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PLANT MICROBE INTERACTIONS

A Plant-Fungus Bioassay Supports the Classification of Quinoa (*Chenopodium quinoa* Willd.) as Inconsistently Mycorrhizal

Julianne A. Kellogg[1] · John P. Reganold[1] · Kevin M. Murphy[1] · Lynne A. Carpenter-Boggs[1]

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is becoming an increasingly important food crop. Understanding the microbiome of quinoa and its relationships with soil microorganisms may improve crop yield potential or nutrient use efficiency. Whether quinoa is a host or non-host of a key soil symbiont, arbuscular mycorrhizal fungi (AMF), is suddenly up for debate with recent field studies reporting root colonization and presence of arbuscules. This research seeks to add evidence to the mycorrhizal classification of quinoa as we investigated additional conditions not previously explored in quinoa that may affect root colonization. A greenhouse study used six AMF species, two AMF commercial inoculant products, and a diverse set of 10 quinoa genotypes. Results showed 0 to 3% quinoa root colonization by AMF when grown under greenhouse conditions. Across quinoa genotypes, AMF inoculant affected shoot dry weight ($p = 0.066$) and height ($p = 0.031$). Mykos Gold produced greater dry biomass than *Claroideoglomus eutunicatum* (27% increase), *Rhizophagus clarus* (26% increase), and within genotype CQ119, the control (21% increase). No treatment increased plant height compared to control, but *Funneliformis mosseae* increased height compared to *C. eutunicatum* (25% increase) and *Rhizophagus intraradices* (25% increase). Although quinoa plants were minimally colonized by AMF, plant growth responses fell along the mutualism-parasitism continuum. Individual AMF treatments increased leaf greenness in quinoa genotypes 49ALC and QQ87, while *R. clarus* decreased greenness in CQ119 compared to the control. Our research findings support the recommendation to classify quinoa as non-mycorrhizal when no companion plant is present and inconsistently mycorrhizal when conditional colonization occurs.

Keywords Arbuscular mycorrhizal fungi · Quinoa · Bioassay · Host-microbe interactions

Introduction

Quinoa (*Chenopodium quinoa* Willd.) belongs to a family generally considered non-mycorrhizal (Amaranthaceae). Non-mycorrhizal plants have been defined as plants with roots that are highly resistant to colonization by arbuscular mycorrhizal fungi (AMF) and, more specifically, mycorrhizal structures do not form in the roots when fungal inoculant is present [1]. Knowledge of the non-mycorrhizal status of Chenopodiaceae and Cruciferae crops dates as far back as the 1800s [2], is assumed common knowledge, and is therefore often not cited in articles. However, plant species of the Amaranthaceae family (including those of the former Chenopodiaceae family) have been shown to be infected by AMF [2, 3]. The phenomenon of non-host plant species being infected by AMF has led to the development of clarifying terms for such plants: "inconsistently mycorrhizal," "weakly susceptible," and, more recently, "conditional rudimentary arbuscular mycorrhizal" [1, 3, 4]. Broadly speaking, mycorrhizal host status of plant families cannot be considered binary. In fact, the plant-host and mycorrhizal interaction exists along a continuum for some plant species, mainly due to conditional colonization [1, 3, 4].

The importance of determining whether or not a plant species such as quinoa is a host of AMF lies in the significance of the fungal symbiont. AMF are symbiotic soil microorganisms that benefit colonized plants with drought tolerance [5], alleviation of salt stress [6], disease resistance [7], and increased uptake of important nutrients such as phosphorous [8]. A better understanding of quinoa's AMF host status may have

✉ Julianne A. Kellogg
julianne.kellogg@wsu.edu

[1] Department of Crop and Soil Sciences, Washington State University, Johnson Hall, PO 646420, Pullman, WA, USA

implications for quinoa's success as a food crop. As it stands, quinoa has been classified as a plant species with Glomalean Fungus Colonization (GFC) [1]; that is, it is considered a non-mycorrhizal plant that can be colonized by AMF along with other endophytes and saprophytes as the plant's root system ages [1, 9]. A GFC designation relies on evidence showing a presence of vesicles and hyphae, with arbuscules being absent from the plant's roots. However, recently published research on quinoa root colonization by AMF suggests the GFC status may be incorrect [10, 11].

Field studies quantifying quinoa root colonization by AMF show varying rates of infectivity. Although field studies are not a definitive test for the host status of a plant species, they can demonstrate conditional colonization. The highest reported rate of root infectivity of mature quinoa plants by AMF in a field study is 31% [10], seen in one year (2016) of the 3-year study. The two previous years of the study found 17% (2014) and 9% (2015) colonization of quinoa roots. Arbuscules were observed, indicating a possible, but not proven, exchange of nutrients. An earlier field study also quantified quinoa root colonization by AMF at a moderate level of 19% from field samples collected at plant maturity [11]; colonization quantification included arbuscules, vesicles, and intraradical hyphae. A field study located in the Andean Bolivian highlands, a region where quinoa is commonly cultivated, found much lower root colonization rates of less than 2% in plants collected at a mid-season plant growth stage [12]; no arbuscules were observed. Such field studies demonstrate a range in infectivity of quinoa roots by AMF and may disprove the GFC designation, instead supporting an 'inconsistently mycorrhizal' designation. However, these studies are not conclusive in determining whether quinoa is an AMF host species or not.

