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Influence of Mycorrhizae and Irrigation on Growth and Mineral Uptake by Corn (*Zea mays* L.) Seedlings in a Calcareous Soil[1]

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Abstract— A pot experiment was conducted to determine the influence of arbuscular mycorrhizal (AM) fungus *Glomus aggregatum* and four levels of water application on early stage of growth and nutrient uptake of corn seedlings (*Zea mays* L.) in a calcareous soil (Lithic Ustorthents). Mycorrhizal inoculation significantly increased dry biomass of shoots and roots, leaf width, chlorophyll contents, and uptake of minerals N, P, K, Mg, Ca, Fe, Mn, B, Cu, and Zn regardless of irrigation level. Water treatments influenced leaf width, dry biomass of shoots and roots, and mineral uptake of N, P, K, Mg, Ca, Mn, and Zn, but not chlorophyll content and uptake of Fe, B, and Cu. Nonmycorrhizal plants with the lowest water exhibited the least plant growth.

Introduction

Arbuscular mycorrhizal (AM) fungi occur on virtually all higher vascular plants (Koide & Schreiner 1992, Tester et al. 1987). AM fungi absorb essential plant nutrients from the soil and translocate the nutrients to roots of plants on which they are symbionts (Gerdemann 1975, Hayman 1982). AM fungi increase plant tolerance to drought conditions (Jayne & Quigley 2014, Augé 2001, Sylvia & Williams 1992), possibly due to extended hyphal distribution in soil for increased water and nutrient uptake (Smith & Read 1997) in crops such as wheat (*Triticum aestivum* L.) (Al-Karaki et al. 2004, Allen & Boosalis 1983, Ellis et al. 1985), soybean (*Glycine max* L.) (Safir et al. 1972; Busse & Ellis 1985), onion (*Allium cepa* L.) (Nelsen & Safir 1982), and pepper (*Capsicum annuum* L.) (Mena-Violante et al. 2006, Waterer & Coltman 1989). In a three-season study, AM inoculated sweet corn (*Zea mays* L.) increased grain yield and biomass under drought field conditions (Sylvia et al. 1993).

A strain of *Glomus aggregatum* was isolated and investigated in Hawaii where the AM fungus was reported to benefit tropical trees grown in an Oxisol, an acid soil in Hawaii (Manjunath & Habte 1989). At lower P levels of soil, a tree species, *Acacia mangium* (Willd.), had increased *G. aggregatum* colonization on roots when soil pH levels increased from 4.3 to 5.0 (Habte & Soedarjo 1996). When grown in rock phosphate, *G. aggregatum* inoculated *Leucaena leucocephala* (Lam.) de Wit had significantly greater shoot and root dry weights (Manjunath & Habte 1989). In Brazil, *G. aggregatum* was frequently present in intensively cultivated fields, suggesting that the fungus was tolerant of agricultural practices and persisted in cultivated soils (Sieverding 1990). Four endemic Hawaiian species were tested with *G. aggregatum* inoculation in soils with different levels of P in a pot experiment (Gemma et al. 2002). At low P levels, inoculation increased leaf size, leaf tissue P

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uptake, and root biomass. The dependency on mycorrhizae was greater when plants were grown in low-P soils.

In Guam, *G. aggregatum* introduced from Hawaii was tested in an experiment with corn grown in basalt sand in pot culture earlier. Inoculation of corn with the AM fungus resulted in improved plant vigor, canopy height, leaf size, plant dry mass, and root formation (Marutani & Dela Cruz 2000). In order to develop cultural practices utilizing the AM fungus in sustainable agriculture, further studies are needed to evaluate the effectiveness of *G. aggregatum* in Guam's tropical island environment.

A commonly cultivated soil on Guam is characterized as a low fertile calcareous soil with a pH greater than 7.0 (Demeterio et al. 1986). The limitations of this soil include poor nutrient availability, shallow depth, and poor water-holding capacity (Young 1988). To this soil, the application of inorganic fertilizer and supplemental water is a common practice for crop production (Young 1988). The objective of this study was to determine the growth response and mineral uptake of corn seedlings grown in a calcareous soil inoculated with *G. aggregatum* with four levels of water application.

