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Genotypic variation in the response of chickpea to arbuscular mycorrhizal fungi and non-mycorrhizal fungal endophytes

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Abstract

Plant roots host symbiotic arbuscular mycorrhizal (AM) fungi and other fungal endophytes that can impact plant growth and health. The impact of microbial interactions in roots may depend on the genetic properties of the host plant and its interactions with root-associated fungi. We conducted a controlled condition experiment to investigate the effect of several chickpea genotypes (*Cicer arietinum* L.) on the efficiency of the symbiosis with AM fungi and non-AM fungal endophytes. Whereas the AM symbiosis increased the biomass of most of the chickpea cultivars, inoculation with non-AM fungal endophytes had a neutral effect. The chickpea cultivars responded differently to co-inoculation with AM fungi and non-AM fungal endophytes. Co-inoculation had additive effects on the biomass of some cultivars (CDC Corrine, CDC Anna, and CDC Cory), but non-AM fungal endophytes reduced the positive effect of AM fungi on Amit and CDC Vanguard. This study demonstrated that the response of plant genotypes to an AM symbiosis can be modified by the simultaneous colonization of the roots by non-AM fungal endophytes. Intraspecific variations in the response of chickpea to AM fungi and non-AM fungal endophytes indicate that the selection of suitable genotypes may improve the ability of crop plants to take advantage of soil ecosystem services.

Key words: *Cicer arietinum* L., plant genotype, symbiosis, plant breeding, soil fungi

Introduction

Plant roots form mutualistic associations with specific soil microorganisms, and those associations can alleviate biotic and abiotic environmental stresses (Gianinazzi-Pearson 1996). Enhancing the beneficial components of a plant's root microbiome could improve the efficiency of plant production. Arbuscular mycorrhizal (AM) fungi form symbioses with more than 80% of plant species and provide them with mineral nutrients, mainly phosphate (Hodge et al. 2000), resistance to root pathogenic fungi (Newsham et al. 1995; Linderman 2000) and drought (Augé 2001) particularly under P-limiting conditions, in exchange for photosynthetically fixed C (Bago et al. 2000). Non-AM fungal endophytes are highly diverse microorganisms that asymptotically colonize plant roots (Jumpponen 2001). These fungi often release secondary metabolites that influence host plant physiology and interact with other fungal species, including phytopathogens (Schulz et al. 2002; Sumarah et al. 2011). Certain non-AM fungal endophytes develop mutualistic associations that can promote plant growth, especially under stressful conditions such as drought (Mandyam and Jumpponen 2005).

The symbioses that plant roots form with soil fungi span along a continuum from mutualistic to parasitic associations. The extent and efficiency of the associations that AM fungi and non-AM fungal endophytes form with plants depend largely on the host plant, the fungal isolate and environmental conditions (Monzon and Azcón 1996; Saikkonen et al. 1998; Klironomos et al. 2003; Andrade-Linares et al. 2011; Sousa et al. 2012; Berrutti et al. 2016).

The symbiosis formed between plants and soil fungi varies in form and effectiveness depending on the plant species and even on the genotype of the same species (Weishampel and Bedford 2006; Singh et al. 2012; Taylor et al. 2015; Cobb et al. 2016). Cheplick (2008) proposed that the effect of plant genotype can override the effect of fungal endophytes on the outcome of a microbial association with roots. The development of the AM symbiosis is initiated and regulated by a variety of root phytochemicals (Biate et al. 2015). The phytochemical profile of plants varies with genotypes and this

is also true for chickpea (Ellouze et al. 2012). Cultivars of crop plants may differ in architectural features such as root length and density, and those differences can influence the uptake of soil nutrients and the formation and function of root symbioses (Baon et al. 1994; Römer et al. 1988; Bryla and Koide 1998; Kashiwagi et al. 2006). Plants with good inherent ability to extract soil P are generally less responsive to the AM symbiosis (Bryla and Koide 1998).

Plant response to inoculation with different AM fungi also depends on the compatibility between AM fungal species and soil properties. Some strains were shown to trigger a better plant response in soil rich in nutrients and organic matter, while others performed best in relatively poor soils or in soils of medium fertility (Herrera-Peraza et al. 2011).

