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Mycorrhizal influence on protein and lipid of durum wheat grown at different soil phosphorus levels

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Abstract Root colonization by arbuscular mycorrhizal fungi (AMF) may affect protein and lipid composition of plants by altering P nutrition or by eliciting other metabolic responses in the host plant. This study was conducted to determine the effects of an AMF and soil P on seed protein and lipid contents and yield of two genotypes of durum wheat (*Triticum durum* L.). Plants were grown in a greenhouse using soil: sand mixes with different levels of P, and with or without the AMF *Glomus mosseae* [(Nicol. and Gerd.) Gerd. and Trappe]. Percentage AMF root colonization decreased as P added to soil increased. The wheat genotype CR057 had higher AMF root colonization but lower seed P and protein concentrations than CR006. Without added soil P, protein concentration was significantly lower and lipid concentration and seed dry weight higher in arbuscular mycorrhizal (AM) than in nonAM plants. Seed lipid and protein contents were highly correlated with P content of plants. In nonAM plants, seed lipid and protein contents were low with no added soil P and did not differ with added soil P. Seed protein/lipid (Pro/L) concentration ratios of AM plants were higher than those of nonAM plants only when no P was added to the soil. The data indicate different patterns of seed P accumulation and different relationships between seed P and protein and lipid in AM and nonAM plants. Thus, both the presence and degree of AMF root colonization affected seed lipid metabolism in these durum wheat genotypes.

Key words *Glomus mosseae* · Lipid · Phosphorus · Protein · Seed · *Triticum durum*

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Introduction

Arbuscular mycorrhizal fungi (AMF) have fundamental effects on host plant biochemistry and physiology (Koide and Schreiner 1992; Smith and Gianinazzi-Pearson 1988). Specific proteins (Gianinazzi-Pearson and Gianinazzi 1989), amino acid fractions (Tawaraya and Saito 1994; Tawaraya et al. 1994), lipids (Bethlenfalvay et al. 1994, 1997), reducing sugars (Tawaraya et al. 1994), and secondary metabolites (Morandi and Gianinazzi-Pearson 1986) are produced by host plants in response to AMF root colonization. Such changes in plant tissues indicate that seeds of mycotrophic plants may also be modified, not only in terms of biomass produced but in the relative abundance of storage products such as proteins, lipids, and starch (Bethlenfalvay et al. 1994, 1997; Lu and Koide 1991). Even though wheat seeds are often considered to be a starchy food, these seeds contain other valuable nutritive compounds (e.g., proteins and lipids) in significant amounts (Al-Karaki and Ereifej 1998; Johnson et al. 1978).

Seed lipid and protein composition are not only heritable traits, but depend to a large extent on environmental conditions (Burton 1987; Wilson 1987). Since AMF influence expression of genetic differences in host plants (Toth et al. 1984), AMF may also affect seed physiology. However, information on AMF effects on seed composition is limited, possibly because most experiments with AMF are conducted in pot cultures, where plants are not usually grown to maturity and where root-volume constraints limit AMF effectiveness (Koide 1991a). The most pronounced aspect of AMF effectiveness has been enhanced P nutrition of host plants (Al-Karaki and Al-Raddad 1997; Koide 1991b;

Kothari et al. 1991; Marschner and Dell 1994; Trimble and Knowles 1995). However, the plant P status can influence seed protein/lipid (Pro/L) balances (Bethlenfalvay et al. 1997; Sawan et al. 1988). Thus, seed Pro/L ratios appear to be affected by AMF due to enhanced P uptake (Bethlenfalvay et al. 1997) or through elicitation of changes in seed Pro/L metabolism independent of plant P status. The objective of this study was to determine the effects of an AMF on seed development and protein and lipid contents of durum wheat grown at different levels of added soil P.

