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Mycorrhizal Fungi Effects on Nutrient Composition and Yield of Soybean Seeds

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ABSTRACT

Nutrient composition and yield of soybean [*Glycine max* (L.) Merr] seeds are heritable traits affected by environmental factors. This study determined the effects of arbuscular-mycorrhizal (AM) fungi on seed protein, lipid, and phosphorus (P) composition and yield in soybean grown under a high nitrogen (N) regime. Plants were grown in pot cultures without AM fungi in P-fertilized (+P) or unfertilized (-P) soil, or in -P soil inoculated with one of the AM fungi *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe (Gm), *Glomus etunicatum* Becker and Gerd.(Ge), or *Gigaspora rosea* Nicol. and Schenck (Gr). Seed yields of +AM plants, as a group, were halfway between those of the +P and -P plants. Seed size was highest in Gm plants. Differences in protein concentrations between Ge and Gr and the other treatments were highly significant. Seed P and protein concentrations were not significantly correlated ($p=0.162$), but a highly significant ($r=-0.949$) negative correlation between seed P and lipid concentrations was observed. Phosphorus concentration was highest and that of lipids lowest in +AM plants. Seed yield and nutrient composition were independent of the intensity of root colonization. The seed protein/lipid ratio was highly correlated with seed P concentration and was significantly higher for +AM plants, as a group, than

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for both +P and -P -AM plants. Differences in seed dry weight, size, seed/stem ratio, P content, and protein concentration among +AM plants showed mycorrhiza-specific host responses. These responses suggest that AM fungi can modify soybean seed development and chemical composition.

INTRODUCTION

Colonization of roots by arbuscular mycorrhizal (AM) fungi alters the development and physiology of soybean plants. Improved P nutrition is the best known of these effects. Others are changes in relative root, shoot, leaf, and nodule development, in the allocation of nutrients to plant organs and to bacterial and fungal endophytes, in symbiotic functions such as carbon dioxide (CO_2) and N_2 fixation, and in the host's response to stress (Bethlenfalvay and Newton, 1989). The AM fungi influence whole-plant processes by regulating plant nutrient status (Espinoza-Victoria et al., 1993) or by eliciting symbiosis-specific production of metabolites independently of plant nutrition (Koide and Schreiner, 1992; Pacovsky and Fuller, 1988).

While AM effects on vegetative growth are well documented, little is known of seed responses to AM root colonization, perhaps because work with AM fungi is usually done in pot cultures without growing plants to maturity (Bethlenfalvay et al., 1994). Yet, seed yield and nutrient composition and the relationship between protein and lipid concentrations are the traits of commercial interest in soybean (Hurburgh et al., 1990).

The physiological basis for phenotypic differences in seed protein and for the inverse relationships between protein and oil concentration (Hartwig and Kilen, 1991) or protein concentration and yield (Hanson, 1991) is little understood (Burton et al., 1995). Seed protein concentration is an inherited trait influenced by the environment (Burton, 1989) and may depend less on the genotype of the embryo than on that of the plant on which the seeds develop (Singh and Hadley, 1968). Thus, whole-plant processes [e.g., carbon (C) and N fixation and allocation], biochemical control (e.g., hormonal regulation), or environmental effects (e.g., nutrient availability) may interact to determine the nutrient composition and yield of soybean seeds (Burton et al., 1995; Mengel et al., 1987). Mycorrhizal fungi modify all of these processes and may also influence seed development. In view of selective preferences between soybean and specific AM-fungal isolates from complex AM-fungal communities (Johnson et al., 1991), identification of host-endophyte combinations and their effects on each other is of interest and consequence to soybean production.

The purpose of this study was to compare seed composition of nonAM soybean plants grown in high- or low-P soil with those of plants colonized by different isolates of AM fungi, and to determine if changes in seed yield and protein and lipid concentration relate to colonization by AM fungi or to P nutrition.

MATERIALS AND METHODS

Experimental Design and Statistics

The experiment consisted of five treatments with five replications and was repeated. Nonsymbiotic plants were grown in fertilized (AM, +P) or nonfertilized (-AM, -P) low-P soil without AM fungi. Symbiotic plants were colonized by one of three AM fungi (Ge, Gm, or Gr). The experimental units (potted plants) were arranged randomly on the greenhouse bench. Differences between the repeated data sets were significant for only one of the seven response variables. The data were, therefore, combined and evaluated together by analysis of variance, orthogonal contrasts, and linear regression. Actual probability values are shown, where appropriate, to permit individual interpretation of significance (Nelson, 1989).