The formation and function of an AM symbiosis can be influenced by other fungal endophytes. These fungi were previously shown to interact with AM fungi, modifying the level of root colonization by AM fungi and the efficacy of the symbiosis (Müller 2003; Verbruggen et al. 2013; Wezowicz et al. 2017). Plant genetics is believed to control the interaction between endophytic and mycorrhizal fungi in a symbiosis (Rengel 2002), however studies have yet to explore that aspect.

Chickpea is a high-value crop used in wheat-based cropping systems in the semi-arid prairies of Canada (Miller et al. 2002). In this area, improving the performance of chickpea as a host would be beneficial. Improving the AM symbiosis can improve plant production (Pellegrino and Bedini 2014). An effective symbiosis can be attributable to an effective AM fungal symbiont (Pellegrino et al. 2011; Pellegrino and Bedini 2014), but also to an effective host plant (Ellouze et al. 2015). Identifying chickpea cultivars that form efficient symbioses with native AM fungi and other fungal endophytes could improve the fitness of chickpea crops in an environment and enhance the performance of the cropping system. Different genotypes of chickpea produce different arrays of bioactive root phytochemicals and selectively promote microbial communities in the rhizosphere (Yang et al. 2012; Ellouze et al. 2013). Genotypic variation is a necessary consideration in the selection of plant varieties

that form highly efficient symbioses with AM and non-AM fungi. Therefore, here we conducted a greenhouse experiment to test the hypothesis that non-AM fungal endophytes influence differently the function of the AM symbiosis in different chickpea genotypes.

Materials and Methods

Experimental design

Thirteen chickpea cultivars with different phenotypes (Table S1) and genotypes (Diapari et al. 2014) were subjected to one of four inoculation treatments: (1) AM fungi; (2) non-AM fungal endophytes; (3) a mixture of AM fungi and non-AM fungal endophytes; or (4) autoclaved inoculants (control). Pots were arranged in a randomized complete block design with four replicates in the greenhouse.

Source of the AM fungal and non-AM fungal endophytic communities

The AM fungi material was *Diversispora eburnea* (3244B), *Claroideoglomus etunicatum* (2639A), and *Glomus* sp. (4350D). These AM fungi are native to cultivated soils in the province of Saskatchewan, Canada. The non-AM fungal material was *Trichoderma harzianum* (P134 D1 11) and *Mortierella alpina* (P156 D2 50), likewise isolated from Saskatchewan soils. These isolates were selected because of their growth-promoting effects on chickpea as identified in a previous study (Bazghaleh et al. 2015). All of the isolates used belong to the collection of the Soil Microbiology Laboratory of the Quebec Research and Development Centre, Agriculture and Agri-Food Canada (Quebec City, QC, Canada).

Preparation of AM fungal inoculants

The AM fungal isolates were propagated on maize (*Zea mays* L.) in 16-L pots filled with calcined montmorillonite clay (Pro's Choice Sports Field Products, Chicago, IL, USA). The maize seeds were surface-sterilized by successive immersion in 95% ethanol for 30 s, sterile distilled water for 30 s,

2.5% sodium hypochlorite (Javex) for 2 min, and sterile distilled water for 2 min. The seeds were germinated on moist filter paper in Petri dishes prior to use. The maize seedlings were inoculated with 100 spores of *D. eburnea*, *C. etunicatum*, and *Glomus* sp. Each AM species was propagated in three pots of maize each containing one plant. The maize was grown in a greenhouse under 16/8 h day/night conditions at 24/16 °C. Supplemental lighting was provided during the daytime with high-intensity discharge lamps (Alto 400 W low-pressure sodium; Philips, Somerset, NJ, USA). The plants were watered with distilled water as needed and fertilized with a modified Long Ashton nutrient solution containing, per liter, 554 mg KCl, 200 mg $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 244 mg MgSO_4 , 520 mg $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 1.7 mg MnSO_4 , 0.25 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.30 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3.0 mg H_3O_3 , 5.0 mg NaCl, 0.09 mg $(\text{NH}_4)_6 \text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, and 32.9 mg NaFe-EDTA. The plants were harvested 12 weeks after emergence. The roots were washed free of rooting media and cut into 1-cm fragments. The root fragments and the growth medium for the AM fungal pot culture were pooled and hand-mixed. The AM fungal spores were extracted from three representative samples of each AM fungal culture by the sucrose centrifugation and flotation method (Walker et al. 1982), then collected on a 125- μm sieve, and counted using a compound microscope. Amounts of each mixture that contained approximately 50 spores were used as AM fungal inoculants. Thus, the AM fungal inoculants used in this study consisted of a mixture of root fragments and growth substrates containing approximately 150 spores, that is, 50 spores of each of the three AM fungal species: *D. eburnea*, *C. etunicatum*, and *Glomus* sp.