Controlled potted plant studies are a more direct test of host status. An early study observed quinoa root colonization rates of 2–3% from greenhouse potted plots grown with onion plants in soil inoculated with *Glomus fasciculatus* [2]. However, a closely related species to quinoa, *Chenopodium album*, resulted in up to 5% root colonization by AMF in the presence of onion. Quinoa plants in inoculated pots without companion onion plants did not show any root colonization by AMF. Another early study found limited (less than 5%) AMF presence in quinoa roots of potted plants in soil inoculated with *Glomus deserticola* [13]. These quinoa plants were subjected to a foliar sublethal application of the herbicide simazine which may have increased root exudates of sugar and amino acids and thereby promoted AMF root colonization. These studies were not designed to provide evidence to claim a host status, but they do describe conditional colonization.

Conditional colonization of non-mycorrhizal plants can be caused by the presence of a mycorrhizal host companion plant, as described above, but the required conditions may also include a specific plant genotype (i.e., variety) or the species of AMF. Mycorrhizal colonization rate of roots and response to colonization is genotype dependent for some crops [14–16], emphasizing the need to screen for genotypes with observable increases in colonization rates by AMF. Modern wheat varieties have been shown to have a reduced capacity to develop a symbiotic relationship with AMF [17, 18]. In sorghum, open pollinated varieties produced more vegetative biomass and grain per plant than commercial hybrids in non-fertilized mycorrhizal soil [19]. Domesticated sunflower varieties have reduced root colonization by AMF when compared to wild sunflower accessions, leading to the conclusion that plant breeding methods may affect a plant variety's capacity to be colonized by AMF [20]. The plant characteristic of high root colonization by AMF may not be selected for when plant selection is performed in phosphorus-rich soils or because traits that confer higher levels of colonization are low in heritability. To determine whether genotype and AMF species are possible conditions for AMF colonization of quinoa, a controlled potted experiment is required.

Understanding of the quinoa genotype by AMF species interaction is of interest to plant breeders. Quinoa projects at Washington State University include development of varieties for organic systems [21, 22]; selection is conducted in low-input organic farming conditions, helping to avoid unintentional reduced symbiotic relationships with soil microorganisms. Pre-breeding projects that determine the mycorrhizal status of quinoa and whether the relationship is genotype dependent can guide parental selection, increasing the efficiency of current and future quinoa breeding projects. The primary objectives of this research were to study (1) the effect of quinoa genotype and AMF inoculant on quinoa root colonization rates in a controlled greenhouse setting and (2) the growth response of quinoa to AMF by measuring leaf greenness, shoot dry weight, and plant height at harvest.

Methods

Plant and Fungal Material

Ten quinoa accessions were selected from the US Department of Agriculture (USDA) North Central Regional Plant Introduction Station of the US National Plant Germplasm System in Ames, Iowa (Table 1). Christensen et al.'s [23] characterization of genetic diversity within the USDA quinoa germplasm informed genotype selection to represent the broad genetic diversity of the USDA genotypes. Genotypes from four distinct subgroups were included in this study: (1) the northern Andean highlands of Ecuador and Peru, (2) the

Table 1 List of quinoa genotypes (accessions) used in the study

Genetic cluster[a]	Quinoa genotype	Accession #	Origin
Northern Highland	K'ello	PI 510533	Peru
	Rosa Junin	PI 596498	Cusco, Peru
Southern Highland	CQ119	PI 614919	Oruro, Bolivia
	QQ87	PI 614884	Jujuy, Argentina
	R-132	PI 478418	Potosí, Bolivia
Lowland	QQ61	PI 614888	Bío Bío, Chile
	QQ74	PI 614886	Maule, Chile
	QQ101	PI 614883	Jujuy, Argentina
Ballón collection	49ALC	AMES 13726	–
	Kaslaea	AMES 13745	–

[a] Accessions are grouped by Christiansen et al.'s [23] genetic clusters. Accessions were sourced from the USDA North Central Regional Plant Introduction Station of the US National Plant Germplasm System in Ames, Iowa

southern Andean highlands of Bolivia, Argentina, and extreme northeast Chile, (3) the Chilean lowlands, and (4) the Ballón collection that may represent a distinct ecotype, the Yungas region of the eastern slopes of the Bolivian Andes [23].

Isolates of AMF species *Funneliformis mosseae*, *Septoglomus deserticola*, *Claroideoglomus eutunicatum*, *Rhizophagus clarus*, *Rhizophagus diaphanus*, and *Rhizophagus intraradices* were purchased from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) at West Virginia University in Morgantown, WV, USA (Table 2). The previous host plant of the AMF species from INVAM was sudangrass (*Sorghum sudanense*). All INVAM inoculants included hyphae, spores, and roots in calcined clay. The control inoculant was purchased from INVAM and included non-inoculated sudangrass roots in calcined clay. The commercial products Mykos Gold (Reforestation Technology International, Gilroy, CA) containing *R. intraradices* and MycoApply Micronized Endo (Mycorrhizal Applications, Grants Pass, OR) containing *R. intraradices*, *F. mosseae*, *C. eutunicatum*, and *G. aggregatum* were included in the trial to observe root infection and growth response to readily available commercial products.