Materials and Methods

Production Of *Glomus aggregatum* Crude Inoculum

In order to conduct thesis Experiments 1 and 2, crude inoculum of *Glomus aggregatum* had to be produced. The crude starter inoculum of *G. aggregatum* was obtained from Dr. Mitiku Habte, a soil microbiologist, at the University of Hawaii. It arrived in crushed basalt that included fungal spores, hyphae, and pieces of roots of corn (*Zea mays* L.). Recommended procedures were conducted to increase inoculum (M. Habte, personal communication).

Commercial crushed basalt sand was sifted using a 2 mm aperture sieve, and then placed in a drying oven (VWR International West Chester, PA) at 90˚C for one week to eliminate native mycorrhizal fungi, and plant pathogens (M. Habte, personal communication). This process essentially 'sterilizes' the basalt. This method was also used in Experiments 1 and 2 for the same reason. The term 'sterile' is used because it best characterizes the high level of disinfection that is achieved with this method.

Sterile basalt and crude starter inoculum were mixed at a 1:1 ratio and placed in six one-gallon plastic pots. Corn seeds, either cv. 'Local White Corn' and 'Hawaiian Supersweet 9A', were disinfected with 0.3% sodium hypochlorite (NaOCl) for 15 min., thoroughly rinsed 3 times with distilled water, and then pre-germinated in Petri dishes on moistened filter paper for three days. Two corn seedlings with radicle development were planted into each pot, and watered daily with distilled water for one week. A modified Hoagland's solution I (Habte & Osorio, 2001) was then applied to all pots on five consecutive days per week for 12 weeks. One-hundred mL of the solution per pot was the application rate; thereby, totaling 500 mL per week. The amount of phosphorus was reduced to 8 mg•L^{1} which was recommended to promote mycorrhizal growth (Habte & Osorio, 2001). To maintain good plant growth, additional distilled water was added when media became dry.

After 12 weeks of growth, shoots and excise roots were removed from basalt media. Crude inoculum consisted of collected basalt and hyphae. The crude inoculum was stored in sterile plastic containers, which has a 2-year shelf life (M. Habte, personal communication).

Soil Preparation And Fungal Treatment

One hundred kg of Guam cobbly clay soil (clayey, gibbsitic, nonacid, isohyperthermic Lithic Ustorthents) was collected from the Guam Agricultural Experiment Station in Yigo (144º 55' E. longitude and 13º 33' N. latitude) at depths of 0-12 cm. The soil was sifted using a 2 mm aperture sieve and placed in a drying oven (VWR Company, Weschester, PA) at a constant of 90˚C for 7 days to eliminate native mycorrhizal fungi. This soil was used in the experiment and a 100-g representative

sample of the soil was air-dried, and soil nutrient analysis was conducted using standard procedures recommended by the Soil and Plant Testing Laboratory, University of Guam (Motavalli et al. 1996). Soil pH was determined using a 1:1 (v/v) soil/water paste and a pH meter (Walsh & Beaton 1973). Organic matter (%) was determined by the Walkley-Black method (Jackson 1958). Available P was determined by the Olsen method with a Bausch and Lomb Spectronic 21 spectrophotometer (Thermo Spectronic, Rochester, NY). Exchangeable K, Ca, Mg, Mn, Fe, Zn, and Cu were determined by an atomic absorption spectrophotometer (Varian Australia Pty Ltd., Victoria, Australia). The analytical characteristics of the soil (Table 1) indicated that it had very high concentration of Ca (5678 mg kg^{-1} dry soil) and low P (25 mg kg^{-1} dry soil).