Preparation of non-AM fungal inoculants

Trichoderma harzianum and *M. alpina* were propagated in half-strength potato dextrose broth. The cultures were grown for 36 h on a Thermolyne Big Bill orbital shaker (Barnstead International, Dubuque, IA, USA) at 80 rpm. The liquid culture of each fungal endophyte was filtered under sterile conditions, and 2 g of each species was mixed with 1 L of sterile distilled water on a magnetic stirring plate and immediately used to inoculate the designated chickpea plants.

Pot establishment and growing conditions for experimental plants

Chickpea cultivars were grown in 4-L pots containing pasteurized (90 °C, 1 h) Orthic Brown Chernozem soil (Aridic Haploborroll in the USDA classification system) (Table 1). The seeds were surface-sterilized as described above. The seeds were germinated in the dark at 25 °C for 72 h on moist, sterile filter paper in Petri dishes. Seven germinated seeds were planted in each pot. All germinated seeds and planting holes were treated with 1.5 g of a peat-based *Mesorhizobium ciceri* inoculant (Nitragin Nitrastick GC; Nitragin Inc., Brookfield, WI, USA). At sowing, 120 g of the AM fungal inoculant containing 150 spores was mixed with soil in the rooting zone. The non-AM fungal endophytes were applied as 2 mL of the fungal suspension using a pipette. Dually inoculated plants were treated with the AM and non-AM fungal inoculants, as described above. The control treatment consisted in autoclaved (121°C, 15 psi, 20 min) inoculants of the AM and non-AM fungi. The chickpea plants were grown for 90 days under the same greenhouse conditions as described above for the maize plants used to propagate the AM fungi. The chickpea plants were watered with distilled water as needed and fertilized weekly with 100 mL of the modified Long Ashton nutrient solution described above.

Data collection

At harvest, the chickpea shoots were cut at ground level, dried at 65 °C in a forced-air dryer to constant weight, and then ground. The roots were separated from the soil by washing under tap water, cut into 4-cm fragments, and mixed. A subsample of the roots was cleared in 10% KOH and stained with 5% Schaeffer black ink in vinegar (Vierheilig et al. 1998) for the assessment of fungal colonization using the gridline intercept method (Giovannetti and Mosse 1980). Root colonization level was assessed at 400× magnification under a compound microscope. The hyphae of AM and non-AM fungi were distinguished from each other based on their morphological features. Arbuscular

mycorrhizal fungal hyphae are non-septate and contain vesicles or arbuscules, whereas non-AM fungal hyphae are often septate and do not contain vesicles or arbuscules.

Subsamples of the ground plant shoots were digested in $\text{H}_2\text{SO}_4/\text{Se}/\text{Na}_2\text{SO}_4$ (Varley 1966). The digests were analyzed for N (Noel and Hambleton 1976) and P concentration (Milbury et al. 1970) on a segmented flow autoanalyzer (Technicon AAI system; Technicon Industrial Systems, Tarrytown, NY, USA) and for K concentration by atomic absorption spectrometry (Anonymous 1987). Another series of subsamples was digested with $\text{HClO}_4/\text{HNO}_3$ (Jones 1991) and analyzed for Fe, Mg, Zn, and Mn contents by atomic absorption spectrometry.

Statistical analysis

Data were tested for normality using the Shapiro–Wilk test, and non-normal data were log-transformed before analysis. Two-way analysis of variance (ANOVA) was conducted to test the significance of cultivar, inoculation, and the interaction of these two factors on shoot biomass, nutrient concentrations in plant tissues, and level of root colonization. The Tukey–Kramer HSD (honest significant difference) post hoc test was used for comparison of treatment means. Correlation analysis was conducted between plant performance indicators using the package agricolae (de Mendiburu 2010) in R software (V. 2.15.2). Plant response to inoculation was calculated as the difference between each inoculation treatment and the control. A principle component analysis (PCA) plot was computed with PC-ORD software (V. 4.34) to illustrate the similarities and differences in the response patterns of the cultivars tested. Patterns of response to the different inoculation treatments of the chickpea cultivars were compared using the multi-response permutation procedure with pairwise comparisons in PC-ORD software (V. 4.3.4). The threshold used to reject or accept a null hypothesis was $\alpha = 0.05$.

