heritable trait (Lackie et al. 1988; Mercy et al. 1990). To determine whether mycorrhizal infection influences host plant responses to drought stress, growth parameters under well-watered and water-stressed conditions were monitored in wheat genotypes with distinct differences in drought resistance.

Materials and methods

A greenhouse experiment was conducted at 28 ± 3 °C under natural illumination during the spring of 1995. Wheat plants were grown in a silty clay soil (fine, mixed, thermic, Typic Xerochrept) mixed with sand (soil : sand, 1 : 1, v/v). Soil properties before mixture with sand were 6.5% sand, 45% silt, 48.5% clay, 1.21% organic matter, pH 8.1 (soil : water, 1 : 1), 8 mg P (NaHCO3-extracted) per kg soil and Mn 23.5, Fe 12.8, Zn 1.6, Cu 1.8 (5 mM DTPA extracted) mg kg–1 soil. The soil mix was fumigated with methyl bromide under air-tight plastic sheets for 3 days and the fumigant allowed to dissipate for 10 days. The soil mix was dispensed into plastic containers (5.5 kg soil per pot) for plant growth. No P was added to the soil. Half of the pots received *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (Al-Raddad 1993) by an inoculum placed 4 cm deep in 10-cm diameter holes in the center of the pots. The AM inoculum consisted of root fragments [AM-colonized chickpea (*Cicer aritinum* L.) roots] and adhering spores mixed with soil to provide 33 chlamydospores per 100 g air-dried soil. Control treatments received no AM inoculum.

Seeds of the durum wheat genotypes CR006 (drought sensitive) and CR057 (drought resistant) (Jaradat 1992) were planted near the center of each pot. One week after emergence, seedlings were thinned to four per pot. Plants were watered daily until drought stress treatments were initiated 3 weeks after planting. Drought stress was imposed by withholding water from pots until a soil water potential of –0.12 MPa was achieved. Thereafter, water was maintained at this level by weighing pots daily and adding appropriate amounts of water. Pots with well-watered plants were maintained at a soil water potential of –0.05 MPa (near field capacity). Soil water potential was determined in the soil mix from a moisture retention curve using a pressure plate apparatus, and soil water content was determined by weighing samples before and after drying at 110 °C for 24 h.

The experiment was terminated by severing shoots from roots 75 days after emergence (head emergence; stages 10.1 to 10.5 on Feeke's scale). Shoots were seperated into leaves, stems, and flower heads, washed with distilled water, oven dried at 80 °C for 24 h and weighed. Roots were rinsed free of soil, cut into 1-cm fragments and thoroughly mixed. Representative fresh samples (1 g) were removed for determination of root AM colonization and total root length. The remaining roots were dried and weighed.

Root samples for determination of root colonization were cleared with 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue in lactophenol as described by Phillips and Hayman (1970), and microscopically examined for colonization by determining percentage root segments containing arbuscules plus vesicles using a gridline intercept method (Bierman and Linderman 1981). The total root length was determined using the gridline intersect method of Newman (1966). Roots used to determine colonization and root length were dried, weighed, and added to the total.

Dried leaf, stem and head flower samples were ground in a cyclone laboratory mill to pass through a 0.5-mm sieve, weighed, ashed overnight at 550 °C in a muffle furnace, and the ash suspended in 2 M HCl for determination of mineral nutrients. Phosphorus was determined colormetrically (Watanabe and Olsen 1965) and Zn, Cu, Mn, and Fe by atomic absorption spectroscopy.

The experiment was randomized in complete blocks with two drought stress levels (well watered and water stressed), two AM inoculum treatments (inoculated and uninoculated), and two wheat genotypes to give a $2 \times 2 \times 2$ factorial with four replications. Data were statistically analyzed by analysis of variance with the MSTATC PROGRAM (Michigan State University, East Lansing, Mich., USA). Probabilities of significance were used to test for significance among treatments and interactions, and LSDs ($P < 0.05$) were used to compare means.

Results

No AM colonization was noted in the roots of control plants. Mycorrhizal infection of both wheat genotypes was reduced by drought stress (Table 1). Under well-watered but not water-stressed conditions, the roots of the drought-resistant genotype CR057 showed a significantly higher AM colonization than the roots of the drought-sensitive genotype CR006 (Table 1).