Materials and methods

A silty clay soil (fine, mixed, thermic, Typic Xerochrept) was mixed with washed cement grade sand (soil:sand, 1:1, v:v), enclosed in air-tight plastic bags, and fumigated with methyl bromide for 3 days. The bags were opened to the atmosphere and frequently stirred to dissipate methyl bromide for 10 days, after which the soil mix was put in plastic pots for plant growth (5 kg soil mix per pot). The properties of the soil before mixture with sand were 6.5% sand, 45.0% silt, and 48.5% clay; 1.2% organic matter; pH 8.1; and 0.26 mmol P kg^{-1} ($NaHCO_3$-extractable) (Watanabe and Olsen 1965). Phosphorus was added to soil mixes at 0.0, 0.8, and 1.6 mmol P kg^{-1} as KH_2PO_4 before planting. Half of the pots received the AMF *Glomus mosseae* [(Nicol. and Gerd.) Gerd. and Trappe] by placing 20 g (moist weight) of inoculum 3 cm deep in 10-cm-diameter holes in the center of the pots prior to planting. The AMF inoculum added to pots consisted of soil and root fragments and spores from AMF-colonized chickpea (*Cicer arietinum* L.) roots (610 chlamydospores kg^{-1} dry soil). Control treatments received no AMF inoculum.

Seeds of durum wheat (*Triticum durum* L. genotypes CR057 and CR006) were planted in each pot (above the AMF inoculum or where AMF inoculum would have been) and placed in a greenhouse for growth with natural light at 28 ± 6 °C (January-May). Photosynthetic photon flux density at plant height ranged between 800 and 1530 μmol m^{-2} s^{-1} throughout the growing period. To assure sufficient nutrients for plant growth, supplements of 25.1 N (6 nitrate: 1 ammonium), 7.2 K, 6.2 Ca, 1.8 S, and 1.6 Mg in mmol kg^{-1} soil mix, and 50 B, 49 Fe as ferric hydroxyethylethylenedi-aminetriacetate, 18 Mn, 4.6 Zn, 1.6 Mo, and 1.2 Cu in μmol kg^{-1} soil mix were added at weeks 4 and 7 after seeding. One week after emergence, seedlings were thinned to 4 per pot; the roots of discarded plants were left in the soil to avoid removal of AMF inoculum. Plants were watered to field capacity with tap water 2–3 times per week.

After plant heads matured and started to dry at 13 weeks, stems were severed 1–2 cm above soil level and air dried in the greenhouse for 2 weeks. Heads were threshed manually and seeds were oven-dried and weighed. Roots were washed free of soil and representative fresh samples (1 g) were removed for mycorrhizal infection determination. Dried seed samples were ground to pass a 0.5-mm sieve in a cyclone laboratory mill.

Seed protein (Kjeldahl N × 6.25) and crude fat (ether extract) were determined according to AOAC (1984). Phosphorus was determined according to Watanabe and Olsen (1965). Root samples for determination of AMF colonization were cleared with 1.78 M KOH and stained with 0.52 mM trypan blue in lactophenol (Phillips and Hayman 1970). Percentage root segments containing arbuscules and/or vesicles was determined using a gridline intercept method (Bierman and Linderman 1981).

The experimental design was a randomized complete block with 4 replications, including 3 P levels, 2 AMF treatments (AM vs nonAM) and 2 wheat genotypes. Data were evaluated by analysis of variance using the MSTATC PROGRAM (Michigan State University, East Lansing, Mich.). Probabilities of significance were used to test significance among treatments and interactions, and LSD values ($P \leq 0.05$) were used to compare means.

Results

Wheat plants grown without added soil P had relatively high AMF root colonization (~50%) which decreased as added soil P increased (~10% at the highest level of added soil P) (Table 1). Significant P × AMF and P × genotype interactions were noted for AMF root colonization. Genotype CR057 generally had higher AMF root colonization than CR006.

Wheat seed dry weight and P acquisition increased in both genotypes as added soil P level increased for both AM and nonAM plants (Table 1). Differences between AM and nonAM plants for seed dry weight were only significant for plants of both genotypes grown with no added P. Seed P concentrations and contents increased with each increment increase of added soil P for both AM and nonAM plants. Seed P concentrations and contents were generally greater for AM than for nonAM plants, except at the highest level of added soil P where the converse parameters was recorded (Table 1). The seed dry weights of the two genotypes did not differ at any level of added soil P whether plants were AM or nonAM, although, genotype CR006 had generally higher P concentrations than CR057 in both AM and nonAM plants. A significant P × AMF interaction was noted for seed dry weight and seed P concentration and content.

