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## Growth response of lentil and wheat to *Glomus clarum* NT4 over a range of P levels in a Saskatchewan soil containing indigenous AM fungi

Accepted: 22 February 1997

**Abstract** The growth responses of lentil (*Lens esculenta* L. cv. Laird) and two wheat cultivars (*Triticum aestivum* L. cv. Laura and Neepawa) to *Glomus clarum* NT4 in soil containing indigenous arbuscular mycorrhizal fungi (AMF) and fertilized with phosphorus at different (0, 5, 10, 20 ppm) levels was studied in a growth chamber. Soil was inoculated with a monospecific culture of *G. clarum* NT4 to provide an inoculant:indigenous AMF ratio of ca. 1:100. The shoot and root growth, and AMF colonization levels of NT4-inoculated lentil were significantly ( $P \leq 0.05$ ) greater than the appropriate control plants in the unfertilized soil at 48 days after planting (DAP). At 95 DAP, NT4 inoculation had significantly increased the shoot dry weight ( $P \leq 0.08$ ) and AMF colonization ( $P \leq 0.05$ ) of lentil plants receiving 5 mg P kg<sup>-1</sup> soil, whereas 20 mg P kg<sup>-1</sup> soil reduced the shoot growth of NT4-inoculated plants. The NT4 inoculant had no effect ( $P \leq 0.05$ ) on shoot P content, but increased ( $P \leq 0.08$ ) the P-use efficiency of lentil plants receiving 5 mg P kg<sup>-1</sup> soil. In contrast to the inoculant's effect on lentil, NT4 generally had no positive effect on any of the parameters assessed for wheat cv. Laura at any P level at 48 or 95 DAP. Similarly, there was no positive effect of NT4 on shoot or root growth, or AMF colonization of wheat cv. Neepawa plants at any P level at 48 DAP. However, NT4 inoculation increased the grain yield of Neepawa by 20% ( $P \leq 0.05$ ) when fertilized with 20 mg P kg<sup>-1</sup> soil. This yield increase was associated with a significant ( $P \leq 0.05$ ) reduction in root biomass and a significant ( $P \leq 0.05$ ) increase in the grain P content of inoculated plants. Thus, NT4 appears to have a preference for the Neepawa cultivar. Our results show that lentil was more dependent on mycorrhizae than wheat and responded to an AMF inoculant even in soil containing

high levels of indigenous AMF. It might, therefore, be possible to develop mixed inoculants containing rhizobia and AMF for field production of legumes.

**Key words** *Glomus clarum* NT4 · Lentil · Wheat · Indigenous AMF · Phosphorus

### Introduction

Arbuscular mycorrhizal fungi (AMF) form an obligate association with most plant roots and can influence crop production. The importance of AMF in crop production lies in their ability to stimulate plant growth in soils with limited amounts of available P, where the AMF external mycelium provides a larger surface area for increased absorption of P (Jakobsen et al. 1992). Furthermore, the ability of AMF to enhance plant growth is influenced by their specific interaction with the host-plant or cultivar (Azcon and Ocampo 1981). In the case of legumes, interactions between AMF and rhizobia also can influence the tripartite symbiosis and plant growth (Ianson and Linderman 1993).

Talukdar and Germida (1993a) isolated several species of AMF that readily colonized field-grown wheat and lentil roots. One of these isolates, *G. clarum* NT4, was found in all the field sites sampled, and significantly enhanced the yield of lentil and spring wheat in "sterilized soil" lacking AMF (Talukdar and Germida 1994). However, interactions between different members of the AMF community in soil may range from neutral to distinct interference (Wilson 1984), and their impact on plant growth may be positive or negative. Therefore, depending upon the composition and effectiveness of individual members of the indigenous AMF community, a plant's growth response to an AMF isolate in sterilized soil may vary from that observed in normal soil. Here we assessed the impact of *G. clarum* NT4 on the growth response of lentil and spring wheat in soil containing indigenous AMF and different levels of P fertilizer.