Plant Fungus Bioassays

A silt loam surface soil was collected from a certified organic farm in Lewiston, Idaho. The soil had a pH of 5.4, 4.0% organic matter, and extractable nutrient concentrations of 29 mg kg^{-1} of P, 1015 mg kg^{-1} of K, 2.2 mg kg^{-1} of Mg, 28.2 mg kg^{-1} of Mn, 37.9 mg kg^{-1} of NO_3^--N, and 7.7 mg kg^{-1} of NH_4^+-N. Using methods adapted from those used by INVAM [24], soil was passed through a 4-mm sieve and mixed with sand and perlite at a 1:1:1 volumetric ratio. This soil/sand/perlite growth medium (SSP) underwent pasteurization at 80 to 82°C for 30 min and was dried and rewetted three times on greenhouse benches at Washington State University in Pullman, WA. The resulting SSP had a pH of 7.7, 1.2% organic matter, and extractable nutrient concentrations of 3 mg kg^{-1} of P, 376 mg kg^{-1} of K, 2.0 mg kg^{-1} of Mg, 35.7 mg kg^{-1} of Mn, 10.7 mg $kg^{-1}$of NO_3^--N, and 17.1 mg kg^{-1} of NH_4^+-N. No fertilizer was added to the SSP. D40H Deepot Cone-tainers (Steuwe & Sons, Tangent, OR, USA) were sterilized with 10% sodium hypochloride water solution. Cotton balls were used to plug cone-tainer holes to prevent SSP and inoculant loss during watering events.

Each sterilized cone-tainer was filled with 325 ml of SSP, followed by 100 ml of AMF inoculant diluted 1:20 with SSP, and topped with 50 ml of SSP (Fig. 1). On 12 December 2015, five quinoa seeds, sterilized with a 5% sodium hypochloride water solution, were pressed into the final SSP layer and covered with a 50-ml layer of autoclaved vermiculite to reduce moisture loss. The quinoa in each cone-tainer was thinned to

Table 2 Sources of the eight arbuscular mycorrhizal fungi inoculants and control materials

AMF treatment	Code	Source	Accession #/product name
Control	Cn	INVAM	–
Rhizophagus intraradices, *Glomus aggregatum*, *Funneliformis mosseae*, *Claroideoglomus eutunicatum*	Ma	Mycorrhizal applications	MycoApply-Endo
Rhizophagus intraradices	Mg	Reforestation technologies international	Mykos Gold
Claroideoglomus eutunicatum	Ce	INVAM	BR 218
Funneliformis mosseae	Fm	INVAM	UT 101
Rhizophagus clarus	Rc	INVAM	BR 854
Rhizophagus diaphanus	Rd	INVAM	BR 608B
Rhizophagus intraradices	Ri	INVAM	UT 126
Septoglomus deserticola	Sd	INVAM	VZ 103B

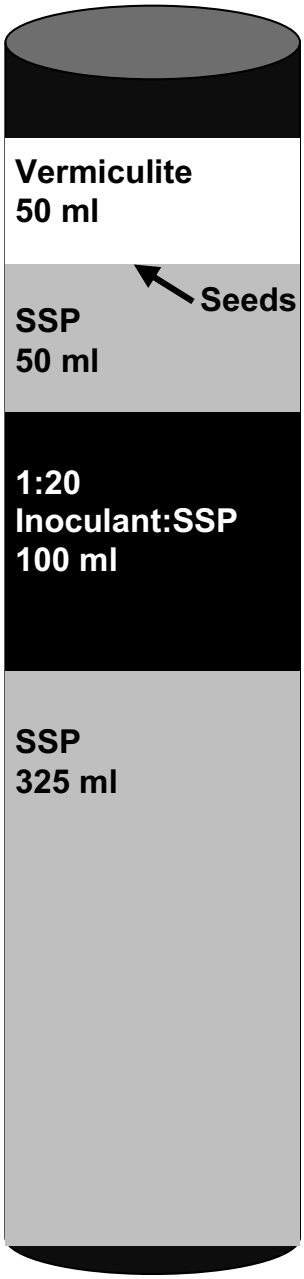

Fig. 1 Method for filling Stuewe & Sons D40-H Deepot. Dimensions are 6.4 cm wide and 25.4 cm long; volume is 656 ml

one plant, after plants grew six true leaves. There were five replicates of each genotype by inoculant combination. The greenhouse was maintained at 28°C during the day and 20°C at night. Photoperiod was initially set to 10 h daylight and increased to 12 h daylight 9 days after planting when quinoa seedlings were observed to require additional daylight; 1000-watt metal halide bulbs measuring a minimum of 168 μmol during low natural light days in the greenhouse provided supplemental light intensity.

During emergence, plants were watered uniformly to maintain SSP volumetric water content (VWC) of approximately 30 to 50%. Once plants were established and thinned, the trial was watered to maintain SSP volumetric water content of approximately 20 to 30%. Eight cone-tainers planted identical to the trial were randomly located within the trial and fitted with soil moisture sensors (Decagon Model 5TM, METER Group, Pullman, WA, USA) to guide the watering schedule.