Seed dry weight enhancement due to AMF was highest for plants grown without added soil P, lower at 0.8 mmol and not significant at 1.6 mmol added P kg^{-1} soil. There was a trend to lower yield in AM plants at 1.6 mmol added P kg^{-1} soil (Fig. 1). Changes in seed P concentration relative to soil P were similar to those for seed dry weight, except that the differences were greater for plants grown at 0 and 0.8 mmol added P kg^{-1} soil than at 1.6 mmol added P kg^{-1} soil (Fig. 1).

Seed protein and lipid concentrations diverged most for AM and nonAM plants grown with no added soil P. Seed protein concentrations were significantly lower and lipid concentrations significantly higher in AM than in nonAM plants only when plants were grown without added P (Table 2). In addition, seed protein concentrations were apparently higher in CR006 than in CR057, although the differences were only significant for plants grown with no added soil P. The seed Pro/L concentration ratios of both genotypes were higher for nonAM than AM plants at no added soil P (Table 2). However, no significant differences between AM and nonAM plants were noted in Pro/L concentration ratios for either genotype when P was added to soil. Significant P × AMF interactions were noted for seed protein and lipid contents.

Seed P, protein, and lipid contents varied with soil P for both AM and nonAM plants (Tables 1, 2), showing significant interactions with AMF status and P fertilization. Seed P content was highly correlated with seed lipid and protein contents in both AM and nonAM plants (Fig. 2). The contents of seed protein ($r = 0.85$

Table 1 Percentage AMF root colonization, seed dry weight, and seed P concentration and content of nonAM and AM wheat genotypes grown at different levels of added soil P. Data represent the mean ± standard error of 4 replicates

P level mmol kg^{-1}	AMF treatment	Genotype	AM root colonization %	Seed dry weight g $plant^{-1}$	Seed P concentration mg g^{-1}	Seed P content mg $plant^{-1}$
0.0	NonAM	CR057	0 ± 0	1.43 ± 0.18	1.38 ± 0.05	2.0 ± 0.3
		CR006	0 ± 0	0.99 ± 0.18	1.68 ± 0.18	1.6 ± 0.2
	AM	CR057	57 ± 4	2.42 ± 0.45	1.74 ± 0.06	4.2 ± 0.8
		CR006	47 ± 3	1.85 ± 0.11	2.12 ± 0.16	3.9 ± 0.3
0.8	NonAM	CR057	0 ± 0	4.90 ± 0.49	2.11 ± 0.09	10.4 ± 1.3
		CR006	0 ± 0	4.88 ± 0.31	2.61 ± 0.15	12.9 ± 1.0
	AM	CR057	31 ± 3	5.75 ± 0.22	2.39 ± 0.24	13.6 ± 1.1
		CR006	24 ± 3	5.48 ± 0.31	2.67 ± 0.21	14.4 ± 1.5
1.6	NonAM	CR057	0 ± 0	6.68 ± 0.19	3.52 ± 0.11	23.6 ± 1.4
		CR006	0 ± 0	6.04 ± 0.39	4.25 ± 0.26	25.9 ± 3.1
	AM	CR057	11 ± 2	6.10 ± 0.99	3.44 ± 0.26	21.5 ± 5.0
		CR006	9 ± 1	5.56 ± 0.27	3.64 ± 0.30	20.1 ± 2.0
LSD (0.05)			5	0.93	0.55	5.6
Significance						
P level (P)			**	**	**	**
AMF			**	NS	NS	NS
P × AMF			**	*	*	*
Genotype (G)			**	NS	**	NS
P × G			NS	NS	NS	NS
AMF × G			**	NS	NS	NS
P × AMF × G			NS	NS	NS	NS