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## Materials and methods

### AMF inoculum preparation

A monospecific culture of *G. clarum* NT4 was produced on a maize (*Zea mays* L. cv. Early Bantam) host grown in 2 kg of sterile soil:sand mix (1:1) for 90 days as described previously (Talukdar and Germida 1993b). The monospecific culture was maintained at 5 °C and contained ca. 2200 total infective NT4 propagules per 50 g as determined by a Most Probable Number assay (Porter 1979).

### Soil

The soil was a sandy loam collected from a summerfallow-wheat rotation (1992–1993) field at Outlook, Saskatchewan. The nutrient status (mg kg<sup>-1</sup>) of soil was as follows: NO<sub>3</sub>-N 6.6; available P 5; K 386; SO<sub>4</sub>-S 7.0; Cu 1.42; Fe 12.6; Zn 3.8; Mn 12.2; and B 0.74. The soil had a pH of 7.2, and contained 3.0% organic matter. Air-dried soil was passed through a 4-mm sieve, and 2 kg potted in 15-cm-diameter pots. The total infective AMF propagule density of the air-dried soil was ca. 330 per 50 g soil (i.e., 13200 per pot).

### Treatments set up for lentil

Eight treatments were assessed: (1) soil, no P fertilizer, (2) soil, no P fertilizer + *G. clarum* NT4, (3) soil + 5 mg P kg<sup>-1</sup> soil, (4) soil + 5 mg P kg<sup>-1</sup> soil + *G. clarum* NT4, (5) soil + 10 mg P kg<sup>-1</sup> soil, (6) soil + 10 mg P kg<sup>-1</sup> soil + *G. clarum* NT4, (7) soil + 20 mg P kg<sup>-1</sup> soil, (8) soil + 20 mg P kg<sup>-1</sup> soil + *G. clarum* NT4. Before seeding, each pot was amended with 0.14 g of KNO<sub>3</sub> and 0.33 g of K<sub>2</sub>SO<sub>4</sub> in 300 ml of water to provide 10 mg kg<sup>-1</sup> of NO<sub>3</sub>-N and 30 mg kg<sup>-1</sup> of SO<sub>4</sub>-S. Phosphorus fertilizer (KH<sub>2</sub>PO<sub>4</sub>) was mixed in 50 ml of water and added at the above rates (i.e., 0, 5, 10 or 20 mg P kg<sup>-1</sup> soil). The potted soil was allowed to equilibrate for 7 days at 25 °C in a growth chamber.

All inoculated pots received 132 NT4 propagules (3 g inoculum) placed 5 cm from the soil surface, which resulted in a NT4:indigenous AMF ratio of ca. 1:100. Control pots received 3 g of autoclaved (121 °C, 15 psi, 60 min) inoculum. Seeds were not inoculated with rhizobia, and any nodulation of lentil plants was due to native rhizobia in the soil.

Each pot was seeded with four lentil (cv. Laird) seeds, and thinned to two seedlings after emergence. Plants were grown in a growth chamber under the conditions described by Talukdar and Germida (1994). Soil was maintained at 70% MHC by the periodic addition of water. Pots were randomized within the growth chamber and repositioned biweekly. Plants were harvested at 48 and 95 days after planting (DAP), and shoot and root dry weights (48 h at 70 °C) determined. The percentage of root length colonized by AMF was determined using the gridline intersect method ( $\times 100$ ) (Giovannetti and Mosse 1980). Shoot and grain samples were digested with H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> and analyzed for total P and N content (Thomas et al. 1967). The P-use efficiency (PUE) of lentil and the wheat cultivars was calculated according to Raju et al. (1990).

### Treatments set up for wheat

The experimental protocol, fertilizer P and NT4 treatments for wheat cv. Laura and cv. Neepawa were similar to that for lentil, except for the following modifications: (1) each pot was amended with 0.72 g of KNO<sub>3</sub> and 0.33 g of K<sub>2</sub>SO<sub>4</sub> in 300 ml of water to provide 50 mg NO<sub>3</sub>-N kg<sup>-1</sup> and 30 mg SO<sub>4</sub>-S kg<sup>-1</sup> before seeding, (2) all the pots were seeded with four seeds of each wheat cultivar Laura or Neepawa. Ten days after seedling emergence, pots were amended with 50 mg NO<sub>3</sub>-N kg<sup>-1</sup> soil.