To assess quinoa growth response to AMF species, the following measurements were taken: (1) non-destructive leaf greenness measurements of the three youngest fully expanded leaves were averaged using the portable SPAD 502 Plus chlorophyll meter (Konica Minolta, Japan) on weeks 10 and 11 of the trial, (2) shoot dry weight, and (3) plant height at harvest. Leaf greenness, shoot dry weight, and plant height have been previously used to assess the effects of abiotic stress on quinoa [25, 26]. Leaf greenness in quinoa has been shown to increase with increased total leaf nitrogen [25]. Shoot dry weight (biomass) and height are frequently used as measures of plant growth response to AMF. In studies investigating the effect of abiotic stress on quinoa physiological responses and seed quality, stressors that decreased quinoa plant biomass also decreased seed yield [27] and, when biomass was not affected, neither was seed yield [26]. Seed yield was not measured in our study because we aimed to harvest the quinoa plants prior to plant senescence. Analysis of variance (ANOVA) of this factorial study considers quinoa genotype to be a main effect, although our interest is in AMF inoculant effect and genotype by inoculant interaction. Quinoa genotypes included in this study differed in physiology and phenology including number of days to maturity. Plant measures of height, dry biomass, and leaf greenness in the last two weeks of the trial captured genotypes maturing at different rates.

Harvest Protocol

The experiment was destructively harvested on 20 February 2016 after 11 weeks of growth. Harvest was conducted once several genotypes reached full maturity. Growth stage varied across genotypes on week 11. Two genotypes had set seed and were beginning to senesce (QQ101, QQ74). Five genotypes were flowering (49ALC, CQ119, Kaslaea, QQ61, QQ87). Three genotypes had completed inflorescence emergence at the time of harvest (K'ello, R-132, Rosa Junin).

All 449 plants grown in the trial were harvested (one plant failed to emerge), with each harvested plant being a replicate of a quinoa genotype and AMF treatment combination (5 replicates, 90 combinations). The plant shoots were cut from the root mass at the soil line. Shoots were dried for 2 days at 80°C and dry weights were recorded. The root mass was carefully removed from the SSP by washing under running tap water and rinsed with deionized (DI) water. Roots were blotted dry and root lengths chopped into 2 to 3 cm pieces. For each plant, twenty to thirty random root sections were stored in a single biopsy cassette in DI water at 4°C.

Root Staining Protocol and Mycorrhizal Colonization Assessment

Roots were cleared with 10% KOH for 10 min at 90°C and stained with 5% black Sheaffer ink in vinegar for 3 min at 95°C [28]. After assessing mycorrhizal colonization rates of a replicate of each genotype by AMF species treatment, a subsample was restained with ink and vinegar for an additional 10 min. A separate subsample was restained by acidifying in 2% hydrochloric acid and staining with a water, glycerin, and lactic acid solution (1:1:1), with trypan blue (0.05%, *w*/*v*) as the dye [29]. For at least one replicate of each genotype and AMF inoculant combination, a minimum of 20 root sections were mounted on microscope slides and assessed using a slide-intersect method [30]. The presence or absence of arbuscules, coils, hypha, spores, or vesicles was observed along 100 intersects at ×200 and ×400 magnifications to estimate the percentage of root length colonized.

Data Analyses

The bioassay was a split-plot design, with the AMF inoculant as the whole plot in a completely randomized design. Quinoa genotypes were nested within AMF inoculant as the subplot. Five replicates were planted, and plant heights, leaf greenness values, and shoot dry weights were measured for each replicate. Quinoa root colonization by AMF was assessed for a minimum of one replication, randomly selected from the pool of five replications. The function "power.prop.test" in R software [31] was used to determine the required number of observations to statistically differentiate root colonization of AMF inoculated quinoa plants vs. non-inoculated plants. In the literature available at the time of data collection, the greatest percentage of quinoa root colonization documented was 19% [11]. Barley, a crop typically considered low in mycorrhizal dependency, is colonized at rates of 8 to 29% in low P soils [16]. The expected % root colonization of the quinoa control plants is zero. Using a confidence level of 95% and power of 80%, comparing the proportions 0 to 19% requires a sample size of 36 while comparing 0 to 8%, requires a sample size of 93. In this study, we analyzed a subsample of 100 randomly selected root samples including at least one replication of each quinoa genotype by AMF inoculant combination (10 controls plus 80 treatments).

ANOVA using a linear mixed effects model fit by residual maximum likelihood was carried out for leaf greenness, shoot dry weight, and plant height using R software and packages "lme4" [32] and "lmerTest" [33]. Plant growth response measures were the independent variables in the models with quinoa genotype and AMF treatment as dependent variables. Quinoa genotype and AMF treatment were treated as fixed effects. Error was estimated using replicate as a random effect. Multiple comparisons of means were performed using Tukey contrasts with the "multcomp" package [34].

Results

Leaf Greenness

Leaf greenness varied among the quinoa genotypes on weeks 10 and 11 (Table 3). Mean leaf greenness estimates across all AMF inoculants for the quinoa genotypes ranged from SPAD meter values of 33.0 (QQ101) to 40.5 (K'ello) on week 10 (Fig. 2) and from 29.2 (QQ101) to 38.3 (K'ello) on week 11. AMF treatment did not have a statistically significant effect on leaf greenness ($p = 0.645$); post hoc analysis was not conducted.