Table 1 Effects of arbuscular mycorrhizal (*AM*) fungi and water-stress treatments on root colonization, dry matter (*DM*) of leaf, stem, flower head, total above ground and root, and total root length (*RL*) of wheat plants

Treatment	AM status	Wheat genotype	Root colonization %	Leaf DM	Stem DM	Flower DM	Total DM	Root DM	RL cm/plant
				g/plant					
Nonstressed	nonAM	CR057	0	2.17	3.69	2.65	8.5	1.14	431
		CR006	0	2.42	3.01	2.21	7.6	1.25	498
	AM	CR057	47	3.06	6.00	3.02	12.1	1.61	541
		CR006	33	3.37	4.99	2.07	10.4	1.99	601
Stressed	nonAM	CR057	0	1.49	1.11	1.44	4.0	0.89	319
		CR006	0	1.21	0.96	1.16	3.3	0.79	338
	AM	CR057	20	1.74	2.03	1.63	5.4	1.05	371
		CR006	15	1.85	1.60	1.36	4.8	1.14	436
LSD (0.05)			9	0.26	1.83	0.52	1.1	0.34	117
Significance									
Water stress (WS)			**	**	**	**	**	**	**
AM			**	**	**	NS	**	**	**
WS × AM			**	NS	**	NS	**	*	NS
Genotype (G)			*	*	NS	**	**	NS	NS
WS × G			NS	NS	NS	NS	NS	NS	NS
AM × G			*	NS	NS	NS	NS	NS	NS
WS × AM × G			NS	NS	NS	NS	NS	NS	NS

* Significant at P 0.05
** Significant at P 0.01
NS Not significant

Total and root dry matter yields increased after inoculation of soil with *G. mosseae* (Table 1); however, AM inoculation had no positive effect on total root length or flower head dry matter yield in either genotype. Wheat vegetative (stem and leaves) and reproductive (flower heads) growth, root dry matter, and total root length were reduced by drought stress in both mycorrhizalm and nonmycorrhizal plants (Table 1). CR057 had higher flower head and total dry matter yields but lower leaf dry matter than CR006 in both mycorrhizal and nonmycorrhizal plants. No significant differences in stem and root dry matter or total root length between genotypes occurred after AM inoculation.

The phosphorus status of leaves, stems, and flower heads showed that P uptake was stimulated by AM inoculation in both genotypes, independent of water availability (Tables 2, 3). Drought stress reduced the P content of leaves, stems, and flower heads in both mycorrhizal and nonmycorrhizal plants. A significant drought stress $\times$AM interaction was noted for P content in leaves, stems and whole plants but not in flower heads (Table 3). Genotypic differences in P content due to AM inoculation were noted in stems and flower heads only under well-watered conditions (Tables 2, 3). CR006 had more P than CR057 in stems, while CR057 had more P than CR006 in flower heads.

Leaf, stem, and flower head Zn content declined under drought-stress conditions regardless of AM inoculation in both genotypes (Table 2). AM inoculation significantly increased the Zn content of leaves and stems but not flower heads (Table 3). A significant difference between genotypes for Zn content was noted only in flower heads, where CR057 accumulated mor Zn than CR006 under well-watered conditions (Tables 2, 3).

Mn and Cu contents of leaves, stems, and flower heads were higher in mycorrhizal than nonmycorrhizal plants (Tables 2, 3). The drought treatment significantly decreased the Mn and Cu contents of flower heads and stems and Mn content of leaves, but had no effect on the Cu content of leaves. The genotypes differed in Mn content of leaves and flower heads and Cu content

Table 2 Effects of AM fungi and water-stress treatments on P, Zn, Mn, Cu, and Fe contents of wheat plants