## Statistics

Treatment combinations were a 2  $\times$  4 factorial, each replicated five times. Data were analyzed using the ANOVA procedure and means separated using the least significant difference (LSD) test in SAS (SAS 1990). Correlation coefficients were calculated using Pearson's partial correlation analysis using PC-SAS. Unless indicated, all treatment means were considered significantly different at  $P \leq 0.05$ . Values for percentage mycorrhizal colonization of both lentil and spring wheat roots were subjected to angular transformation before statistical analysis.

## Results

### Effect of *G. clarum* NT4 on lentil

#### *Dry matter production and nutrient uptake*

The growth response of uninoculated lentil plants to increasing amounts of P fertilizer was generally positive at 48 DAP (Table 1). In contrast, amending the soil with up to 10 mg of P kg<sup>-1</sup> did not significantly affect the early vegetative growth of the NT4-inoculated plants. However, NT4 significantly increased early growth of shoots in the unamended soil such that inoculated lentil plants yielded 48% more shoot biomass than the corresponding control. This yield was approximately equal to uninoculated control plants receiving 5–10 mg P kg<sup>-1</sup> soil.

Grain yield comparisons were not made at 95 DAP because pod formation and maturity were not uniform. However, total above-ground biomass (i.e., pods and shoot material) was measured. The growth response of uninoculated lentil plants to P at 95 DAP was not significantly different up to a 10 mg kg<sup>-1</sup> amendment. The NT4 inoculant significantly ( $P = 0.08$ ; LSD = 1.14 g) enhanced the total shoot dry weight of plants in soil receiving 5 mg P fertilizer kg<sup>-1</sup> soil, yielding approximately the biomass of uninoculated plants receiving 20 mg P kg<sup>-1</sup> soil (Table 1). The addition of 20 mg P kg<sup>-1</sup> soil significantly increased the shoot biomass of uninoculated plants but was detrimental to the growth of NT4-inoculated plants. Early root biomass of plants increased significantly in response to P fertilization, but not in response to the NT4 inoculant (data not shown). There was no significant difference between any treatments at 95 DAP. Root nodules were observed on all the lentil plants regardless of P level, inoculation treatments, or sampling interval, but nodule parameters were not determined.

The NT4 inoculant had no effect on the shoot P content of plants at any P level (Table 1). In addition, there were no significant differences between the PUE of control and inoculated plants at any P level except 5 ppm, where the NT4 inoculant increased PUE ( $P = 0.08$ ). Neither the NT4 inoculant nor the P fertilizer affected the N content of lentil shoots (data not shown).

**Table 1** Mean ( $n=5$ ) shoot dry weight, % AMF-colonized root, and shoot P content of lentil plants inoculated with *Glomus clarum* NT4 after 48 and 95 days growth in a P-deficient soil

Fertilizer P (mg/kg soil)	Inoculant	Shoot dry weight (g/pot)				AMF colonized root (%)		Shoot P content (mg/pot) 95 DAP
		48 DAP	% Change	95 DAP	% Change	48 DAP	95 DAP	
0	Control	1.10d		5.95c		29de	21b	27.4d
	NT4	1.63c	+48	6.18c	+4	53a	22b	29.2cd
5	Control	1.71abc		6.32bc		29de	22b	37.2bc
	NT4	1.64c	-4	7.47ab	+18	39b	28a	44.3ab
10	Control	1.57c		5.60c		33bcd	18b	26.8d
	NT4	1.69bc	+8	5.71c	+2	37bc	22b	27.9d
20	Control	1.96a		8.00a		23e	19b	48.3a
	NT4	1.93ab	-2	6.12c	-31	30cde	21b	40.9ab

amended with different levels of P. Means with the same letter within a column are not significantly different at  $P \leq 0.05$  (DAP days after planting)