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; NS not significant

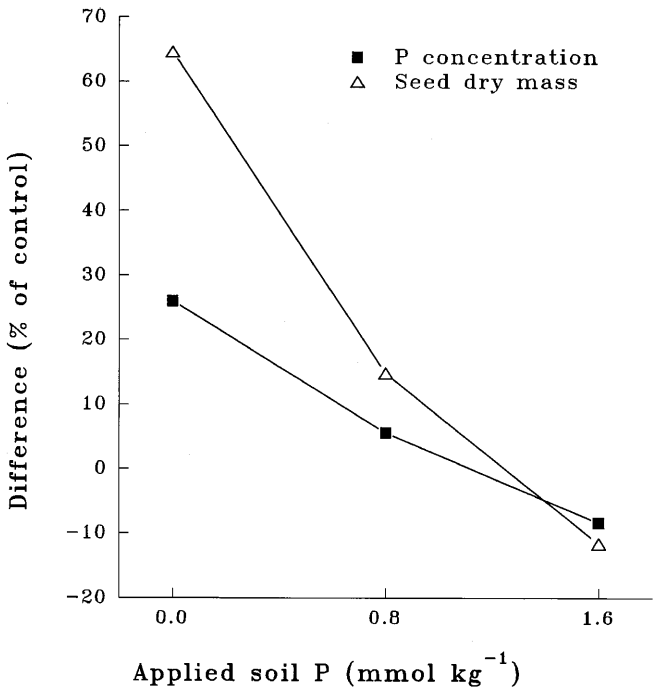

Fig. 1 Seed dry weight and seed P concentration of AM wheat genotypes grown at different levels of added soil P. Values are expressed as percent differences from the control [(AM–nonAM)/nonAM] × 100. Data represent the mean results for two genotypes

for AM and $r = 0.92$ for nonAM) and lipid ($r = 0.90$ for AM and $r = 0.91$ for nonAM) (each significant at $P \leq 0.01$) changed gradually in both genotypes with change in seed P content, and continued to increase with the level of added soil P. Seed protein and lipid contents were low without added soil P, doubled at 0.8 mmol added P kg^{-1} soil, and did not increase further at 1.6 mmol added P kg^{-1} soil. No genotypic differences were noted for seed P, protein, or lipid content for plants grown at different levels of added soil P. Significant differences between AM and nonAM plants were noted only for seed lipid content (Table 2).

Discussion

Substantial diversion of host plant photosynthate to fungal lipids in mycotrophic associations may be accompanied by characteristic alterations in host plant lipid metabolism and redistribution of carbon among lipid and nonlipid fractions of various tissues (Lösel 1980). Changes recorded in seed lipid composition of host plant roots colonized with AMF as a substitute for P fertilizer indicate that genetically-based mechanisms are important in AMF host responses, changes in P nutrition, and relief from P deficiency (Bethlenfalvay et al. 1994, 1997; Pacovsky and Fuller 1987). Although soil mineral nutrition affects seed lipid content less than that of vegetative tissues (Hitchcock and Nichols 1971), environmental conditions can affect seed protein and lipid composition (Dornbos and Muller 1992; Kuiper

Table 2 Seed protein and lipid concentration and content and Pro/L concentration ratio of nonAM and AM wheat genotypes grown at different levels of added soil P

P level	AMF treatment	Genotype	Protein		Lipid		Pro/L ratio
mmol kg^{-1}			concentration mg g^{-1}	content mg plant^{-1}	concentration mg g^{-1}	content mg plant^{-1}	
0.0	NonAM	CR057	140±2	201±28	21.2±1.5	30±4	6.68±0.46
		CR006	152±5	167±26	19.9±1.2	22±2	7.73±0.36
	AM	CR057	136±3	313±64	23.9±1.9	54±12	5.77±0.35
		CR006	140±2	259±13	23.5±0.7	43±5	6.00±0.17
0.8	NonAM	CR057	130±4	639±77	19.2±1.6	96±16	6.84±0.33
		CR006	135±4	657±34	20.5±1.5	99±7	6.67±0.35
	AM	CR057	128±4	734±18	20.9±0.8	120±4	6.13±0.10
		CR006	132±1	725±37	19.7±0.9	108±9	6.80±0.29
1.6	NonAM	CR057	124±3	824±16	19.0±1.1	127±5	6.53±0.26
		CR006	131±3	791±65	20.9±0.8	126±11	6.30±0.30
	AM	CR057	121±8	704±71	20.7±1.0	123±17	5.85±0.29
		CR006	127±2	669±31	21.2±1.3	112±10	6.07±0.33
LSD (0.05)			10	133	3.2	28	0.86
Significance							
P level (P)			**	**	*	**	NS
AMF			*	NS	*	*	*
P× AMF			NS	**	NS	*	*
Genotype (G)			**	NS	NS	NS	NS
P× G			NS	NS	NS	NS	NS
AMF× G			NS	NS	NS	NS	NS
P× AMF× G			NS	NS	NS	NS	NS