Results

The roots of the control plants were free of fungal colonization, as expected. The roots of the inoculated plants were all colonized, and the level of colonization (combined AM and non-fungal endophytes) of the dually inoculated plants exceeded the level of the singly inoculated plants in four of the 13 chickpea genotypes tested: CDC Anna, CDC Corrine, CDC Cory, and CDC Leader (Table 2).

Effects of cultivar and inoculation on chickpea performance indicators

Chickpea cultivar, inoculation treatment and their interactions significantly influenced plant biomass, root colonization percentage, and shoot N, P, and Mg concentrations (Table 3). Concentrations of K, Fe, Mn and Zn in shoots were unaffected by inoculation or cultivar (Table 3). The significant interactions between fungi and cultivar that was observed for all impacted variables clearly indicate that the chickpea cultivars responded differently to the symbiotic fungal communities.

Inoculation with AM fungi increased the biomass of all chickpea genotypes relative to the controls, except for CDC Cory (Table 2). Co-inoculation with AM and non-AM fungal endophytes increased the biomass of CDC Anna and CDC Corrine beyond the effect of AM inoculation alone but inhibited the biomass of Amit and CDC Vanguard (Table 2). Co-inoculation of AM and non-AM fungi reduced the concentration of P in CDC Anna and CDC Luna and the concentration of Mg in CDC Anna and CDC Vanguard in comparison with AM inoculation alone (Table 2). Plant N concentration was never influenced by inoculation (Table 2).

A significant correlation between root colonization and plant biomass was only observed in dual inoculation treatment ($p < 0.001$).

Response patterns of chickpea cultivars

The PCA reveals different patterns of plant response to the root-associated fungal symbionts. For

example, Amit, CDC Cory, and CDC Orion responded more positively to inoculation with AM fungi only than the other cultivars (Fig. 1), Amit and CDC Frontier showed a positive response to inoculation with non-AM fungal endophytes only (Fig. 2), and CDC Xena, CDC Nika, CDC Cabri, CDC Vanguard and CDC Orion responded negatively to non-AM fungal endophytes. CDC Cory and CDC Anna, and CDC Frontier showed a positive response to dual inoculation (Fig. 3). Furthermore, the multi-response permutation procedure revealed differences in the patterns of response to inoculation treatments among the chickpea cultivars ($P < 0.0001$). Pairwise comparisons demonstrated the differences in the overall response of each cultivar to inoculation with AM fungi, inoculation with non-AM fungal endophytes, and dual inoculation (Table S2).

Discussion

Our study revealed variations in the response of 13 cultivars of chickpea to communities of AM fungi and non-AM fungal endophytes commonly found in chickpea fields. This intraspecific variation points to the possibility of selecting genotypes that form efficient symbioses with naturally occurring soil fungi, but also indicates that genetic selection may inadvertently alter the ability of plants to benefit from services naturally provided by the soil ecosystem. Intraspecific variations naturally occur in symbiosis-specific genes and genes that regulate the physiology and morphology of the host plant. These genes interact and result in a specific response to a microbial symbiosis (Estaún et al. 1987; Linderman and Davis 2004; Balestrini and Lanfranco 2006). Incorporating new biomarkers into plant selection may be necessary to take advantage of the potential of efficient symbioses (Hohmann et al. 2016).

We observed that, at least in terms of biomass, some chickpea cultivars—in particular CDC Corrine and CDC Anna—were more responsive to fungal symbioses than other cultivars. This finding suggests that the responsiveness of chickpea to fungal symbioses could be due to genetic selection (Moreno and Cubero 1978; Singh 1997). Selection for high-yielding varieties, which traditionally

takes place on fertile substrates, could lead to the loss of genes, phytochemicals and/or other features that are necessary for the formation of efficient symbioses. Plant selection for disease resistance could also result in selection of reduced ability to form symbioses owing to common pathways in the regulation of symbiosis and disease resistance (Toth et al. 1990). Hence, breeding programs targeting high yield and disease resistance may have inadvertently selected some of the chickpea genotypes that respond poorly to fungal symbiosis.