Biological Materials and Soil

Soybean [*Glycine max* (L.) Merr, cv. Hobbit] seeds were pre-germinated, selected for uniformity, planted in 1.5-L plastic pots, and thinned from three to one plant per pot. Pots were filled with a sandy-loam soil (bank of the Willamette River near Corvallis, OR), mixed with sand (1:1, v:v), and pasteurized (75°C, three h). The soil (pH 6.5) contained 71% sand, 20% silt, and 9% clay, and nutrients ($g \cdot kg^{-1}$) as: ammonium-nitrogen (NH_4-N), 1.9; nitrate-nitrogen (NO_3-N), 24.1; P [sodium bicarbonate ($NaHCO_3$)-extractable], 0.01; P (total), 0.5; potassium (K), 176; calcium (Ca), 8.8; magnesium (Mg), 3.5; sulfur (S), 0.8; and micronutrients ($mg \cdot kg^{-1}$) boron (B), 0.1; copper (Cu), 2.4; iron (Fe), 70.0; manganese (Mn), 5.1; and zinc (Zn), 0.8.

Soil inocula of the AM fungi *G. mosseae* [INVAM # CA110 (International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, Division of Plant and Soil Sciences, University of West Virginia, Morgantown, WV 26526-6057)], *G. etunicatum* (INVAM # UT 183-1), and *G. rosea* (INVAM # FL 105) were used to colonize plants of the Gm, Ge, and Gr treatments, respectively, as described by Schreiner et al. (1997). A saturating amount of the inoculum (3,000 spores or sporocarps per pot plus AM root and hyphal fragments) were used, obviating the need for infectivity testing in this long-term experiment. A suspension of the soil microflora from the AM inocula and from unsterilized local soil sieved free of AM propagules was applied to both +AM and -AM soils.

Growth Conditions

Plants were grown in a greenhouse at Corvallis, Oregon (November 1994 to March 1995). The mature seeds were harvested after 135 d of growth. Automatic controls kept temperatures between 18 and 28°C. Sunlight was supplemented by 1,000 W phosphor-coated metal halide lamps (General Electric) providing 16 h of photosynthetically active radiation (500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) at soil-surface level.

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, 1 g·kg^{-1} soil] was added to the soil of the -AM, +P treatment as P fertilizer. Plants were watered from below (saucers) with tap water for three weeks. Then a nutrient solution consisting of (mM) calcium nitrate [$Ca(NO_3)_2$], 1; ammonium nitrate (NH_4NO_3), 1; potassium sulfate (K_2SO_4), 1, magnesium sulfate ($MgSO_4$), 1/4; and micronutrients (≈one-quarter-strength Hoagland solution) was applied. The solutions were replenished as the saucers became dry. The larger plants of the +P and Ge treatments required more moisture than plants of the other treatments and were given tap water between nutrient applications to provide all plants with the same amount of nutrients. The N concentration of the solution was increased to 16 mM on day 43 (onset of flowering). No nutrients were applied during the week prior to harvest while the pods were drying to discourage vegetative growth.

Harvest and Assays

Roots obtained in soil cores 8 d before harvest were washed, cleared [5% potassium hydroxide (KOH), w:v, 30 min, 90°C], and stained with trypan blue in lacto-glycerol (0.05%, 10 min, 90°C). Root colonization (%) by the fungi was estimated by the grid-line intersect method (Giovanetti and Mosse, 1980). Seeds were permitted to dry in the pods for one week after harvest. Since most leaves had fallen by harvest, total shoot weights were not obtained. Stems were weighed to provide a comparative measurement of vegetative versus reproductive tissues. Nutrient determinations of senescent vegetative tissues were not made due to the inherent variability of such data.

TABLE 1. Plant traits. Soybeans were grown in P-fertilized (+P) or unfertilized (-P) soil, or in -P soil but colonized (Col) by the arbuscular mycorrhizal fungi *Glomus mosseae* (Gm), *Glomus etunicatum* (Ge), or *Gigaspora rosea* (Gr). Numbers are the means ±SE of 10 replications. Values followed by the same letter are not significantly different ($p>0.05$).

Treatment	Seed				Root
	Dry wt g plant^{-1}	Number	Average dry wt (g)	Seed/stem ratio	Col (%)
+P	23.2 ±0.9 a	149 ±7 a	0.157 b	0.89 ±0.02 c	0
Gm	14.4 ±0.4 b	86 ±3 b	0.168 a	1.32 ±0.06 a	75.9 ±1.4 a
Ge	13.0 ±0.4 c	84 ±2 b	0.154 b	0.92 ±0.04 c	43.7 ±5.9 b
Gr	12.9 ±0.3 c	83 ±3 b	0.156 b	1.22 ±0.05 ab	76.1 ±1.8 a
-P	8.1 ±0.2 d	53 ±2 c	0.155 b	1.15 ±0.04 b	0

Seed protein, crude fat, and P concentrations were determined from the pooled seeds of each replicate plant by Western Laboratories, Modesto, CA, according to the methods described in the Official Methods of Analysis, paragraphs 7.015, 7.056, and 7.123, respectively (AOAC, 1980).