Additional inferences were made from the leaf greenness data using means and 95% confidence intervals (CI); data are compatible with any value of μ within the CI but relatively incompatible with any value outside the CI [35] (Fig. 2). Conservative interpretation of the CI for leaf greenness on week 10 revealed differences between AMF treatments for several of the quinoa genotypes; i.e., CI did not overlap. Three of the AMF treatments differed from the control for several quinoa genotypes. For 49ALC, *R. diaphanus* and *R. intraradices* treatments resulted in plants greener than the control. For QQ87, the *R. diaphanus* treatment resulted in plants greener than the control. For CQ119, the *R. clarus* treatment resulted in plants less green than the control and the Mykos Gold and *R. diaphanus* treatments.

Shoot Dry Weight

Shoot dry weight varied significantly among quinoa genotypes and AMF inoculant had a marginally significant effect ($p = 0.066$) (Table 3). Mean shoot dry weight across all AMF inoculants for the quinoa genotypes ranged from 1.1 g (R-132) to 1.6 g (QQ61) (Fig. 3). Mean shoot dry weight across all quinoa genotypes for the AMF inoculants ranged from 1.2 g (*R. clarus*) to 1.5 g (Mykos Gold). With no significant interaction between AMF inoculant and quinoa genotype, post hoc analyses were conducted. No inoculant was statistically significantly different from the control, but the Mykos Gold treatment produced plants with greater dry weight than *C. eutunicatum* ($p = 0.051$, 27% increase) and *R. clarus* ($p = 0.068$, 26% increase).

Further inferences were made from the shoot dry weight data using means and 95% CI; interpretation of the CI revealed differences between AMF treatments for several of the quinoa genotypes (Fig. 3). For CQ119, the Mykos Gold treatment resulted in plants with higher shoot dry weights than

Table 3 ANOVA table for leaf greenness on weeks 10 and 11, shoot dry weight, and plant height at harvest

Effects[a]	DF	SS	MS	*F* value	*p* > *F*
Leaf greenness on week 10					
AMF treatment	8	69.6	8.700	0.754	0.6448
Quinoa genotype	9	1887.1	209.676	18.159	<2e−16*
AMF treatment: quinoa genotype	72	839.9	11.665	1.010	0.462
Leaf greenness on week 11					
AMF treatment	8	141.0	17.62	0.898	0.528
Quinoa genotype	9	3336.8	370.75	18.894	<2e−16*
AMF treatment: quinoa genotype	72	1112.5	15.45	0.788	0.889
Shoot dry weight					
AMF treatment	8	0.429	0.054	2.067	0.066****
Quinoa genotype	9	14.111	1.568	60.419	<2e−16*
AMF treatment: quinoa genotype	72	1.903	0.026	1.018	0.445
Plant height at harvest					
AMF treatment	8	274.8	34.36	2.466	0.031***
Quinoa genotype	9	8067.5	896.39	64.331	<2e−16*
AMF treatment: quinoa genotype	72	803.0	11.15	0.800	0.872

[a] Analysis used a linear mixed effects model fit by residual maximum likelihood with Satterthwaite approximation for degrees of freedom to show the decomposition of fixed effects

****0.1, ***0.05, **0.01, *0.001—significance codes

the control and the MycoApply-Endo, *C. eutunicatum*, *R. clarus*, and *R. diaphanus* treatments. For K'ello, the *R. clarus* treatment resulted in plants with lower shoot dry weights than the Mykos Gold, *F. mosseae*, and *R. diaphanus* treatments.

Plant Height at Harvest

Plant height at harvest differed among quinoa genotypes and AMF inoculants (Table 3). Mean height across all AMF inoculants for the quinoa genotypes ranged from 25.6 cm (R-132)