### Mycorrhizal colonization

The different shapes of arbuscules and vesicles within the root cortex were an indication that different AMF endophytes had colonized lentil roots in both the control and NT4-inoculated treatments. Colonization of uninoculated plant roots by indigenous AMF at 48 DAP ranged from 17–33%, with the highest % colonization observed in plants receiving 10 mg P kg<sup>-1</sup> soil (Table 1). The NT4 inoculant significantly increased the percentage of the root length colonized by AMF in soils receiving  $\leq 5$  mg kg<sup>-1</sup> soil at 48 DAP. However, there were no significant differences in colonization between inoculated and uninoculated plants at the other levels of added P. The NT4 inoculant had no significant effect on AMF colonization of roots assessed at 95 DAP, except in soil receiving 5 mg P kg<sup>-1</sup> soil (Table 1). Nevertheless, there was a significant ( $P=0.065$ ) positive correlation ( $r=0.93$ ) between the shoot biomass of NT4-inoculated plants and the AMF colonization of roots at 95 DAP.

### Effect of *G. clarum* NT4 on wheat

#### Dry matter production and nutrient uptake

The uninoculated and inoculated Laura plants responded differently to the addition of P fertilizer during the early (i.e., 48 DAP) vegetative growth stage (Table 2). Plants inoculated with NT4 produced less shoot biomass in response to P, except at the highest fertilizer rate. The responses of control and inoculated plants at 95 DAP followed a pattern similar to that at 48 DAP. There was no significant difference in root biomass between control and inoculated Laura plants at any P level (data not shown). Neither P fertilizer nor the NT4 inoculant affected the grain yield of Laura plants (Table 4). Furthermore, the NT4 inoculant had no effect on the shoot or grain P content or PUE of Laura at similar rates of added P (Tables 2 and 4).

The early shoot growth of both uninoculated and inoculated Neepawa plants increased with the addition of P such that plants receiving 20 mg P kg<sup>-1</sup> soil produced maximal shoot biomass (Table 3). The NT4 inoculant depressed early shoot growth of Neepawa at all P levels by 8–14%. At 95 DAP, the vegetative growth of NT4-inoculated plants at low P levels responded in a

**Table 2** Mean ( $n=5$ ) shoot dry weight, % AMF-colonized root, and shoot P content of wheat cv. Laura plants inoculated with *G. clarum* NT4 after 48 and 95 days growth in a P-deficient soil

Fertilizer P (mg/kg soil)	Inoculant	Shoot dry weight (g/pot)				AMF colonized root (%)		Shoot P content (mg/pot) 95 DAP
		48 DAP	% Change	95 DAP	% Change	48 DAP	95 DAP	
0	Control	1.32bc		2.10bc		19a	14de	2.31d
	NT4	1.19c	-10	1.89c	-10	28a	18bcd	2.69d
5	Control	1.56abc		2.40abc		18a	22ab	6.66ab
	NT4	1.25c	-20	1.95c	-19	26a	24a	4.21cd
10	Control	1.66ab		2.03c		23a	12e	4.50bcd
	NT4	1.30bc	-22	2.27bc	+12	15a	16cde	6.27abc
20	Control	1.83a		2.63a		18a	20abc	7.03a
	NT4	1.92a	+5	2.44ab	-9	18a	16cde	5.93abc

amended with different levels of P. Means with the same letter within a column are not significantly different at  $P \leq 0.05$  (DAP days after planting)

**Table 3** Mean ( $n=5$ ) shoot dry weight, % AMF-colonized root, and shoot P content of wheat cv. Neepawa plants inoculated with *G. clarum* NT4 after 48 and 95 days growth in a P-deficient

soil amended with different levels of P. Means with the same letter within a column are not significantly different at  $P \leq 0.05$  (DAP days after planting)

Fertilizer P (mg/kg soil)	Inoculant	Shoot dry weight (g/pot)				AMF colonized root (%)		Shoot P content (mg/pot) 95 DAP
		48 DAP	% Change	95 DAP	% Change	48 DAP	95 DAP	
0	Control	0.98bc		2.82bc		16bc	23a	1.27c
	NT4	0.90c	- 9	2.67c	- 5	25a	24a	1.13c
5	Control	1.30ab		3.05abc		19ab	26a	1.67bc
	NT4	1.22abc	- 8	2.81bc	- 8	18bc	17a	1.90bc
10	Control	1.44a		3.07abc		17bc	25a	3.30b
	NT4	1.24abc	-14	3.20ab	+ 3	21ab	22a	3.28b
20	Control	1.58a		3.09abc		19ab	21a	5.30a
	NT4	1.40a	-12	3.46a	+12	12c	23a	3.45b