Inoculation of plants with specific fungal endophytes singly had a neutral to positive effect on plant biomass, as it was reported previously (Mayerhofer et al. 2013). Inoculation with only AM fungi increased the biomass of chickpea in 12 genotypes, relative to the controls. However, the co-inoculation of AM fungi and non-AM fungal endophytes had contrasting effects on plant biomass in different chickpea genotypes. This observation suggests that the non-AM fungal endophytes may interact with AM fungi and therefore affect the outcome of the AM symbiosis. Fungal endophytes may influence other fungal species through competition between extraradical mycelia for nutrients and colonization sites (Green et al. 1999; Pozo and Azcón-Aguilar 2007) may trigger morphological changes in roots (Malinowski et al. 1999; Shores and Harman 2008); they may release or alter various secondary metabolites inside the roots (Peipp et al. 1997; Vinale et al. 2008; Sumarah et al. 2011; Miller et al. 2012); and they may interact with plant hormonal signaling and modify the proteome and metabolism of the plant (Harman et al. 2004; Shores et al. 2005; Vassilev et al. 2006; Gravel et al. 2007).

The co-inoculation of AM fungi and non-AM fungal endophytes can have a different effect on the hormonal profile of plants than that produced by inoculation with AM fungi or non-AM fungi separately (Martínez-Medina et al. 2011). *Trichoderma harzianum* is able to change the systemic production of specific plant metabolites and antibiotics (Yedidia et al. 2003) such as salicylic and jasmonic acids (Sticher et al. 1997; Pieterse et al. 2003). It is known that the level of salicylic acid in

roots is negatively correlated with the level of AM root colonization (Medina et al. 2003; Vierheilig 2004). In addition, the salicylic acid level was found to be lower in mycorrhiza-responsive plants than in mycorrhiza non-responsive plants (Blilou et al. 1999). As a requirement for the formation of mycorrhizal symbiosis, AM fungi trigger a reduction in plant salicylic and jasmonic acids (Medina et al. 2003). The suppression of these defensive hormones by AM fungi or their stimulation by *T. harzianum* in particular chickpea genotypes may be involved in the establishment and function of mycorrhizal symbiosis. Moreover, changes in the levels of phytohormones caused by the interactions between AM fungi and non-AM fungal endophytes may directly affect plant physiology and biomass production.

We have shown that the interaction between AM fungi and non-AM fungi can be modified by the host plant in a tripartite symbiosis. *Trichoderma harzianum* was previously found to promote the colonization of roots by *Glomus mosseae* and increase plant biomass in cucumber (Chandanie et al. 2009), but it had no effect on root colonization or plant biomass in melon (Martínez-Medina et al. 2011). The effect of fungal endophytes on AM symbiosis may also depend on the genotype of the host plant (Tucci et al. 2011). In their study, Tucci et al. (2011) found that *T. harzianum* triggered the salicylic acid pathway only in AM-responsive tomato genotypes. Since the level of salicylic acid controls the colonization of roots by AM fungi, genotypic variations in the induction of salicylic acid by *T. harzianum* could influence the colonization of the roots as well as the outcome of the AM symbiosis (Tucci et al. 2011).

Our study is the first to report that chickpea cultivars respond differently to co-inoculation with AM fungi and non-AM fungal endophytes. The cultivars CDC Vanguard and Amit responded positively to AM fungi in the absence of non-AM fungal endophytes, but did not respond when simultaneously exposed to the non-AM fungal endophytes. In contrast, fungal endophytes added to the positive response observed in CDC Corrine, CDC Cory, and CDC Anna. This result shows that

ubiquitous endophytic fungi found in Saskatchewan soils in which chickpea crops are grown could potentially influence the formation and function of the AM symbiosis of chickpea. It is noteworthy that differences in the response of chickpea cultivars to non-AM fungal endophytes were observed using a community of only two fungal species. Since the roots of plants are exposed to a wide diversity of fungal species in crop fields, chickpea cultivars can be expected to exhibit even greater variation in their response to AM symbiosis, as shown earlier (Tavasolee et al. 2011; Pellegrino and Bedini 2014).