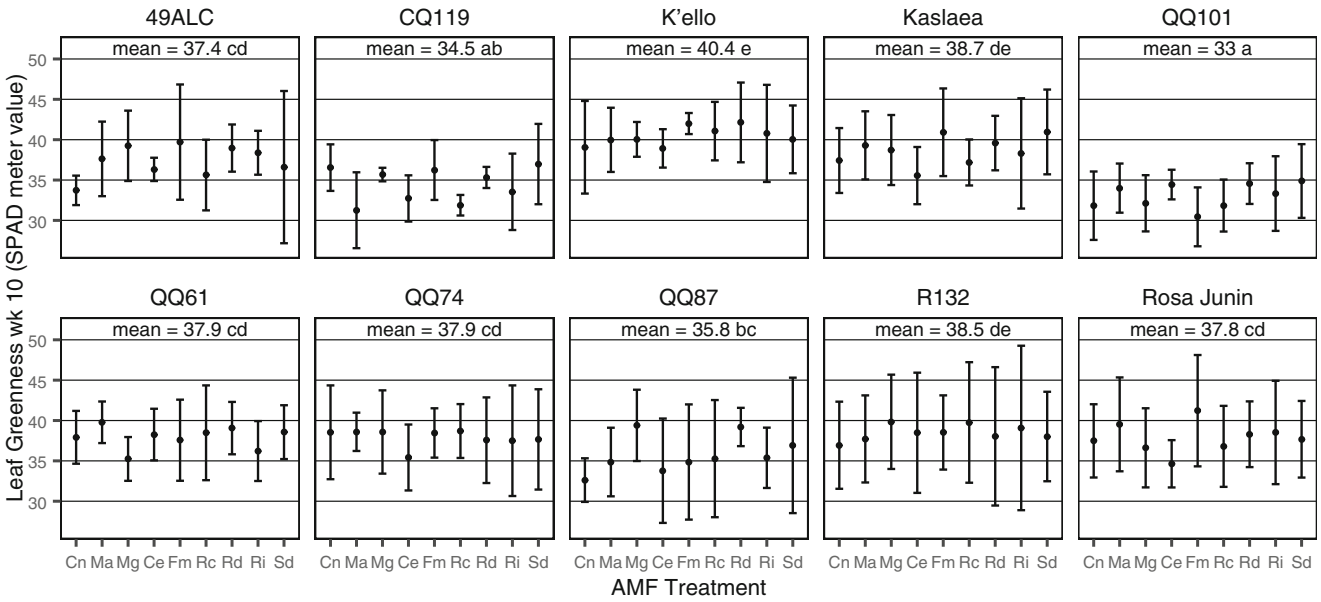

Fig. 2 Leaf greenness (SPAD meter value) of quinoa genotypes inoculated with nine different AMF treatments at week 10 of the trial. AMF treatment codes are listed in Table 2. The mean of five replicates (*point*; 95% CI) is reported. Quinoa genotype means with at least one common letter are not significantly different

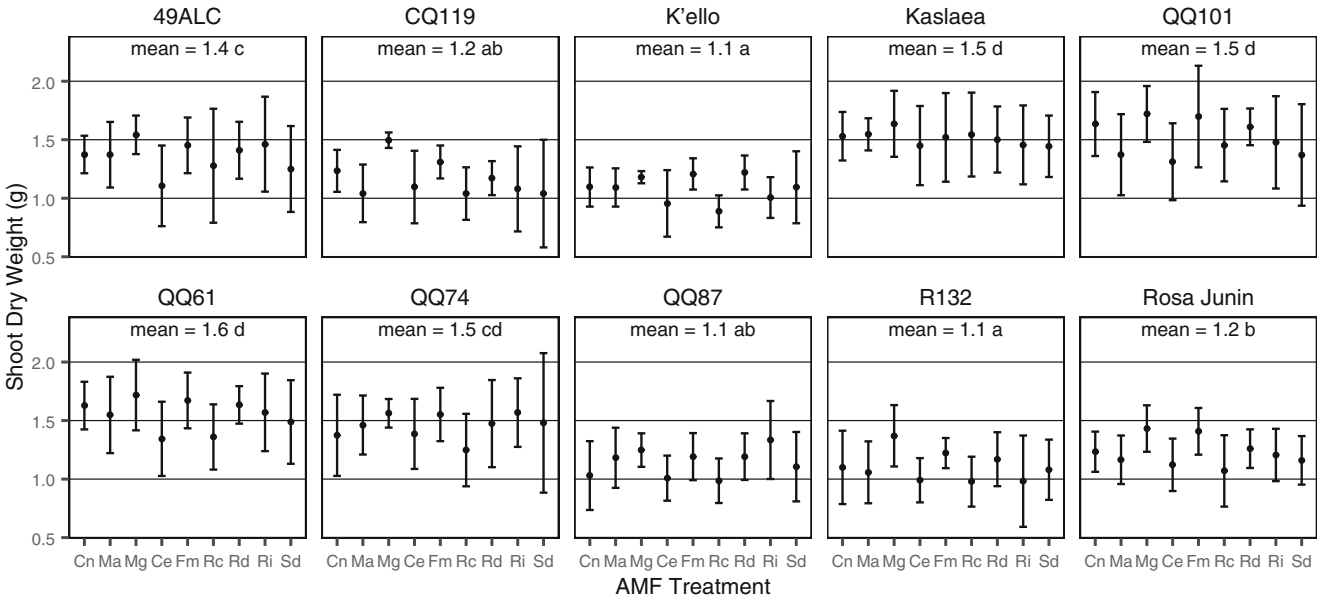

Fig. 3 Shoot dry weight (g) of quinoa genotypes inoculated with nine different AMF treatments. The mean of five replicates (*point*; 95% CI) is reported. Quinoa genotype means with at least one common letter are not significantly different

to 39.8 cm (Kaslaea) (Fig. 4). Mean height across all quinoa genotypes for the AMF inoculants ranged from 28.9 cm (*C. eutunicatum*) to 36.3 cm (*F. mosseae*). There was no significant interaction between AMF inoculant and quinoa genotype; post hoc analysis on plant height data revealed only the AMF species isolate *F. mosseae* produced plants greater in height than *C. eutunicatum* ($p = 0.084$; 25% increase) and *R. intraradices* ($p = 0.097$; 25% increase). Plant heights of AMF-inoculated plants were not different from the non-mycorrhizal control. Further inferences were made from the shoot dry weight data using means and 95% CI; Rosa Junin plants inoculated with Mykos Gold were taller than plants inoculated with *C. eutunicatum*, *R. diaphanus*, *R. intraradices*, or *S. deserticola* but did not differ from the control (Fig. 4).