RESULTS

Phosphorus fertilization (+P) or its absence (-P) had large effects on seed yield (Table 1). Average seed yield of the three AM treatments (13.4 g·plant^{-1}) was 65% higher than that of the -P plants, and 42% lower than that of the +P plants. The Gm plants produced the most seed mass among the +AM plants and had the largest seeds (seed dry wt/seed number) among all treatments (Table 1). The Gm plants also had the highest seed/stem ratio, a measure of resource allocation. Differences in this ratio among +AM plants (e.g., Ge versus Gm, 43%) as well as between plants of the -AM treatments (+P versus -P, 29%) indicated that AM fungi affect carbon (C) partitioning in a manner similar to that of P availability.

Intensive colonization of the Gm and Gr roots showed high morphological compatibility between host and endophyte (Table 1). This condition coincided

TABLE 2. Seed nutrient composition. Soybean were grown in P-fertilized (+P) or unfertilized (-P) soil, or in -P soil but colonized (Col) by the arbuscular mycorrhizal fungi *Glomus mosseae* (Gm), *Glomus etunicatum* (Ge), or *Gigaspora rosea* (Gr). Means ±SE of 10 replications.

Treatment	Seed nutrient composition			
	Protein	Lipid	Phosphorus	Protein/lipid
	Concentration (mg g^{-1})			
+P	354.2 ±3.5 b	205.8 ±13.3 b	2.35 ±0.09 b	1.72 b
Gm	358.6 ±5.0 b	173.8 ± 5.7 c	2.61 ±0.11 a	2.06 a
Ge	376.5 ±4.5 a	184.1 ± 7.5 bc	2.40 ±0.04 b	2.04 a
Gr	369.0 ±3.3 a	189.8 ±13.4 bc	2.50 ±0.09 ab	1.94 a
-P	359.8 ±2.6 b	250.0 ± 6.2 a	2.11 ±0.07 c	1.43 c
	Content (g seed^{-1})			
+P	8.2 ±0.3	4.8 ±0.3	0.054 ±0.001	1.71
Gm	5.1 ±0.2	2.5 ±0.1	0.037 ±0.001	2.04
Ge	4.9 ±0.2	2.4 ±0.1	0.031 ±0.001	2.04
Gr	4.8 ±0.1	2.5 ±0.1	0.032 ±0.001	1.92
-P	3.0 ±0.1	2.0 ±0.1	0.017 ±0.001	1.50

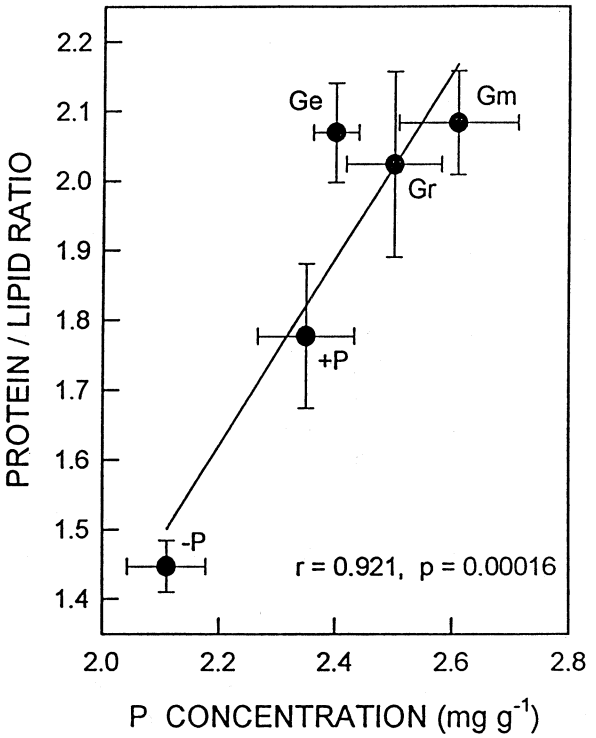

FIGURE 1. The protein/lipid concentration ratio as a function of P concentration in soybean seeds. Plants were grown in P-fertilized (+P) or unfertilized (-P) soil, or in -P soil but colonized by the arbuscular-mycorrhizal fungi *Glomus mosseae* (Gm), *Glomus etunicatum* (Ge), or *Gigaspora rosea* (Gr). Data points represent the means ±SE of 10 replications.

with functional compatibility (favorable AM growth response, Ravnskov and Jakobsen, 1995), expressed here by the high seed/stem ratios of the Gm and Gr plants. Conversely, both colonization and the seed/stem ratio were low in the Ge plants.