This study is a first step toward elucidating plant genetic factors that control the multipartite symbiosis that forms between chickpea and AM fungi and non-AM fungal endophytes. The knowledge acquired through research on this topic may lead to the development of chickpea cultivars that form beneficial associations with indigenous fungal resources. We believe that the ideotype of chickpea successfully regulates microbial associations that can increase the productivity of the plant through the effective utilization of soil resources.

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Figures legends

Fig. 1 Biplot of the principal component analysis (PCA) of the response pattern of chickpea cultivars to inoculation with arbuscular mycorrhizal (AM) fungi. The pattern of response considers the response (o) of biomass, root colonization, and concentrations of N, P, and Mg in plant tissues ($n = 4$). The AM fungal species used were *Diversispora eburnea*, *Claroideoglossum etunicatum*, and *Glomus* sp.

Fig. 2 Biplot of a principal component analysis (PCA) of the response pattern of chickpea cultivars to inoculation with non-arbuscular mycorrhizal (AM) fungal endophytes. The pattern of response considers the response (o) of biomass, root colonization, and concentrations of N, P, and Mg in plant tissues ($n = 4$). The non-AM fungal species used were *Trichoderma harzianum* and *Mortierella alpina*

Fig. 3 Biplot of a principal component analysis (PCA) of the response pattern of chickpea cultivars to inoculation with a mixture of arbuscular mycorrhizal (AM) fungi and non-AM fungal endophytes. The pattern of response considers the response (o) of biomass, root colonization, and concentrations of N, P, and Mg in plant tissues ($n = 4$). The AM fungal species used were *Diversispora eburnea*, *Claroideoglossum etunicatum*, and *Glomus* sp. The non-AM fungal species used were *Trichoderma harzianum* and *Mortierella alpina*.

Table 1. Physical and chemical characteristics of the soil^a used in the greenhouse experiment.

Texture	pH ^b	EC ^b (mS cm ⁻¹)	NH ₄ -N (mg kg ⁻¹)	NO ₃ -N (mg kg ⁻¹)	PO ₄ -P (mg kg ⁻¹)	K (mg kg ⁻¹)	OC ^c (mg kg ⁻¹)	Total N (mg kg ⁻¹)
Loamy sand	6.48	0.48	19.72	14.13	21.92	357	0.57	0.08

^aSoil was collected from a farm located 25 km northwest of Swift Current, SK, Canada (50°20.554' N 108°2.252' W)

^b1:1 soil:water (McKeague, 1978)

^cOrganic carbon (Baccanti and Colombo, 1992)

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7 **Table 2.** Level of root colonization, biomass production and concentrations of P, N, and Mg in the shoots of chickpea cultivars^a inoculated
8 with autoclaved inoculant (control; C), non-arbuscular mycorrhizal (AM) fungal endophytes^b (E), AM fungi^c (A), and a mixture of AM fungi
9 and non-AM fungal endophytes (AE) in a greenhouse experiment.