Treatment	AM Status	Genotype	Plant part	P mg/plant	Zn	Mn	Cu μg/plant	Fe
Nonstressed	nonAM	CR057	Leaf	1.6	81	69	20	221
			Stem	3.8	191	41	26	193
			Head	7.8	176	64	24	263
			Total	13.2	448	174	70	677
		CR006	Leaf	1.7	75	73	25	275
			Stem	5.4	151	51	18	183
			Head	6.3	102	48	20	187
			Total	13.4	327	172	63	645
	AM	CR057	Leaf	3.4	148	101	29	348
			Stem	8.1	209	72	46	415
			Head	10.8	171	88	31	297
			Total	22.2	528	262	106	1059
		CR006	Leaf	4.9	140	126	35	401
			Stem	11.2	235	65	42	303
			Head	7.9	106	54	25	160
			Total	24.0	480	245	102	864
Stressed	nonAM	CR057	Leaf	0.9	67	49	21	210
			Stem	1.3	64	22	12	72
			Head	3.8	75	40	13	232
			Total	6.0	201	111	46	513
		CR006	Leaf	0.7	60	50	15	204
			Stem	1.2	43	22	9	61
			Head	3.6	61	27	13	178
			Total	5.5	164	99	37	443
	AM	CR057	Leaf	1.4	89	71	31	352
			Stem	2.4	75	33	21	130
			Head	5.5	73	52	19	250
			Total	9.2	236	156	71	732
		CR006	Leaf	2.3	95	90	32	391
			Stem	2.7	65	29	19	106
			Head	5.3	79	33	15	213
			Total	10.3	239	152	66	710
LSD (0.05)			Leaf	1.5	41	24	11	118
			Stem	2.4	58	20	12	89
			Head	1.8	35	19	5	81
			Total	3.4	81	36	20	160

Table 3 Significance of differences in mineral (P, Zn, Cu, Mn, Fe) contents of different plant parts and total plant contents resulting from AM fungi and water-stress treatments of wheat plants

Plant part	Source of variation	P	Zn	Cu	Mn	Fe
Leaf	Water stress (WS)	**	*	NS	**	NS
	AM	**	**	**	**	**
	WS × AM	*	NS	NS	NS	NS
	Genotype (G)	NS	NS	NS	*	NS
	WS × G	NS	NS	NS	NS	NS
	AM × G	NS	NS	NS	NS	NS
	WS × AM × G	NS	NS	NS	NS	NS
Stem	Water stress (WS)	**	**	**	**	**
	AM	**	*	**	**	**
	WS × AM	**	NS	*	NS	NS
	Genotype (G)	*	NS	NS	NS	NS
	WS × G	NS	NS	NS	NS	NS
	AM × G	NS	NS	NS	NS	NS
	WS × AM × G	NS	NS	NS	NS	NS
Head	Water stress (WS)	**	**	**	**	NS
	AM	**	NS	**	*	NS
	WS × AM	NS	NS	NS	NS	NS
	Genotype (G)	*	**	*	**	*
	WS × G	*	**	NS	NS	NS
	AM × G	NS	NS	NS	NS	NS
	WS × AM × G	NS	NS	NS	NS	NS
Total plant	Water stress (WS)	**	**	**	**	**
	AM	**	**	**	**	**
	WS × AM	**	NS	NS	NS	NS
	Genotype (G)	NS	*	NS	NS	NS
	WS × G	NS	NS	NS	NS	NS
	AM × G	NS	NS	NS	NS	NS
	WS × AM × G	NS	NS	NS	NS	NS

* Significant at P 0.05
** Significant at P 0.01
NS Not significant

of flower heads; CR057 had higher values than CR006 under well-watered conditions (Table 2).

Iron content was not affected by drought stress in leaves or flower heads, but was higher in stems in well-watered conditions (Tables 2, 3). AM inoculation increased the Fe content of leaves and stems but had no effect on Fe content of flower heads. CR057 plants accumulated more Fe than CR006 in flower heads under well-watered conditions. The overall effects of AM colonization on the growth of stressed and nonstressed plants are summarized in Table 4.