manner similar to Laura plants, and Neepawa biomass was reduced by 5–8%. In contrast, at high P levels the shoot biomass of NT4-inoculated plants was enhanced by 3–12%. As in the case of the cv. Laura, the NT4 inoculant had no effect on the shoot growth of Neepawa plants at equivalent P levels (Table 3). Although neither the NT4 inoculant nor P fertilizer affected the early root growth of Neepawa, NT4 increased the root biomass of plants receiving  $\leq 5$  ppm of P, and decreased the root biomass of plants receiving  $> 5$  ppm of P compared with control plants at 95 DAP (data not shown). P fertilizer had no effect on the grain yield response of Neepawa plants. However, the NT4 inoculant significantly increased the grain yield of Neepawa plants by 20% in soil fertilized with 20 mg P kg<sup>-1</sup> soil (Table 4). The NT4 inoculant had no significant positive effect on shoot P content or PUE of Neepawa at any added P level (Table 3). However, it significantly increased the grain P content of Neepawa plants receiving  $\geq 10$  mg P kg<sup>-1</sup> soil, but not the PUE of any treatment (Table 4).

#### Mycorrhizal colonization

As in the case of lentil plants, both Laura and Neepawa cultivars were colonized by various indigenous AMF in the Outlook soil. Mycorrhizal colonization of uninocu-

lated control Laura plants was 12–22% over the 95-day study irrespective of soil P levels, indicating that additions of P fertilizer had no significant impact on overall activity of indigenous AMF. Colonization levels at 48 or 95 DAP were not very different between the control and NT4-inoculated plants at any given P level (Table 2). In contrast, the NT4 inoculant enhanced AMF colonization of Neepawa plants in the unamended soil, and reduced colonization in soils receiving 20 mg P kg<sup>-1</sup> at 48 DAP (Table 3). However, at 95 DAP, there were no significant differences between the AMF colonization levels of the NT4-inoculated and control plants.

#### Discussion

Recently, Talukdar and Germida (1994) showed that a *G. clarum* NT4 inoculant enhanced growth and/or yield of lentil and wheat in a sterile soil:sand mix. The present results indicate that *G. clarum* NT4 can significantly increase the shoot dry matter production of lentil ( $P \leq 0.08$ ) and the yield of wheat cv. Neepawa ( $P \leq 0.05$ ) in non-sterile soil containing AMF and other indigenous microorganisms. Furthermore, as previously demonstrated for other AMF (Schubert and Hayman 1986), the positive effect of NT4 was dependent on specific interactions with the host crop and the level of

**Table 4** Mean ( $n=5$ ) grain yields and grain P contents of two spring wheat cultivars inoculated with *G. clarum* NT4 after 95 days growth in soil amended with different levels of P fertilizer.

Means with the same letter within a column are not significantly different at  $P \leq 0.05$

Fertilizer P (mg/kg soil)	Inoculant	Grain yield (g/pot)				Grain P content (mg/pot)	
		Laura	% Change	Neepawa	% Change	Laura	Neepawa
0	Control	1.71a		2.18c		14.68abc	9.04c
	NT4	1.39a	-8	2.14c	- 2	9.71c	8.96c
5	Control	1.52a		2.52abc		12.20bc	11.93b
	NT4	1.56a	+3	2.32bc	- 8	12.89bc	11.09b
10	Control	1.53a		2.39abc		12.54bc	11.97b
	NT4	1.53a	0	2.65ab	+11	12.73bc	13.90a
20	Control	1.95a		2.30bc		21.32a	11.23b
	NT4	1.91a	-2	2.75a	+20	17.85ab	13.86a

available soil P. In contrast to wheat, lentil responded dramatically to NT4 when present at a level only 1/100th that of the indigenous AMF community.