Inoculation		Cultivar												
		Am	Al	Fr	Le	Lu	Or	Xe	An	Ca	Ni	Cr	Va	Co
P (g kg ⁻¹)	C	2.6ab	2.4b	2.9a	2.6a	2.4b	2.2ab	2.0a	2.4b	2.4a	2.1ab	2.0b	2.4a	2.4a
	E	2.3b	2.4b	2.3ab	2.5ab	2.5ab	2.1b	2.0a	2.6ab	2.4a	2.0b	2.6a	2.2ab	1.9b
	A	2.6ab	3.1a	2.2b	2.2b	2.8a	2.4a	2.1a	3.0a	2.1b	2.4a	2.6a	2.1a	2.0ab
	AE	3.0a	2.7ab	2.4ab	2.3ab	2.4b	2.2ab	2.2a	2.3b	2.2ab	2.1ab	2.4a	2.3ab	2.4a
N (g kg ⁻¹)	C	47.1a	44.6a	52.3a	45.7a	41.1b	41a	44.4	48.3ab	41.1a	42.2a	43a	44.2a	46.7a
	E	41.7a	45.7a	44.1b	42.9a	41.9b	41a	45.0	44.5b	41.0a	44.2a	43.1a	44.8a	39.3b
	A	47.2a	47.9a	46.3ab	41.3a	51.6a	42a	45.4	56.1a	43.1a	46.7a	53.5a	45.1a	41.5ab
	AE	44.8a	46.9a	47.3ab	45.5a	43.2ab	44.7a	44.1	46.6ab	43.3a	46.6a	45.2a	42.9a	43.2ab
Mg (g kg ⁻¹)	C	5.3b	4.9b	4.8a	5.6b	4.8b	4.8a	4.8a	5.1b	4.4b	4.4b	4.5c	5.1ab	4.5b
	E	5.4ab	5.3ab	5.0a	5.2b	5.4ab	5.0a	5.2a	5.3b	5.2ab	4.5ab	5.0bc	5.2ab	4.6b
	A	5.8ab	5.8a	5.2a	6.3a	6.0a	5.1a	5.3a	6.3a	5.4a	5.3a	5.5ab	5.6a	5.9a
	AE	6.4a	5.7a	5.1a	6.0a	5.5ab	5.1a	5.9a	5.1b	5.5a	5.2a	5.8a	4.7b	6.0a
Colonization (%)	C	0c	0b	0c	0c	0c	0c	0c	0d	0c	0c	0d	0c	0d
	E	8b	9a	13b	11b	8b	8b	7b	8c	6b	6b	7c	7b	8c
	A	13a	11a	15ab	12b	13a	14a	11a	14b	11a	12a	14b	14a	14b
	AE	14a	12a	19a	15a	15a	17a	12a	23a	15a	14a	24a	13ab	22a
Biomass (g)	C	3.0b	2.7b	3.8b	3.9c	2.7b	3.1b	3.1t	3.1c	2.8b	4.7b	2.7c	2.8b	2.2b
	E	3.7b	3.2b	4.4b	4.5b	3.2b	3.2b	3.2t	3.5c	3.0b	5.1b	3.0c	3.2b	2.8b
	A	4.7a	3.9a	5.7a	5.2a	3.9a	4.0a	3.9a	4.4b	3.8a	5.9a	3.6b	4.8a	3.3ab
	AE	3.4b	3.9a	5.6a	5.1a	3.9a	3.9a	4.1a	5.6a	3.8a	5.8a	4.7a	3.3b	4.3a

10 LS means within a column that are followed by different letters are significantly different at $P \leq 0.05$ ($n = 4$).
11 ^aCultivars tested were Am = Amit, Al = CDC Alma, An = CDC Anna, Ca = CDC Cabri, Cr = CDC Corrine, Co = CDC Cory, Fr = CDC Frontier, Le = CDC
12 Leader, Lu = CDC Luna, Ni = CDC Nika, Or = CDC Orion, Va = CDC Vanguard, and Xe = CDC Xena.
13 ^bNon-AM fungal endophytes used were *Trichoderma harzianum* and *Mortierella alpina*.
14 ^cAM fungi used were *Diversispora eburnea*, *Claroideoglomus etunicatum*, and *Glomus* sp.

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Table 3. Probability of effects of chickpea cultivar^a, fungal inoculation^b, and the interaction of these factors on plant biomass, root colonization level, and concentrations of N, P, K, Mg, Fe, Mn, and Zn in plant tissues, according to an analysis of variance ($n = 4$).

Factor	Biomass	Colonization	P	N	K	Mg	Fe	Mn	Zn
Fungi	<0.001	<0.001	<0.001	0.001	0.85	0.01	0.22	0.10	0.57
Cultivar	<0.001	<0.001	<0.001	0.009	0.18	<0.001	0.51	0.13	0.44
Fungi × Cultivar	<0.001	<0.001	0.02	0.002	0.45	<0.001	0.56	0.15	0.33

^aCultivars tested were Amit, CDC Alma, CDC Anna, CDC Cabri, CDC Corrine, CDC Cory, CDC Frontier, CDC Leader, CDC Luna, CDC Nika, CDC Orion, CDC Vanguard, and CDC Xena.

^bInoculation treatments were (1) arbuscular mycorrhizal (AM) fungi (*Diversispora eburnea*, *Claroideoglomus etunicatum*, and *Glomus* sp.), (2) non-AM fungal endophytes (*Trichoderma harzianum* and *Mortierella alpina*), and (3) a mixture of AM fungi and non-AM fungal endophytes. Autoclaved inoculant was used as a control.

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