Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors[1]

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Using mycorrhizal fungi known to colonize wheat, the mycorrhizal dependence of various small grains including modern wheat varieties, primitive wheat lines, and wheat ancestors was studied. With the exception of the United States cultivar Newton and the German cultivars Apollo, Kanzler, and Sperber, dry weight of eight other modern wheats from the United States and Great Britain were increased by 29–100% following inoculation with mycorrhizal fungi. All landraces from Asian collections or early introduced American cultivars were also dependent on the symbiosis, with dry weight increases averaging 169 and 55%, respectively. All wheat ancestors of the AA and BB genomes (except *Aegilops speltoides*) benefitted significantly from the symbiosis, whereas no benefit was observed for ancestors of the DD genome, tetraploid wheats of the AABB or AAGG genomes, or in the hexaploid ancestor *Triticum zhukovskyi* (AAAAGG genome). These differences in mycorrhizal response of the ancestors, lines, and cultivars were highly correlated with root fibrousness ratings. When the fungi used as a combined inoculum in the previous experiment were inoculated individually onto selected plant species or cultivars, 6 of the 10 isolates stimulated growth of *Andropogon gerardii*, a highly dependent grass species, and 8 of the 10 stimulated the growth of 'Turkey' wheat. In contrast, none of the isolates positively affected growth of 'Newton' or 'Kanzler' wheat cultivars, and in fact several fungi decreased the biomass produced by these two cultivars. These studies have demonstrated a strong genetic basis for differences in mycorrhizal dependence among cultivars. A trend for greater reliance on the symbiosis in older cultivated wheats than iin wheat ancestors or modern wheats was also observed. The depression in growth associated with certain mycorrhizal fungi and wheat cultivars demonstrates that colonization of roots does not guarantee benefit from the symbiosis.

Key words: root fibrousness, growth response, vesicular–arbuscular mycorrhizae.

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En utilisant des champignons mycorhiziens dont la capacité à coloniser le blé est connue, les auteurs ont étudié la dépendance mycorhizienne d'espèces à petits grains, telles que des variétés modernes de blé, des lignées primitives de blé, ainsi que les ancêtres du blé. À l'exception du cultivar américain Newton et des cultivars allemands Apollo, Kanzler, et Sperber, les poids secs de huit autres blés modernes des États-Unis et de la Grande Bretagne ont vu leur poids sec augmenter de 29 à 100%, après inoculation avec les champignons mycorhiziens. Touts les lignées de collections asiatiques ou les cultivars américains d'introduction ancienne sont également dépendants de la symbiose, montrant des augmentations de poids secs moyens de 169 et 55%, respectivement. Tous les ancêtres du blé des génomes AA et BB (sauf l'*Aegilops speltoides*) bénéficient grandement de la symbiose, alors que les ancêtres du génome DD, les blés tétraploïdes de génomes AABB ou AAGG, ainsi que l'ancêtre héxaploïde, *Triticum zhukovskyi* (génome AAAAGG). Ces différences de réaction aux mycorhizes chez les ancêtres, les lignées et les cultivars montrent une forte corrélation avec leur classification selon la fibrosité des racines. Lorsque les champignons utilisés en inoculum composite pour l'expérience précédente sont inoculés individuellement sur des espèces ou cultivars sélectionnés, 6 des 10 isolats stimulent la croissance de l'*Andropogon gerardii*, une espèce de graminées fortement dépendante, et 8 sur 10 stimulent la croissance du blé 'Turkey'. Au contraire, aucun de ces isolats n'affecte positivement la croissance des cultivars de blé 'Newton' ou 'Kanzler'; plusieurs champignons diminuent même la biomasse produite par ces deux cultivars. Ces études démontrent qu'il existe une forte base génétique liée à la dépendance mycorhizienne, d'un cultivar à un autre. Les auteurs ont également observé une tendance pour les vieux blés cultivés à dépendre plus fortement de la symbiose que pour les ancêtres du blé ou les blés modernes. La diminution de croissance associée avec certains champignons et certains cultivars de blé démontre que la colonisation des racines ne garantie pas un bénéfice de la symbiose.

Mots clés : fibrosité racinaire, réaction de croissance, mycorhizes à vésicules et arbuscules.

[Traduit par la rédaction]

Introduction

Efforts to assess the importance of mycorrhizal symbiosis in winter wheat (*Triticum aestivum* L.) have focused on the degree of colonization observed in field-grown wheat roots. Root colonization has been used as an indicator of the activity of mycorrhizal symbiosis because growth benefits from mycorrhizae are difficult to assess in the field and because no growth benefit can occur in the absence of colonization. Asai (1934) first suggested that fall-sown cereal crops were not readily colonized by mycorrhizal fungi in the fall. This was confirmed by Jakobsen and Nielsen (1983) in studies in Denmark, Trent *et al.* (1988) for Oklahoma wheats, and Hetrick and Bloom (1983) and Hetrick *et al.* (1984) for wheat grown in Kansas.

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However, fall colonization of wheat was reported in other locations in the United States and Britain (Yocum *et al.* 1985; Buwalda *et al.* 1985; Dodd and Jeffries 1986).