Plastic pots were disinfected with 3% NaOCl, rinsed thoroughly with distilled water, and then air-dried for 48 hours. Each pot was filled with 2 kg of the dried Guam cobbly clay soil. An inoculated treatment (+) received 50 g of *G. aggregatum* crude inoculum per kg of soil. Application of 50 g of crude inoculum per kg of soil usually produces rapid initiation of AMF colonization of targeted plants (Habte & Osorio 2001). A non-inoculated treatment (-) received 50 g of sterile basalt per kg of soil. Crude inoculum and sterile basalt (control) were mixed evenly with upper 10 cm of soil of each pot.

Plant Materials

Hawaiian Supersweet No. 9A corn seeds (*Zea mays* L.) were disinfected with 0.3% sodium hypochlorite (NaOCl) in a 500 mL Erlenmeyer flask on a shaker for 10 min. After being rinsed three times with distilled water, seeds were pre-germinated in Petri dishes. Three germinated seeds were planted in each pot, and then thinned to two plants per pot after one week. Plants were watered with distilled water as needed before water level treatments were begun.

Water Treatment

The experiment began on the 12th day after sowing. Water and nutrient solutions were applied on a 7-day (one week) watering and nutrient schedule for three consecutive weeks. A one-week watering and nutrient schedule consisted of consecutive watering days (Day 1, Day 2, and Day 3) where each pot received 800, 400, 200, or 100 mL of water for treatments W1, W2, W3, and W4, respectively. For plant nutrient applications, 150 mL of modified Hoagland's solution (Habte & Osorio 2001) was applied to all plants in each pot on Day 4 and Day 7 for each of the three weeks. Nothing was applied on Day 5 and Day 6 of the weekly schedule. The total amount of water applied to each pot over the course of the 21-day (three weeks) experiment was 7200 (W1), 3600 (W2), 1800 (W3), or 900 mL/pot (W4). The total amount of nutrient solution applied was 900 mL/pot to all water treatments for the experiment.

Pots were placed on a bench under a clear polyethylene cover sheet (Warp Bros, Chicago, IL) in a nursery. A quantum meter (Spectrum Technologies, Inc. Plainfield, IL) was used to determine the photosynthetically active radiation (PAR) as μmol m^{-1} s^{-1} under the plastic cover and the open field. Plants received an average 73% PAR under the clear plastic housing.

Inoculation Detection

Prior to drying and preparing roots for measurements, root samples of each inoculated treatment were collected, cleared, acidified, stained, and destained in order to properly observe for colonization of *G. aggregatum.* Methods conducted to prepare roots for observation were in accordance with recommended procedures (Habte and Osorio, 2001).

PLANT GROWTH

At the end of the 21-day experiment, the width of the newest mature leaf of each plant was determined, and the average of two plants per pot used for statistical analysis. A chlorophyll SPAD-502 meter (Spectrum Technologies, Inc., Plainfield, IL) was used to determine the average of the estimated chlorophyll in four mature leaves per pot. From each pot, plant shoots of two plants were separated from roots, dried in a drying oven (VWR Company, Weschestder, PA) at 75 ˚C for 48 h to constant weight, and weighed to determine the dry shoot biomass per plant. Soil was carefully removed from roots. The weight of roots was measured after placing in a drying oven at 75 ˚C for 48 h to constant weight. Dried roots were ashed in a muffled furnace (Lindberg/BlueM, Asheville, NC) at 1000 ˚C for 2 h, and dry root biomass was determined by subtracting the weight of soil particles from the weight of roots before ashing root tissues.

MYCORRHIZAL DEPENDENCY

Mycorrhizal dependency of corn at each irrigation regime was determined using the relative field mycorrhizal dependency (RFMD) equation (Anderson & Ingram 1993) as follows:

RFMD = (WTm–WTn)/WTn

where: WTm = the total dry weight of shoots and roots of a mycorrhizal plant.

WTn = the total dry weight of shoots and roots of a non-mycorrhizal plant.