Root Colonization

Quinoa root colonization by AMF was extremely low (≤3%) or none (Table 4) in all treatments. Only hyphae

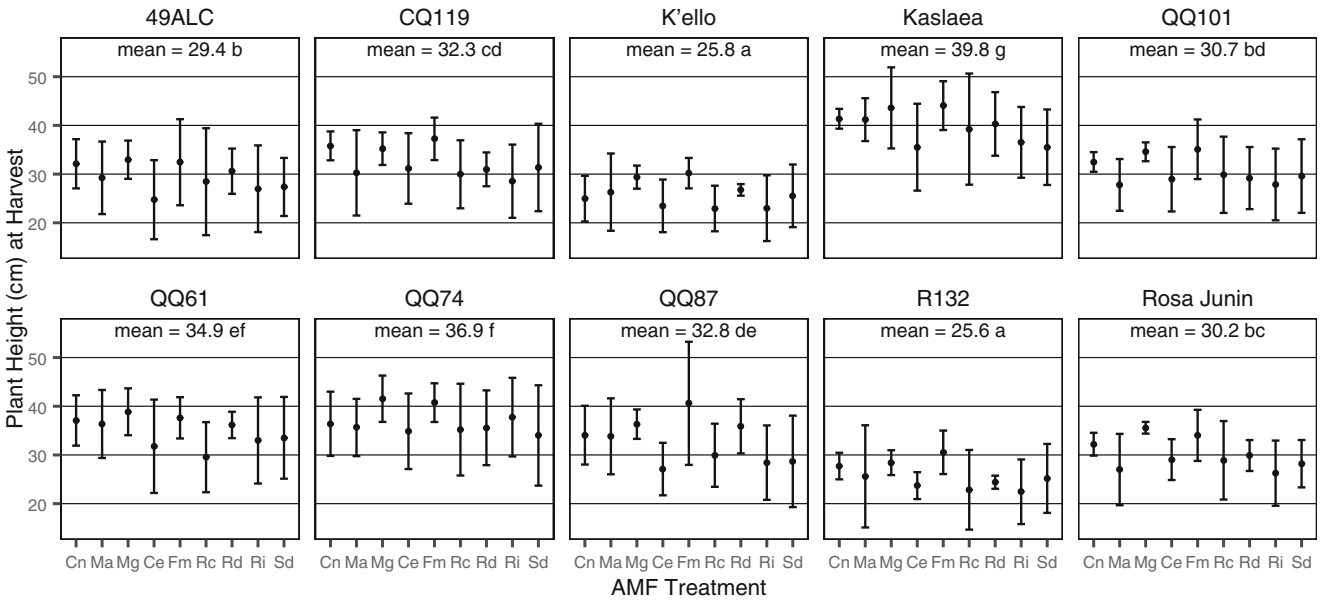

Fig. 4 Plant height (cm) at harvest of quinoa genotypes inoculated with nine different AMF treatments. The mean of five replicates (*point*; 95% CI) is reported. Quinoa genotype means with at least one common letter are not significantly different

Table 4 Mycorrhizal colonization (percent root length) in 10 quinoa genotypes inoculated with 8 AMF species or control[a]

	49ALC	CQ119	Kaslaea	K'ello	QQ101	QQ61	QQ74	QQ87	R-132	Rosa Junin
Cn	0.0	0.0*	0.0	0.0	0.0*	0.0	0.0	0.0	0.0	0.0
Ma	0.0	0.0	0.0	0.0	0.0	**0.33***	0.0	0.0	0.0	0.0
Mg	0.0	0.0	0.0*	0.0*	0.0	0.0	0.0	0.0	0.0	**3.0**
Ce	0.0	0.0	0.0	**1.0**	0.0	0.0	0.0	0.0	**2.0**	0.0
Fm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0*	0.0	0.0
Rc	**3.0**	0.0	0.0	**0.5**	0.0	0.0	0.0	0.0	0.0	0.0
Rd	0.0	0.0	0.0	0.0	**1.0***	0.0	0.0	0.0	0.0*	0.0
Ri	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0**	0.0	0.0
Sd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Only hyphae were observed. A minimum of one replication of each treatment was analyzed. *2 replicates were analyzed, **3 replicates were analyzed. A minimum of 20 root sections per replicate were plated on a 1 cm × 1 cm grid. 100 intersections were observed per replicate

were observed, with no vesicles, arbuscules, or coils. No additional AMF structures were observed following staining with trypan blue. Control plants showed 0% colonization.

Discussion

It is questionable whether a functional AMF relationship was established in our study because only hyphae were observed within the quinoa roots and with extremely low or no quinoa root colonization by AMF. Interestingly, certain AMF treatments affected plant growth response measurements. Differences in leaf greenness, shoot dry weight, and plant height in several of the quinoa genotypes with different AMF inoculants suggest beneficial or detrimental plant-microbe interactions. It is possible we missed AMF-specific structures in the roots, and the low rate of colonization did have an impact on plant response. In support of this hypothesis, Klironomos' study [36] that included multiple plant species showed no relationship between percentage root colonization by AMF and plant growth response. A meta-analysis that investigated the relationship between percentage root colonization and plant response found that increased percentage root colonization resulted in a greater plant response, but only explained 11.8% of the variation in plant biomass [37]. Additionally, the effect on plant response varied greatly across AMF taxa; *Funneliformis* resulted in the greatest effect on plant response. In our study, *Funneliformis mosseae* generally resulted in greater plant growth responses. In another study by Klironomos, no AMF arbuscules or vesicles were found in eight of 64 plant species inoculated with *Glomus etunicatum*, yet the inoculated plants exhibited either increased or decreased biomass compared to non-inoculated plants [38]; one plant species from the Chenopodiaceae family exhibited a minimal increase in biomass over the control (<10%).