The many explanations offered for these apparent contradictions include insufficient or ineffective inoculum (Vierheilig and Ocampo 1991*a*; Hetrick *et al.* 1984), high soil fertility (Hetrick *et al.* 1984; Young *et al.* 1985; Jakobsen and Nielson 1983), or climatic conditions. Low fall soil temperatures could prevent fungal establishment in seedlings (Jakobsen and Nielsen 1983; Hetrick and Bloom 1984; Young *et al.* 1985; Buwalda *et al.* 1985). In fact, Buwalda *et al.* (1985) suggested that differences in rate of mycorrhizal fungus development between winter and spring wheats could be explained by differences in accumulated thermal time. However, differences in thermal time did not explain the variation in root colonization of wheat at several sites in British Columbia (Cade-Menun *et al.* 1991) or in British studies (Dodd and Jeffries 1989).

Physiological differences between plant cultivars could also explain the observed variation in colonization of wheat. In greenhouse experiments, Azcon and Ocampo (1981) demonstrated that mycorrhizal dependence differed among 13 wheat cultivars. Cultivar differences in response to mycorrhizae have also been observed by Young *et al.* (1985) and Vierheilig and Ocampo (1991*a*, 1991*b*). However, it has been demonstrated that some of these cultivar differences are rather transient and can be overcome by repeated inoculation with the fungal symbionts (Vierheilig and Ocampo 1991*b*). Thus, the effect of the cultivar on susceptibility to mycorrhizal colonization and mycorrhizal dependence is not clear. In other plant species including *Zea mays* (Toth *et al.* 1990), *Vigna unguiculata* (Mercy *et al.* 1990), *Medicago sativa* (Lackie *et al.* 1988), *Arachis hypogaea* (Kesava Rao *et al.* 1990), and *Agropyron cristatum* (Jun and Allen 1991), however, cultivar effects on mycorrhizal dependence have been recognized. In the latter of these studies (Jun and Allen 1991), the cultivars compared varied in ploidy level, but no relationship was observed between ploidy level and mycorrhizal response. More recently, the mycorrhizal dependence of wild, primitive, and modern wheat cultivars of different genomes and ploidy levels was compared (Kapulnik and Kushnir 1991). A significant increase in dry weights of mycorrhizal plants was observed in 6 of 27 wheat lines and species, primarily in those lines containing the D genome.

In an attempt to resolve some of the contradictory data on mycorrhizal symbiosis in winter wheat, we obtained mycorrhizal fungi known to colonize wheat from various locations in Europe and North America. We also obtained seed of wheat cultivars known to support colonization by mycorrhizal fungi from Europe and North America. For comparison, the mycorrhizal dependence of a number of wheat ancestors, primitive wheat lines, and modern cultivars was also quantified. Using these inocula and cultivars, the objective of the present studies was to resolve whether there is a genetic basis for the contradictory responses of wheat to mycorrhizal symbiosis.

Materials and methods

Soil preparation

A native prairie soil, Chase silty clay loam, fine montmorillonitic mesic Aquic Argiudoll of pH 6.8 and containing 12 mg · kg^{-1} plant available P (Bray test 1), was collected from Konza Prairie Research Natural Area, Manhattan, Kansas. The soil was steamed at 80°C for 2 h and allowed to cool for 72 h. Plastic pots (6 × 25 cm) were then filled with 500 g (dry weight) of the steamed soil.

Seed sources

Modern and benchmark U.S. cultivars

Seed of winter wheat cultivars Newton, Pawnee, Triumph 64, Scout 66, Marquis, and Wichita were obtained from the Wheat Genetics Resource Center (Department of Plant Pathology, Kansas State University, Manhattan). Seed of the spring wheat (*T. aestivum*) cv. Alondra was provided by C. F. Konzak (Department of Agronomy, Washington State University, Pullman). The latter cultivar was included because it is known to yield well in low-P soils. Winter wheat varieties known to be colonized by mycorrhizae were obtained from H. W. Dehne (Institut fur Pflanzenkrankheiten, Hannover, Germany). These included the cultivars Apollo, Kanzler, and Sperber. Cultivars from Great Britain, namely Avalon, Fredrich, and Rapier, were provided by the National Small Grains Collection (USDA, Agricultural Research Service, Aberdeen, Idaho). These were included in the study because British Scientists report high levels of colonization in British wheats.

Modern cultivars of barley, oat, and rye were also tested. The winter barley (*Hordeum vulgare*) cultivars Andrea, Igri, Mammut, Marinka, Tapir, and Trixi provided by H. W. Dehne were included because they are readily colonized by mycorrhizal fungi. Seed of *H. vulgare* cvs. Betzes and Carstens and *Avena sativa* cv. Starter and *Secale cereale* cv. Imperial were provided by the Wheat Genetics Resource Center.

Wheat ancestors, landraces, and early introductions

Seed of four *T. aestivum* landraces (genomes AABBDD), namely CI 8621 (collected in China), PI 87115 (Korea), PI 167549 (Turkey), and PI 245624 (Afghanistan), three landrace cultivars, namely Mediterranean, Purple Straw, and Turkey, introduced into the United States in the 18th and 19th centuries and grown widely as cultivars, 12 diploid wheat ancestors of the AA, BB, and DD genomes, four tetraploid ancestors of the AABB and AAGG genomes, and two hexaploid wheat cultivars with the AABBGG genome were provided by the Wheat Genetics Resource Center.