MINERAL ANALYSIS

Dried shoots of plants were ground, and mineral analyses conducted at a commercial testing lab (A&L Southern Agricultural Laboratories, Pompano Beach, FL). Standard analytical methods (Wolf 1982) were used to determine concentrations of macro elements (N, P, K, Mg, and Ca), and microelements (Fe, Mn, B, Cu, and Zn) in shoots.

EXPERIMENTAL DESIGN AND DATA ANALYSIS

The experiment was conducted using a split plot design with two treatments with four replications. The mainplot was water volume and the subplot was inoculation treatment. Data were analyzed by analysis of variance (ANOVA) using the NCSS 2001 Statistical Software (NCSS 2001). Where appropriate, the least significance difference (LSD) at 0.05 significance level was determined to compare mainplot (water) means, subplot (inoculation) means, subplot treatments at the same mainplot treatment, and subplot treatments at different mainplot means (Gomez & Gomez 1984; Little & Hills 1978). Line graphs were created with software GraphPad Prism v.4 (GraphPad Software, Inc., San Diego, CA). A simple correlation analysis of parameters was performed using the JMP statistical software (SAS Institute, Inc. Cary NC).

Results

Inoculation

Prior to drying and measuring roots, random root samples from each inoculated treatment were observed through a stereo microscope at 40x magnification for the presence of spores and hyphae of *G. aggregatum* (Habte and Osorio, 2001). Numerous spores and hyphae were present in all samples of inoculated plants to confirm successful fungal colonization of roots of plants due to inoculation. AMF colonization/infection of roots levels were not quantified in this experiment.

Plant Growth

AM inoculation significantly influenced shoot biomass, root biomass, leaf width, and SPAD chlorophyll reading of corn seedlings (Table 2). Mycorrhizal plants had the greater mean values of all growth parameters compared to those of non-inoculated plants with about 50 to 500% increase (Fig 1). Water treatment significantly affected all but chlorophyll reading (P=0.62) (Table 2). With the least irrigation (900 mL/pot), leaf width, shoot biomass and root biomass were least regardless of AM inoculation treatments (Fig 1). The interaction between irrigation and inoculation treatment was significant for all but leaf width (Table 2). AM plants obtained the greatest shoot biomass and most vigorous shoot growth with the water application of 3600 mL. At the lowest water level (900mL), AM corn leaves had the highest chlorophyll reading while non-AM plants had the least chlorophyll. As volume of water increased, growth parameters, shoot and root biomass and leaf width increased and then remained at plateau or slightly decreased eventually (Fig 1).

Nutrient Uptake

Water treatment and inoculation affected all measures, but the interaction of two factors was not significant (Table 3). Uptake of both macronutrients (N, P, K, Mg and Ca) and micronutrients (Fe, Mn, B, Cu, and Zn) was significantly increased by mycorrhizal colonization (Tables 3 and 4). At all irrigation levels, AM plants displayed a greater absorption of minerals than non-AM plants (Figs 2 and 3). Water volume significantly influenced uptake of N, P, K, Mg, Ca, Mn, and Zn, but did not affect uptake of minerals Fe, B, and Cu (Table 4). Absorption of N by both AM and non-AM plants was highest at the 1800 mL and decreased as the water level increased to 3600 and then 7200 mL (Fig 2). A similar pattern was observed for absorption of K. Uptake of P, Mg, Ca, Mn and Zn was the least at the lowest irrigation treatment (900 mL) in AM inoculated and non-inoculated plants (Fig 2). The interaction between water and inoculation treatments was not significant for mineral uptakes (Table 3 and 4). Correlation analysis indicated that all growth parameters were positively correlated with uptake of all mineral nutrients (Table 5).

Mycorrhizal Dependency

The range of RFMD of corn seedling ranged from 4.83 to 7.70 and with a mean RFMD of 5.90 for the four water volumes, indicating that corn plants responded to mycorrhizal inoculation by increasing biomass at all four water treatments. There were no significant differences for RFMD in corn at the four water volumes. This may be due to the small number of replications and the high coefficient of variation (CV=47.7%).