Discussion

Plants inoculated with *G. mosseae* had higher total and root dry matter yields than nonmycorrhizal plants. This increase in plant growth was probably indirectly due to mycorrhizal enhancement of P uptake, since mycorrhizal plants also had higher P contents in leaves, stems, and flower heads than nonmycorrhizal plants. Growth increases may be attributed directly to enhanced photosynthesis associated with increased P uptake in plants (Dietz and Foyer 1986). The positive effects of AM on wheat total and root dry matter were reduced by drought stress. Under well-watered conditions, plants

Table 4 Calculated mycorrhizal (AM) increases in total dry matter yield and total nutrient uptake of AM wheat grown under water-stressed and nonstressed conditions

Treatment	Genotype	Total dry matter[a]	P[b]	Zn	Mn	Cu	Fe
Nonstressed	CR057	42	68	18	51	50	56
	CR006	38	79	47	41	64	34
Stressed	CR057	35	53	17	40	61	43
	CR006	45	87	46	53	68	60

[a] Total dry matter (DM) increase $= (DM_{AM} - DM_{nonAM}) \times 100/DM_{nonAM}$
[b] Nutrient uptake (NU) increase $= (NU_{AM} - NU_{nonAM}) \times 100/NU_{nonAM}$

accumulated more dry matter in both vegetative (leaves and stems) and reproductive (flower heads) plant parts than plants grown under drought-stress conditions, regardless of AM inoculation. Reductions in total P accumulation caused by drought stress may have detrimentaly affected plant growth in water-stressed plants. Reproductive growth (flower head dry matter) of CR006 plants inoculated with AM fungus was lower than uninoculated plants under well-watered conditions. This was probably due to delayed flowering of CR006 at harvest (data not shown). Similar effects were observed with cowpea [*Vigna unguiculata* (L.) Walp] under well-watered conditions (Kwapata and Hall 1985).

Plant growth responses to AM inoculation were higher in CR006 than in CR057 under water-stressed but not under well-watered conditions, even though AM colonization was higher in CR057 than in CR006. CR057 was previously found to produce higher grain and straw yields under field conditions (Jaradat 1992) and grew better than CR006. However, in the presence of AM, the differences between CR057 and CR006 in total dry matter were reduced, and differences in stem and root dry matter and total root length were not significant.

It has been suggested that mycorrhiza are relatively more important to plant growth under dry conditions than when soil moisture is plentiful (Michelsen and Rosendahl 1990). The present results support this in the case of the CR006 genotype. However, the biomass increase with CR057 plants due to AM inoculation was lower under drought conditions than with CR006; the converse was true under well-watered conditions. This may reflect the higher drought resistance of uninoculated the plants of CR057.

Many studies have indicated that AM contributes to plant growth via assimilation of immobile soil nutrients (P, Cu, Zn) especially in poor soils (Hayman and Mosse 1971; Bethlanfalvey et al. 1988). In this study, colonized wheat plants had considerably higher aboveground mineral nutrient contents (P, Zn, Mn, Cu, and Fe) than nonmycorrhizal plants under both well-watered and water-stressed conditions. This probably resulted from a greater absorption surface area provided by extensive fungal hyphae (Raju et al. 1990b) and enhanced root growth. Similar results have been reported for other plant species (Ellis et al. 1985; Kwapata and Hall 1985; Bethlenfalvay et al. 1988; Raju et al. 1990a; Manjunath and Habte 1991).

The host plant species, cultivar and growing conditions can influence the effectiveness of AM symbiosis in nutrient uptake (Kwapata and Hall 1985; Mercy et al. 1990; Manjunath and Habte 1991; Jacobsen et al. 1992). From our results, it appears that AM colonization was more effective in increasing P, Zn and Cu uptake under well-watered conditions for the drought-sensitive genotype CR006 than the drought-resistant genotype CR057. Only Mn and Fe uptake were increased by AM fungi more in CR057 than in CR006 under well-watered conditions. However, AM inoculation increased P, Zn, Mn, Cu, and Fe uptake more in CR006 than in CR057 under water-stressed condions. Increased nutrient uptake in response to AM fungal infection was suggested to be a plant strategy for drought-stress resistance by Ruiz-Lozano et al. (1995).

The improved growth and nutrient uptake in wheat plants recorded here demonstrate the potential of mycorrhizal inoculation for the protection from drought stress of wheat grown in semiarid areas of the world.

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