Seedling treatment

All seeds were germinated in 4 × 4 cm plastic pots (four seeds per pot) containing sterile vermiculite. Six days after emergence (2–3 leaf stage), seedlings were vernalized in a 4°C growth chamber for 42 days. An 8-h photoperiod was provided by fluorescent lighting. Following vernalization, 10 seedlings of each species were individually transplanted into pots containing steam-pasteurized soil. One-half of the seedlings of each species were inoculated with vesicular–arbuscular mycorrhizal fungal spores at transplant, whereas the remaining half were not.

Mycorrhizal fungus inoculum preparation

Inoculum for these studies was obtained by mixing spores from a variety of species and isolates known to colonize wheat or native grasses. *Glomus etunicatum* Becker and Gerd. and *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe were obtained from the Konza Prairie Research Natural Area. A *Glomus versiforme* (Karsten) Berch isolate obtained originally from Oregon State University and maintained in pot cultured at Kansas State University was also used. Another isolate of *G. versiforme* and *Glomus intraradix* Schenck and Smith, both known to colonize wheat, were obtained from Dr. L. Peterson (University of Guelph, Guelph, Ont.). Dr. J. Dodd (University of Kent, Canterbury, Kent, U.K.) provided *Glomus geosporum* (Nicol. and Gerd.) Walker, *Glomus monosporum* Gerd. and Trappe, and *G. mosseae* isolates known to colonize wheat. Two isolates of *G. etunicatum*, known to colonize wheat and barley, were obtained from the Institut fur Pflanzenkrankheiten (Hannover, Germany).

All fungi, except the German isolates of *G. etunicatum*, were propagated on asparagus (*Asparagus officinalis* L. cultivar UC72) for 8–12 months prior to use. Spores were isolated from pot cultures by wet sieving, decanting, and sucrose density gradient centrifugation (Daniels and Skipper 1982). Spores were suspended in distilled water and combined to obtain a final spore suspension of 400 spores/mL, with 50 spores of each of the eight species. At transplant 1 mL of

TABLE 1. Total plant dry weight, root fibrousness rating, percent growth response, mycorrhizal dependency, and mycorrhizal root colonization of various cultivars of *Triticum aestivum, Hordeum vulgare, Avena sativa*, and *Secale cereale*

Origin and cultivar	Total dry wt. (g/plant)		Root fibrousness rating	Root colonization (%)	Growth response (%)	Mycorrhizal dependency (%)
	Mycorrhizal	Nonmycorrhizal				
Modern wheat varieties						
United States						
Triumph 64	1.67*	1.10	3.6	29.2	51.8	34.1
Wichita	2.24*	1.12	3.4	24.4	100.0	50.0
Pawnee	2.54*	1.58	4.6	45.0	60.8	37.8
Marquis	2.04*	1.72	4.2	18.2	29.5	22.8
Scout 66	2.52*	1.45	4.6	24.6	73.8	42.5
Newton	1.90	1.66	5.0	25.6	14.5	12.6
Brazil						
Alondra	1.46*	0.78	3.4	71.2	87.2	46.6
Germany						
Apollo	3.16	2.95	5.6	50.8	7.1	6.6
Kanzler	3.48	3.45	5.6	70.8	0.9	0.9
Sperber	3.09	2.92	5.0	32.2	5.8	5.5
Great Britain						
Avalon	3.97*	2.47	5.0	71.2	60.7	37.8
Fredrich	3.25*	2.09	4.6	66.8	55.5	35.7
Rapier	4.19*	2.87	5.0	37.0	46.0	31.5
LSD ($P = 0.05$)	0.645	0.724	0.710	11.95	—	—
Barley						
United States						
Betzes	3.08	3.08	6.5	3.6	0.0	0.0
Carstens	3.28	3.08	5.6	6.0	6.5	6.1
Germany						
Andrea	3.10	3.49	5.0	7.5	−11.2	−12.6
Igri	3.43	3.70	5.6	0.0	−7.3	−7.9
Marinka	2.32	2.52	5.6	5.2	−7.9	−8.6
Tapir	3.78	3.68	7.4	3.8	2.7	2.6
Mammut	3.93*	2.88	5.0	15.0	36.5	26.7
Trixi	3.25*	2.19	5.0	29.4	48.4	32.6
Oat						
Starter	2.58	2.27	4.4	7.2	13.7	12.0
Rye						
Imperial	2.16	2.48	5.6	3.6	−12.9	−14.8
LSD ($P = 0.05$)	0.726	0.677	0.419	6.96	—	—

NOTE: Root fibrousness scale defined in text. Only data for mycorrhizal plants are presented. Differences between wheat varieties or barley, oats, and rye within a column may be determined using the LSD value.

*Means within a plant species are significantly ($P = 0.05$) different as determined by the least significant difference (LSD) test.

spore suspension was pipetted onto roots of seedlings. The German isolates of *G. etunicatum* were obtained in an expanded clay carrier that was used directly as an inoculum source. One gram of the carrier containing spores and hyphal fragments of *G. etunicatum* was added to each seedling that received the previously described mycorrhizal inoculum mixture. Inoculum was added following seedling vernalization to ensure that low vernalization temperatures would not adversely affect fungal species survival or inoculum potential. Also, it has been demonstrated that in our climate, colonization does not normally occur in fall as soil temperatures are declining (Hetrick and Bloom 1983).