AMF are obligatorily dependent on a plant, whereas plant responses to AMF colonization fall along a mutualism-parasitism continuum [39]. In our study, AMF inoculants caused a positive, negative, or no plant response. We also observed that quinoa does not require AMF to establish, grow, and uptake nutrient resources. Our study subjected quinoa to extremely low levels of soil nutrient resources, yet all plants that emerged survived to harvest and some genotypes had begun to set seed. Although ANOVA showed no significant interaction between the quinoa genotype and AMF treatments for any of the plant response measurements, CI's indicated specific positive and negative interactions of genotypes and treatments. Within a quinoa genotype, plant responses fell along the mutualism-parasitism continuum. This parallels Klironomos' findings that the direction and magnitude of plant responses to AMF are dependent on the AMF species and plant species combination [38]. Our study differs in that we observed this phenomenon at the subspecies level in an inconsistently mycorrhizal species. Plant growth responses with no observed root colonization may suggest non-invasive rhizosphere interactions of some AMF species with quinoa.

The lack of root colonization in our study is in agreement with other potted plant studies that showed little to no root colonization of quinoa even when grown with a mycorrhizal plant or treated with herbicide [2, 13]. These findings support a non-mycorrhizal, non-host status for quinoa. Vestberg et al. [11] and Wieme et al. [10] quantified much higher AMF colonization rates with field studies in temperate climates and demonstrated that field-grown quinoa is more susceptible to colonization; from their research, a conditionally mycorrhizal or inconsistently mycorrhizal status may be an appropriate classification for quinoa.

A closer look at the conditions of field studies investigating quinoa root colonization by AMF tells a more nuanced story about conditional quinoa root colonization. Urcelay et al. [12] analyzed roots of field grown quinoa plants harvested at an early growth stage and measured root colonization rates of less than 2%; their plants were grown at 3700 m in Bolivian highland sandy soils low in organic matter. On the other hand, plants in the Vestberg et al. study [11] were grown in Finland in sandy clay soils with 3.3% soil organic matter and the Wieme et al. study [10] was located in Washington State, USA in silt loam soils with approximately 3% organic matter. The Wieme et al. study [10] provided ideal conditions for an inconsistently mycorrhizal plant species to be colonized by AMF: (1) adjacent experimental plots with mycorrhizal plant species; (2) fairly high levels of soil organic matter that may have been colonized by mycorrhizae; and (3) quinoa plants that were sampled at maturity. Quinoa can no longer be classified as GFC because Wieme et al. [10] observed arbuscules in quinoa roots, but the literature supports the classification of quinoa as being inconsistently mycorrhizal.

Conditions present in quinoa's dry, high altitude, and saline center of origin and regions of early cultivation also support the classification of quinoa as an inconsistently mycorrhizal species. Quinoa is considered a facultative halophyte [40], and plants that grow in harsh environments are habitat specialists that often do not require mycorrhizae [9]. Next steps in research to further understand quinoa's association with AMF could include (1) investigating the genetic basis for quinoa's non-host status [4]; (2) determining whether quinoa can contribute to the understanding of the evolutionary history of mycorrhizal symbioses with plants [9]; and (3) probing the causes of quinoa growth responses at low levels of root colonization [38].

A shift to evaluating quinoa plant response to interactions with other fungi, such as those belonging to the genera *Alternaria*, *Bartalinia*, *Cadophora*, *Coniochaeta*, *Fusarium*, *Neonectria*, *Penicillium*, *Phoma*, *Plectosphaerella*, *Rhinocladiella*, and *Sarocladium* found in quinoa roots [41], could shed light on how quinoa can be grown under harsh environmental conditions. Research of the relationship between quinoa and endophytic bacteria is also promising. Endophytic bacteria from the *Bacillus* genus (1) inhabit quinoa seeds, (2) are found in all seedling organs, (3) form a possible obligate host relationship, and (4) may contribute to how quinoa seedlings withstand extreme situations [42]. Native endophytic bacteria have been isolated from the *Bacillus*, *Paenibacillus*, and *Pseudomonas* genera from quinoa plants as well as native fungi from the *Trichoderma*, *Beauveria*, and *Metarhizium* genera from quinoa's rhizosphere [43]; isolates of these groups were tested as quinoa inoculants and those that increased plant length, panicle weight, and grain yield were selected for use in developing a product using technology easily replicated by farmers for improving quinoa crop production.

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Availability of data and material The datasets generated during and/or analyzed in this study are available from the corresponding author upon reasonable request.

Code availability R code is available from the corresponding author upon reasonable request.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Julianne Kellogg. The first draft of the manuscript was written by Julianne Kellogg, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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