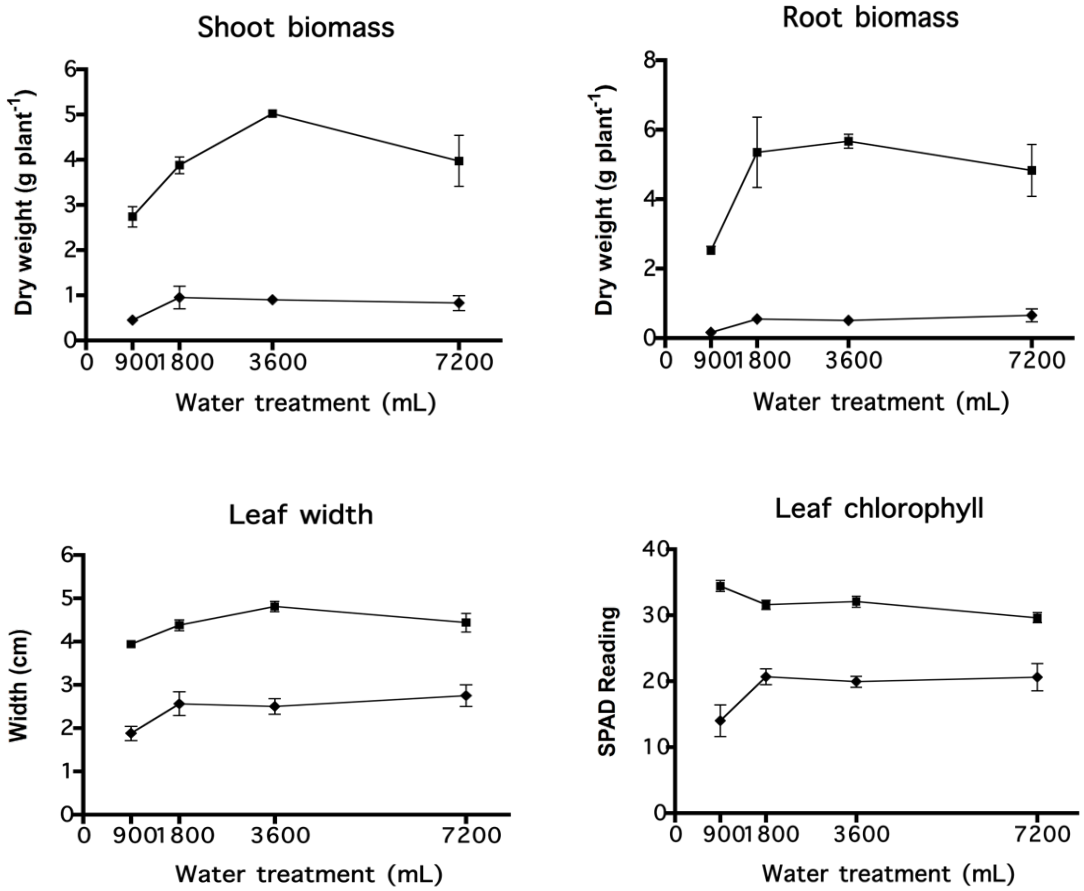

Figure 1. Average of shoot and root biomass (g/plant), leaf width (cm), and chlorophyll SPAD reading of corn seedlings grown in Guam cobbly clay soil, either inoculated (■) or not inoculated (♦) with *Glomus aggregatum*, and provided one of four volumes of water: W1=7200 mL, W2=3600 mL, W3=1800 mL, and W4=900 mL during a 3-week experiment. Crossbars represent standard deviations of means with four replications.