This use of multiple spore sources of known ability to colonize wheat and native grasses was considered the best procedure to ensure that incompatibility between inoculant and plant species or cultivars would not cloud data interpretation. To obtain some measure of the efficiency of the individual inoculum sources, these fungi were inoculated separately onto *Andropogon gerardii* Vitm. (a native prairie grass that responds to a number of mycorrhizal fungi, Hetrick and Wilson 1991) and *T. aestivum* cvs. Kanzler, Newton, and Turkey (landrace). For the German *G. etunicatum* isolates, 2 g of the expanded clay carrier with spores and hyphae were used as inoculum. All other fungi were individually added as a spore suspension (400 spores per pot) to each of five replicate pots for each plant species. As controls, five pots of each plant species were not inoculated.

Experimental design and maintenance

Pots were arranged in a randomized complete block design with five replications per treatment. The plants were maintained in a 12−17°C greenhouse for 6 weeks and subsequently maintained at 20−25°C for the remainder of the study. This variation in green-

TABLE 2. Total plant dry weight, root fibrousness rating, percent growth response, mycorrhizal dependence, and mycorrhizal root colonization of *Triticum aestivum* landraces

Accession	Total dry wt. (g/plant)		Root fibrousness rating	Root colonization (%)	Growth response (%)	Mycorrhizal dependency (%)
	Mycorrhizal	Nonmycorrhizal				
Asian collections						
CI 8621	1.38*	0.59	2.6	70.8	133.9	57.2
PI 87115	1.75*	1.17	4.2	71.0	49.6	33.1
PI 1617549	2.00*	0.46	4.0	63.0	334.8	77.0
PI 245624	1.55*	0.60	3.8	67.8	158.3	61.3
Introduced American cultivars						
Turkey	2.65*	1.57	3.7	31.1	68.8	40.8
Purple Straw	2.30*	1.37	3.8	6.3	67.9	40.4
Mediterranean	2.24*	1.73	4.8	20.4	29.5	22.8
LSD (P = 0.05)	0.688	0.612	0.743	13.62	—	—

NOTE: Root fibrousness scale defined in text. Only data for mycorrhizal plants are presented. Differences between landraces within a column may be determined using the LSD value.
*Means within a landrace are significantly (P = 0.05) different as determined by the least significant difference (LSD) test.

house temperatures was used to simulate field conditions. Plants were watered daily and fertilized biweekly with 0.0625 g Peter's No-Phos Special Fertilizer solution (25:0:25; Robert B. Peters Co., Inc., Allentown, Penn.) dissolved in 50 mL H_2O. Thus, approximately 35 μg/g N and 30 μg/g K were added biweekly to each pot. After 14 weeks, plants were harvested and shoot, root, and total dry weight were recorded. Subsamples of dried roots were stained in trypan blue (Phillips and Hayman 1970) and examined microscopically to assess percent root colonization using a Petri dish scored in 1-mm squares (Daniels *et al.* 1981). Primary root diameters of each plant species were measured microscopically using an ocular micrometer. Four representative primary roots of each plant were measured and averaged to obtain root diameters for each plant species. Primary roots were defined as the large adventitious roots emerging directly from the crown of the plant. The root system coarseness, branching, and number of primary roots were rated using the following scale developed by Hetrick *et al.* (1988): 1 one primary root, few or no lateral branches; 2, few primary roots (2–9), moderately to highly branched; 3, moderate number of primary roots (10–30), sparingly branched, coarse; 4, moderate number of primary roots (10–30), moderately branched, moderately coarse; 5, moderate number of primary roots (10–30), highly branched, abundant fine roots; 6, large number of primary roots (>30), sparingly branched, coarse; 7, large number of primary roots (>30), moderately branched, moderately coarse; and 8, large number of primary roots (>30), highly branched, abundant fine roots.

Statistical analysis

A one-way analysis of variance (ANOVA, $P \leq 0.05$) was performed on shoot, root, and total dry weight, root colonization, root fibrousness rating, and root diameter for each plant species using the SAS statistical package (SAS Institute Inc. 1988). Since shoot and root dry weight were each highly correlated with total dry weight, only total dry weights are presented to simplify data presentation. Root to shoot ratios were also assessed, but no trends were observed and these data are not presented. Growth responses were calculated for each plant species as follows: percent growth response = [(dry weight inoculated − dry weight noninoculated)/dry weight noninoculated] × 100. Mycorrhizal dependence was calculated similarly except the denominator was the dry weight of inoculated rather than noninoculated plants. The relationship between percent growth response and root fibrousness or root colonization was examined for all *T. aestivum* varieties and ancestors using nonlinear regression analysis. Correlation analysis was also used to assess the relationship between root colonization and growth response. In these latter analyses data for the Asian landrace PI 167549 were omitted because it was consistently aberrant.

Results

Modern wheat and barley varieties

With the exception of the cultivar Newton, inoculation with mycorrhizal fungi increased the growth of all the United States wheat cultivars by 29–100%, with root colonization ranging from 18 to 45% (Table 1). Though colonization levels were high in wheat cultivars from Brazil, Germany, and Great Britain, growth was stimulated by mycorrhizae only in the Brazilian and British wheats. When correlation analysis was conducted for root colonization and growth response, there was no significant correlation between these variables.