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; NS not significant

1984). The effects of improved P nutrition on seed lipid content are apparently inconsistent, e.g., pea and soybean showed decreases (Bethlenfalvay et al. 1994, 1997) and cotton increases (Sawan et al. 1988) in plants grown with added soil P.

In this study, the only significant interaction found for most studied parameters was P× AMF, indicating that AMF colonization affected these parameters as soil P availability changed. Responses of seed P accumulation to soil P level and correlations between seed P content and protein and lipid contents in AM plants were not consistent with those for nonAM plants grown at low soil P. Likewise, lack of response of seed Pro/L concentration ratios to P level in nonAM plants, and responses in AM plants, indicated that the mechanisms by which P nutrition affects production of seed proteins and lipids are influenced by symbiotic conditions of plants and not by P nutrition or P availability.

Seed yield and P concentration enhancement by AMF at low soil P was higher than at increasing P levels. Chandraskekara et al. (1995) observed reduced seed yields for AM sunflower (*Helianthus annuus* L.) grown at high soil P. The AMF were more effective in enhancing host plant P uptake at lower levels of soil P.

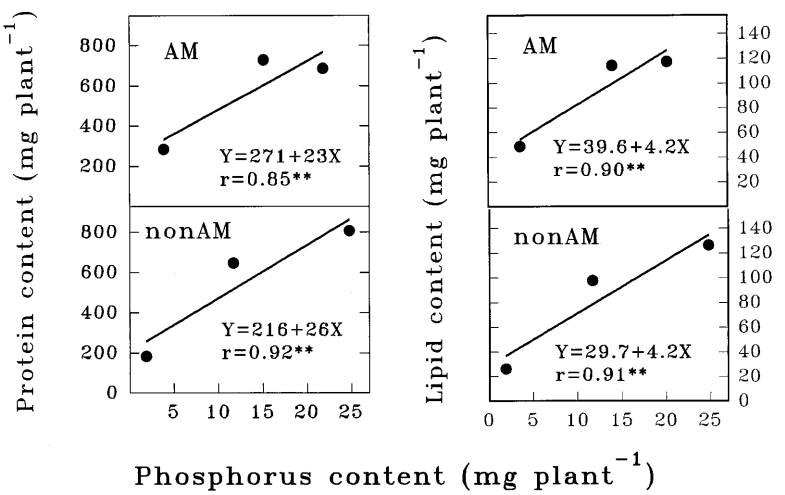

Fig. 2 Regression relationships of lipid or protein contents with P content in seeds of AM and nonAM wheat genotypes. Data represent the mean results for two genotypes

Different relationships between seed lipid and protein contents in AM and nonAM plants indicated that AMF effects on seed composition are distinct from those mediated by P nutrition.

One important factor for selection of wheat cultivars in improvement programs has been high yield rather than improved chemical composition like protein and lipid (Al-Karaki and Ereifej 1998). However, demands for high quality as well as yield of seeds, especially in developing countries, may call for more improvement and refinement of breeding programs. Enhancement of protein and lipid in seeds is determined not only by genetics and environmental variation, but also by their interaction (McKendry et al. 1985). Breeding strategies should consider and account for root AMF interactions and their impact on genetic expression and environmental adaptability of host plants to growth conditions.

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