Talukdar and Germida (1994) found that *G. clarum* NT4 significantly enhanced lentil growth by 43% and grain yield by 57% compared with uninoculated control plants in a sterile soil:sand mix with 10 mg P kg<sup>-1</sup> soil. We found that in a non-sterile soil inoculating lentil with NT4 and fertilizing with up to 5 mg P kg<sup>-1</sup> soil enhanced the total above-ground biomass of lentil compared with control plants by 48% at 48 DAP and 18% at 95 DAP. This increase in shoot biomass was approximately equal ( $P \leq 0.08$ ) to that of control plants receiving 20 mg P kg<sup>-1</sup> soil at 95 DAP, and illustrates the benefits that lentil derived from an AMF association. These results support the findings of Talukdar and Germida (1994) that NT4 can enhance lentil growth. However, the lower response to NT4 in non-sterile soil also supports the suggestion by Wilson (1984) that the presence of more than one AMF species in a host's rhizosphere can lead to antagonistic interactions between the endophytes. The native rhizobia in the Outlook soil appeared to have no effect on the lentil-AMF symbiosis. This may represent inter-endophyte incompatibility between the native rhizobia and AMF (Ianson and Linderman 1993), or show that the native rhizobia were ineffective at fixing nitrogen.

Previously, Talukdar and Germida (1994) showed that inoculation of wheat cv. Laura with NT4 increased the grain yield by 12% in the absence of other AMF. In contrast, our results show no significant effect of NT4 on the growth or yield of cv. Laura in non-sterile soil at any P level. It is possible that *G. clarum* NT4 was "out-competed" by other indigenous AMF which were more parasitic than NT4 in their relationship with the wheat cv. Laura. Alternatively, as suggested by Buwalda and Goh (1982), competitive interactions between the host and the colonizing endophyte(s) may have resulted in a carbon drain to the host irrespective of soil P levels.

In contrast to the effect of *G. clarum* NT4 on cv. Laura in non-sterile soil, NT4 significantly ( $P \leq 0.05$ ) increased the grain yield of Neepawa by 20% with the addition of 20 mg P kg<sup>-1</sup> soil. The addition of low levels of P fertilizer to P-limited soils increased the growth of AMF-inoculated barley (Clarke and Mosse 1981) and onion (Schubert and Hayman, 1986) more than uninoculated plants. The effect of the NT4 inoculant on the growth and yield of wheat cv. Neepawa may be the result of a higher symbiotic efficiency of this endophyte than indigenous AMF colonizing uninoculated plants. Alternatively, as suggested by Barber and Laugham (1967), moderate amounts of P fertilizer may reduce competition for P between plants and microorganisms, thereby enhancing the positive impact of NT4. The yield increase of wheat cv. Neepawa was directly associated with an increase in the uptake of P by grain tissues, as observed for barley (Saif and Khan 1977).

As in previous studies using NT4 in sterile soil (Talukdar and Germida 1994), lentil responded more to

the NT4 inoculant than wheat. It is well known that plants with a fibrous root system (e.g., wheat) depend less on mycorrhizae for growth than plants with a coarse root system (e.g., lentil) (Baylis 1975). Furthermore, plant growth response to AMF inoculation varies among cultivars of the same host, such as wheat (Azcon and Ocampo 1981). For example, Azcon and Ocampo (1981) studied the mycorrhizal dependency of 13 wheat cultivars for nutrient assimilation and growth and found that some cultivars responded positively to *G. mosseae* colonization whereas others were unresponsive. In support of this, we found that the mycorrhizal effectiveness was much greater for Neepawa plants than for Laura. These differences between wheat cultivars may reflect AMF interactions at the various P levels, potentially resulting in altered external mycelium development.

Inoculation of crops such as legumes with AMF can significantly enhance plant dry matter production and (potentially) yields in soils with a limited P availability. The application of an AMF inoculant delivered by co-inoculating legume seeds with rhizobia and AMF spores or other propagules might be an effective strategy to accomplish this goal. However, the evaluation of an AMF inoculant in the presence of indigenous AMF under normal or moderate fertilizer regimes is important. Studies are underway to develop formulations of microbial consortia comprising rhizobia and AMF for lentil and pea.

**Acknowledgements** This work was supported by grants from the Natural Sciences and Engineering Research Council, the Westerns Grains Research Foundation and the Saskatchewan Agricultural Development Fund. Contribution No. R 782, Saskatchewan Center for Soil Research.

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