Barley cultivars from the United States were not affected by mycorrhizal inoculation, and growth of only two of the six German cultivars was improved by inoculation (Table 1). No growth improvement was evident in the oat and rye cultivars tested. Root colonization levels in barley, oat, and rye were relatively low, particularly in cultivars not affected by mycorrhizae. Consequently, there was a strong correlation (r = 0.89, P <0.001) between root colonization and growth response in these small grains.

Landraces

When the effect of mycorrhizae on growth of Asian or United States wheat landraces was assessed, all produced greater dry weight in the presence of the symbiosis (Table 2). Growth responses were variable and ranged between 49 and 334% in the Asian collections and between 29 and 68% in the introduced United States cultivars. Root colonization levels were significantly higher in the Asian collections than in the introduced United States cultivars; however, there was no significant correlation (r = 0.59, P = 0.22) between root colonization and growth response for the landraces.

Wheat ancestors

Assessment of mycorrhizal fungus effects on wheat ancestors of different genomes revealed that positive growth responses occurred in all ancestors of the AA, BB, and VV genomes except *Aegilops speltoides* (BB genome), which was not improved by mycorrhizal symbiosis (Table 3). No mycorrhizal response was observed in diploid ancestors of the DD genome or in any of the tetraploid wheats (representing either the AABB or AAGG genome) studied. Similarly, no significant response to mycorrhizal symbiosis was observed in the hexaploid wheats

TABLE 3. Total plant dry weight, percent growth response, and mycorrhizal root colonization of wheat (*Triticum aestivum* L.) ancestors or related genomes

Wheat ancestor and cultivar	Total dry wt. (g/plant)		Root fibrousness rating	Root colonization (%)	Growth response (%)	Mycorrhizal dependence (%)
	Mycorrhizal	Nonmycorrhizal				
Diploid (AA)						
Triticum boeoticum	2.92*	1.72	4.0	71.2	69.8	41.1
Triticum urartu	1.75*	0.83	4.2	77.4	110.8	52.6
Triticum monococcum (TA 2712)	2.91*	2.08	4.8	26.6	39.9	28.5
Triticum monococcum (TA 138)	3.92*	2.41	4.2	87.6	62.7	38.5
Diploid (BB)						
Aegilops bicornis	1.93*	0.64	3.0	74.0	201.6	66.8
Aegilops sharonensis	2.12*	1.54	4.6	16.0	37.7	27.4
Aegilops ligustica	2.15*	1.65	4.8	20.2	30.3	23.3
Aegilops longissima	2.45*	1.75	4.0	79.8	40.0	28.6
Aegilops speltoides	1.78	1.79	5.3	28.6	−0.6	−0.6
Diploid (VV)						
Haynaldia villosa	2.27*	1.58	4.0	22.8	43.7	30.4
Diploid (DD)						
Triticum tauschii (TA 1691)	2.44	2.40	5.0	19.4	1.7	1.6
Triticum tauschii (TA 1649)	1.53	1.73	5.8	22.8	−11.6	−13.1
Tetraploid (AABB/AAGG)						
Triticum araraticum	1.57	2.13	6.6	16.0	−26.3	−35.7
Triticum dicoccoides	2.01	1.84	5.4	58.4	9.2	8.5
Triticum turgidum cv. Langdon	2.57	3.15	5.6	11.2	−18.4	−22.6
Triticum timopoheevii	3.71	3.85	7.3	48.4	−3.6	−3.8
Hexaploid (AABBGG/AABBDD)						
Triticum zhukovski	1.73	2.53	5.8	21.6	−31.6	−46.2
Triticum aestivum cv. Newton	1.53	1.99	6.8	20.2	−23.1	−30.1
LSD (P = 0.05)	0.77	0.32	0.53	13.48	—	—

NOTE: Root fibrousness scale defined in text. Only data for mycorrhizal plants are presented. Differences between wheat ancestors within a column may be determined using the LSD value.

*Means within a plant species are significantly (P = 0.05) different as determined by the least significant difference (LSD) test.

Triticum zhukovskyi (AAAAGG genome) or *T. aestivum* cv. Newton (AABBDD genome). However, the absence of growth response in the hexaploid wheat 'Newton' was not typical of hexaploid wheats; cultivars other than 'Newton' responded positively to the symbiosis (Table 1). There was a positive correlation (r = 0.66, P = 0.002) between root colonization and growth response among the ancestors. A schematic diagram of the evolution of wheat is given in Fig. 1. *Haynaldia villosa* of the VV genome, although included as a diploid genome ancestor, is not included in the diagram because it is not believed to have contributed directly to the evolution of modern wheat.

Effect of inoculum isolate

Throughout the previous experiments, a mixture of fungal species was used to ensure that inoculum differences would not affect the cultivar responses. When these species were tested individually on a native grass species (*A. gerardii*), *T. aestivum* cultivars Kanzler and Newton and the landrace Turkey, 5 of the 10 fungal isolates stimulated the growth of *A. gerardii*, and 8 of the 10 isolates stimulated the growth of 'Turkey' wheat (Table 4). In contrast, none of the isolates positively affected 'Newton' or 'Kanzler' wheat and, in fact, several fungal isolates significantly decreased the biomass produced by these wheats. Thus, there was no apparent relationship between growth response and country of origin of the fungal isolate and plant cultivars.