Differences between seed protein concentrations were small (Table 2), but highly significant for two of the +AM treatments versus the +P plants (Ge, $p<0.001$; Gr, $p=0.007$). Protein concentration was not correlated with P concentration ($P>0.05$) and was not affected by P availability in -AM plants (+P versus -P, $p=0.286$).

Lipid concentration varied more widely among the treatments than protein concentration, and tended to be lowest in the +AM plants (Table 2). This resulted in higher protein/lipid ratios in the seeds of +AM plants. The lipid concentration of the -P seeds was higher ($p<0.001$) than that of the other treatments. The lipid

TABLE 3. Differences between AM-treatment traits evaluated by orthogonal contrasts. Numbers are probability values.

Variables	Treatment comparisons		
	Ge vs Gm	Ge vs Gr	Gm vs Gr
Seed			
Dry wt plant^{-1}	0.013	0.953	0.011
Average dry wt	<0.001	0.648	<0.001
P concentration	0.071	0.379	0.334
P content	0.001	0.464	0.004
Seed/stem ratio	<0.001	<0.001	0.190
Protein conc.	0.001	0.156	0.052
Root			
Colonization	<0.001	<0.001	0.986

concentrations of Ge, Gr, and +P seeds were grouped ($p>0.05$), and only Gm seeds had significantly lower lipid concentrations than the +P seeds ($p=0.019$).

Seed P concentrations were highest in the +AM plants (Table 2). High P levels tended to be associated with low lipid levels. Seed P and protein levels were not related, but the seed protein/lipid ratio was significantly ($p<0.001$) correlated with seed P concentration (Figure 1). The +AM values in this relationship were grouped and were greater than both -AM values.

Large differences between -AM and +AM values often mask the differences among +AM treatments in statistical evaluations. Differences due to AM root colonization alone occurred in this experiment and were evaluated by orthogonal contrasts separately from the -AM treatments (Table 3). The data (Tables 1 and 2) and the contrasts showed that in spite of the large difference in root colonization between Ge plants and the Gm and Gr plants, there was no consistent pattern in the differences between plant responses to the fungi. Root colonization was, therefore, not a reliable predictor of plant response.

DISCUSSION

Soybean plants grow as nodulated, AM symbioses. In soils low in N and P, the microsymbionts are the major suppliers of N and P to the association, and the three symbionts are competitive sinks for the products (C, N, and P) of their

symbiotic partners (Bayne et al., 1984). Rhizobium effects on soybean seed nutrition are well-known (Harper, 1987), while those of AM fungi are little-known. Interactions between the microsymbionts are complex, especially under P stress (Bethlenfalvay et al., 1985). Since N_2 fixation is impaired during P deficiency (Sa and Israel, 1995) and is partially alleviated by mycorrhiza-mediated uptake of otherwise unavailable P, we grew our plants under a high fertilizer N-regime. This inhibited (but did not completely eliminate) nodulation, avoided P-nutrition-related N-deficiency effects on seed protein status and permitted focus on AM effects.

The salient finding of our study was the difference between yield and nutrient-composition (protein/lipid ratio) responses of soybean seeds to the -AM or +AM treatments. Fertilization of -AM plants (+P) increased yield well above that achieved by the +AM plants, but +P plants did not respond to improved P availability by exceeding the seed P concentrations or protein/lipid ratios of the +AM plants. These data suggest that the mechanisms that influence seed nutrient composition in -AM soybean are modified in +AM plants by direct host-endophyte interactions, but also indirectly through improved P nutrition.

Although these mechanisms are little-known, relevant work has shown alterations in the lipid metabolism of host-plant tissues that accompany a diversion of photosynthate to fungal lipids in biotrophic associations (Lösel, 1980). Such alterations were found to result in a modification of seed lipid composition and content in AM plants (Pacovsky and Fuller, 1987), and led these authors to suggest that genetically-based mechanisms impact on the AM host response that cannot be explained by AM effects on P nutrition.

Much of the variation in the expression of heritable soybean seed traits is ascribed to the environment (Wilson, 1987). Although AM-fungal effects on soybean cultivars (Heckman and Angle, 1987) and yield (Carling and Brown, 1980) are known, AM fungi are have not been recognized as an environmental factor that affects soybean seeds. Different soils in which soybean may be grown contain different AM-fungal communities (Land et al., 1989) with many isolates (Ellis et al., 1992). While plants are colonized by more than one AM-fungal isolate, preference of soybean for specific isolates from the community (Johnson et al., 1991, 1992), suggests that variation in the composition of the AM mycoflora from field to field may influence variability in soybean seed development.

Knowing how compatible individual members of AM-fungal communities in soybean fields are with their host plants may explain some of the variability in seed yield and nutrient composition. This knowledge could then be used to manage AM fungi to promote desirable plant responses (Miller et al., 1994).

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