Root colonization varied considerably among plants and fungal isolates. Relatively high levels of root colonization were observed occasionally, even when plants did not benefit from the symbiosis. Thus, there was no apparent relationship between growth response and root colonization for *A. gerardii* and *T. aestivum* cultivars inoculated with individual fungal isolates.

Relationship between growth response to mycorrhizae and root fibrousness

When the root systems of wheat cultivars and ancestors were compared, root fibrousness of mycorrhizal and nonmycorrhizal plants of the same species were highly correlated (P = 0.0001). Therefore the ratings for nonmycorrhizal plants were omitted to simplify data presentation. When the comparative fibrousness of the various plant species and cultivars was examined, however, genotypes that responded positively to mycorrhizal symbiosis appeared to have less fibrous root systems than those that did not benefit from mycorrhizae (Tables 1−3). Nonlinear regression analysis of these data (Fig. 2) revealed a negative relationship between percent growth response and root fibrousness (R^2 = 0.719). Plants with greater than 25% growth response to mycorrhizae generally also had root systems with a fibrousness rating below 5. Plants that did not benefit from the symbiosis had root systems with fibrousness ratings of 5.2−7.4. There was no apparent evolutionary relationship between root fibrousness, growth response, and wheat ancestors or modern cultivars. Though primary root diameter has been a good indicator of mycorrhizal dependence in studies with other grasses (Hetrick *et al.* 1988), no trend was evident in the wheat ancestors or cultivars tested in the present studies. Therefore, these data are not presented.

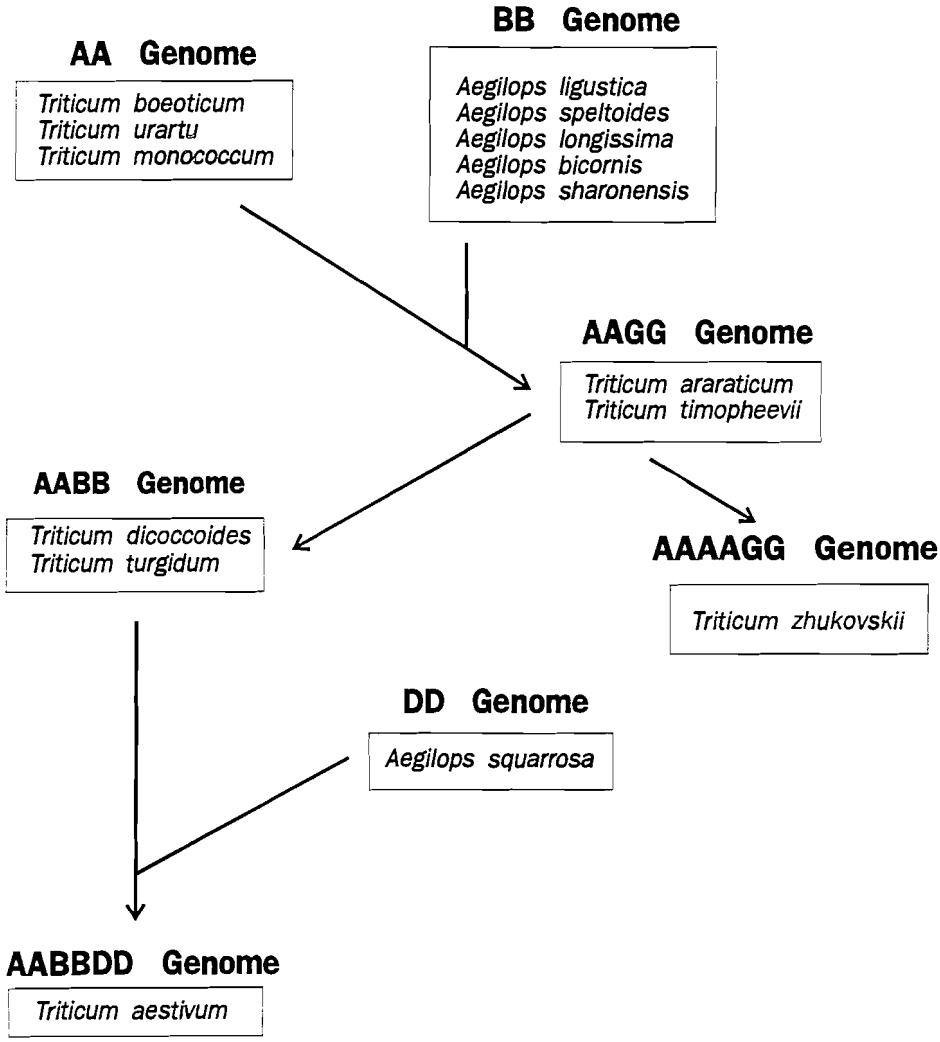

FIG. 1. Schematic diagram detailing the interaction of wheat ancestors in the evolution of wheat.

TABLE 4. Effect of various vesicular–arbuscular mycorrhizal fungal species on dry weight production and root colonization of three cultivars of *Triticum aestivum* and *Andropogon gerardii*

	Total dry wt. (g)				Root colonization (%)			
		T. aestivum				*T. aestivum*		
Inoculum	*A. gerardii*	'Kanzler'	'Newton'	'Turkey'	*A. gerardii*	'Kanzler'	'Newton'	'Turkey'
United States								
G. etunicatum	1.59*	3.05	2.23	3.24*	37.2*a*	46.2*b*	36.8*c*	55.8*ab*
G. mosseae	1.39*	**1.50***	2.11	2.64*	26.0*b*	42.4*b*	44.8*c*	27.6*c*
G. versiforme	0.90*	**2.08***	**1.32***	1.28	15.4*bc*	90.5*a*	61.4*b*	3.0*e*
Great Britain								
G. mosseae	1.33*	3.54	**1.28***	3.29*	25.2*b*	6.4*d*	4.8*d*	28.6*c*
G. geosporum	1.28*	2.45	2.20	3.19*	17.8*b*	7.6*d*	76.8*a*	21.2*cd*
G. monosporum	0.03	**1.27***	2.23	1.57	0*d*	16.5*c*	74.2*a*	2.4*e*
Canada								
G. intraradix	0.24	3.01	**0.98***	3.08*	23.5*b*	2.8*d*	34.8*c*	49.4*b*
G. versiforme	0.08	3.03	2.59	3.09*	4.8*cd*	2.6*d*	15.0*d*	70.8*a*
Germany								
G. etunicatum A	0.14	3.47	2.59	3.16*	0*d*	18.2*c*	3.2*de*	18.8*cd*
G. etunicatum B	0.03	**2.42***	2.46	3.09*	0*d*	18.4*c*	0*e*	25.0*cd*
Noninoculated	0.08	3.75	2.59	1.60	0*d*	0*e*	0*e*	0*e*

NOTE: Means within a column followed by the same letter are not significantly ($P = 0.05$) different as determined by least significant difference (LSD) test. Values that are boldface designate significant ($P = 0.05$) growth depressions compared with noninoculated control.

*Significant ($P = 0.05$) differences between inoculated and noninoculated plants of the same species as determined by the paired *t*-test.

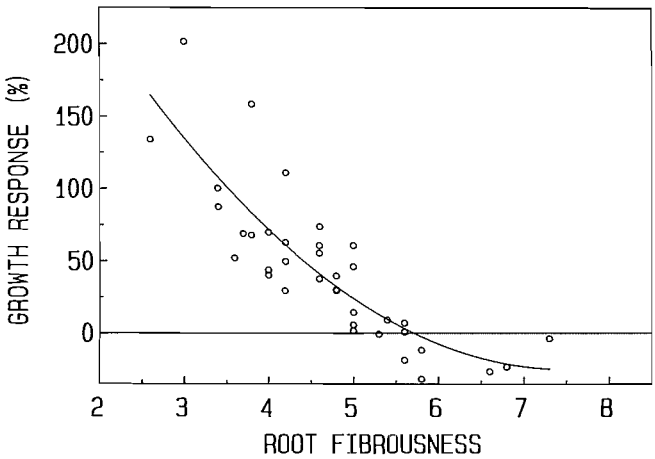

FIG. 2. Nonlinear regression model demonstrating the relationship between percent growth response and root fibrousness of modern wheat varieties, landraces, and ancestors. $y = 11.44x^2 - 156.48x + 512.78$, $R^2 = 0.719$. The scale used to assess root fibrousness is detailed in the text.

Discussion

Considerable past research has focused on whether or not wheat is colonized by mycorrhizae early or late in the growing season, because in the absence of root colonization, the potential for growth improvement does not exist. The present studies have sought to resolve some of the contradictory nature of the past research by inoculating both cultivars known to be colonized and those in which colonization is not readily apparent. Both fungal isolates known to colonize wheat and isolates with unknown efficiency on wheat were used in a combined inoculum to ensure that inoculum density, efficiency, or host specificity would not unduly influence the cultivar responses observed.

The present studies have revealed significant differences in how cultivars respond to mycorrhizal symbiosis. There was also a trend for benefit to be stronger or more consistent in primitive wheat varieties, ancestors, and landraces than in modern cultivars. This was particularly evident in the final experiment in which the fungal isolates were inoculated individually onto *T. aestivum* cultivars Turkey, Newton, and Kanzler (Table 4). A wide range of fungi were able to stimulate growth of the landrace Turkey, whereas none stimulated the growth of the other two modern cultivars. In fact, growth depressions were observed for several of the fungi on these modern cultivars. The United States cultivar Newton and all of the German cultivars tested in the present research were released since 1977, and none showed a response to colonization. However, the British cultivars, also recently released, did show a response. Thus, among more recent cultivars, the effect of mycorrhizal symbiosis on plant growth is less consistent.

The general trend, in which mycorrhizal response was greatest in Asian landraces, less in older, introduced or improved cultivars, and lowest in modern cultivars, is probably not related to their growth habit, since both high and low responses were observed within semidwarf and tall varieties. Instead, this general trend could be related to breeding history. Development of cultivars in this century has been done primarily at experiment stations where use of inorganic fertilizers is common, especially in recent decades. Though mycorrhizal symbiosis can improve the nutritional status of host plants there is also a metabolic cost to the plant for maintaining the symbiosis (Hayman 1983). Therefore, germ plasm selection under fertilized conditions could have reduced the frequency of genes that foster mycorrhizal associations.

A genetic control of plant resistance to mycorrhizae has been described by Duc *et al.* (1989). They observed that colonization of roots by mycorrhizal fungi was completely inhibited in *myc*$^-$ mutants of legumes, regardless of the fungal species used as inoculum. Based on their research, the ability of a plant to form mycorrhizae is apparently under the control of several recessive genes. However, for plants like the grasses studied here, which do form mycorrhizae, there appears to be a further genetic regulation for the extent of colonization and benefit from the symbiosis. This appears to be strongly related to root fibrousness and consequently confers a degree of mycorrhizal dependence on individual cultivars or plant species.

That the degree of mycorrhizal colonization is under genetic control is further indicated by the research of Toth *et al.* (1990) who demonstrated that lower levels of mycorrhizal colonization occurred in corn cultivars that were also somewhat resistant to a wide range of plant pathogenic fungi. They observed a positive relationship between disease susceptibility, mycorrhizal dependence, and smaller root system size. This is particularly interesting because the cultivar Newton, which was colonized but did not benefit from the symbiosis, has been grown extensively throughout the state of Kansas and was released because of its resistance to wheat soil-borne mosaic virus. Since susceptibility to mycorrhizae can be reduced when resistance genes to fungal pathogens are introduced into a cultivar, or as a result of modern breeding strategies for disease resistance (Toth *et al.* 1990), the absence of response to mycorrhizae in this cultivar could result from inclusion of disease-resistance genes.

Though in the grass *A. cristatum* no relationship was observed between ploidy level and mycorrhizal symbiosis (Jun and Allen 1991), in the present study strong relationships between ploidy level and mycorrhizal growth responses were observed. Significant growth responses were observed only in the diploid wheat ancestors of the AA, BB, and VV genomes. No responses were observed in tetraploid wheats, although positive responses can be observed in hexaploid wheats, depending on the cultivar tested. These results differ considerably from those of Kapulnik and Kushnir (1991) who observed more consistent mycorrhizal dependency in the D-genome ancestor, *Triticum tauschii* (=*Aegilops squarrosa* (D genome)) lines than in the others tested. The considerable variation in the genetic makeup of *T. tauschii* (Gill *et al.* 1986) may explain this disparity in results. However, other differences between the results of the present studies and those of Kapulnik and Kushnir (1991) are more difficult to explain and may be related to the host affinities of the fungal species used in the latter study or to the differences in growth medium and fertility levels of the two studies.

A relationship between plant response to mycorrhizae and root morphology was first hypothesized by Baylis (1975) for plants with widely different rooting strategies. More recently, far more subtle differences in root morphology have been related to variation in mycorrhizal dependence (Hetrick *et al.* 1991). In the present studies, differences in response to mycorrhizae were related to subtle differences in rooting strategy observed within wheat cultivars and genetic ancestors

of wheat. In previous studies (Hetrick *et al.* 1991), it was demonstrated that plants that rely heavily on mycorrhizal symbiosis (obligate mycotrophs) have more plastic root morphologies, i.e., they maintain a less fibrous root system in response to colonization by the mycorrhizal symbiont. In facultative mycotrophs such as wheat, however, root morphology appears to be a fixed characteristic, unchanged by mycorrhizal colonization of roots. Therefore, the observed variation in root morphologies among wheat cultivars and ancestors and similarity of morphologies within cultivars or ancestors, whether or not they are mycorrhizal, suggests that the morphological differences observed are genetic characteristics of the plants themselves rather than changes induced by the symbiosis.

The present studies suggest that mycorrhizal dependence is a genetic trait that is strong in some wheat genotypes and absent in others. Since mycorrhizal dependence is an index of plant response that might be anticipated at a particular P level (Plenchette *et al.* 1983), only relative relationships between cultivars can be inferred from this index. The P level of the soil used in the present experiments is relatively low. Therefore, both the degree of growth response and the number of cultivars responding to mycorrhizae probably would decline with increasing P levels in the soil. Further research will be necessary to describe these relationships at higher P levels and under field conditions.

Further research will also be necessary to determine how this mycorrhizal dependence was lost in tetraploid and some hexaploid wheats and retained in others. The greater benefit from mycorrhizal symbiosis conferred on landraces than on some modern cultivars of wheat suggests that mycorrhizal dependence is stronger in older populations of wheat. It is interesting that the benefit to seedling growth conferred on cultivated landraces of wheat is apparently greater than the benefit conferred on wild wheat ancestors. It is possible that monoculture in the absence of heavy fertilization selects for mycorrhizal dependence. Whether selection of wheat lines for low-input agriculture will increase reliance on the symbiosis remains to be studied.

It is clear from the present studies and from those of Kapulnik and Kushnir (1991) that there is no consistent relationship between the degree to which a plant is colonized and the potential for the plant to benefit from colonization. Although in the present studies a positive relationship between these variables was evident for barley and wheat ancestors, no significant relationship was evident for modern wheat cultivars or landraces of wheat. Thus, the observation of colonization in fall or spring in field-grown wheat should not be interpreted to imply beneficial effects on plant growth. In fact, the data presented in Table 4 suggest that it is just as likely that colonization can significantly reduce plant growth. The time at which colonization is initiated is apparently of far less importance than determination of whether any benefit to plant growth accompanies colonization. The significant correlation between root fibrousness and mycorrhizal dependence suggests that the use of a morphological trait such as root architecture is probably a more reliable predictor of plant benefit from the symbiosis than is colonization by the fungal symbiont.

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