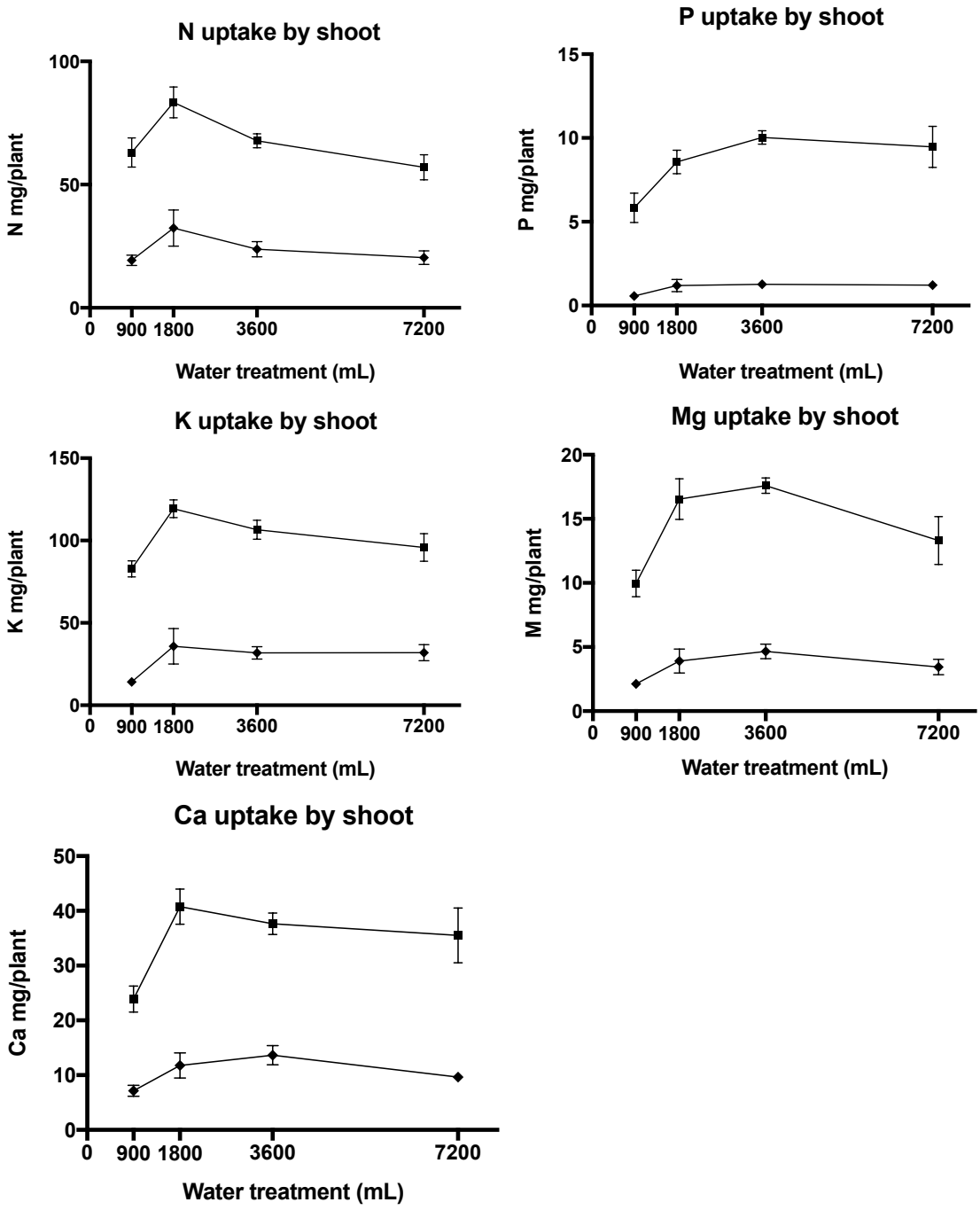

Figure 2. Average of macronutrients N, P, K, Mg, and Ca uptake (mg/plant) by shoots of corn seedlings grown in Guam cobbly clay soil, either inoculated (■) or not inoculated (♦) with *Glomus aggregatum* and provided one of four volumes of water: W1=7200 mL, W2=3600 mL, W3=1800 mL, and W4=900 mL during the 3-week experiment. Crossbars represent standard deviations of means of four replications.

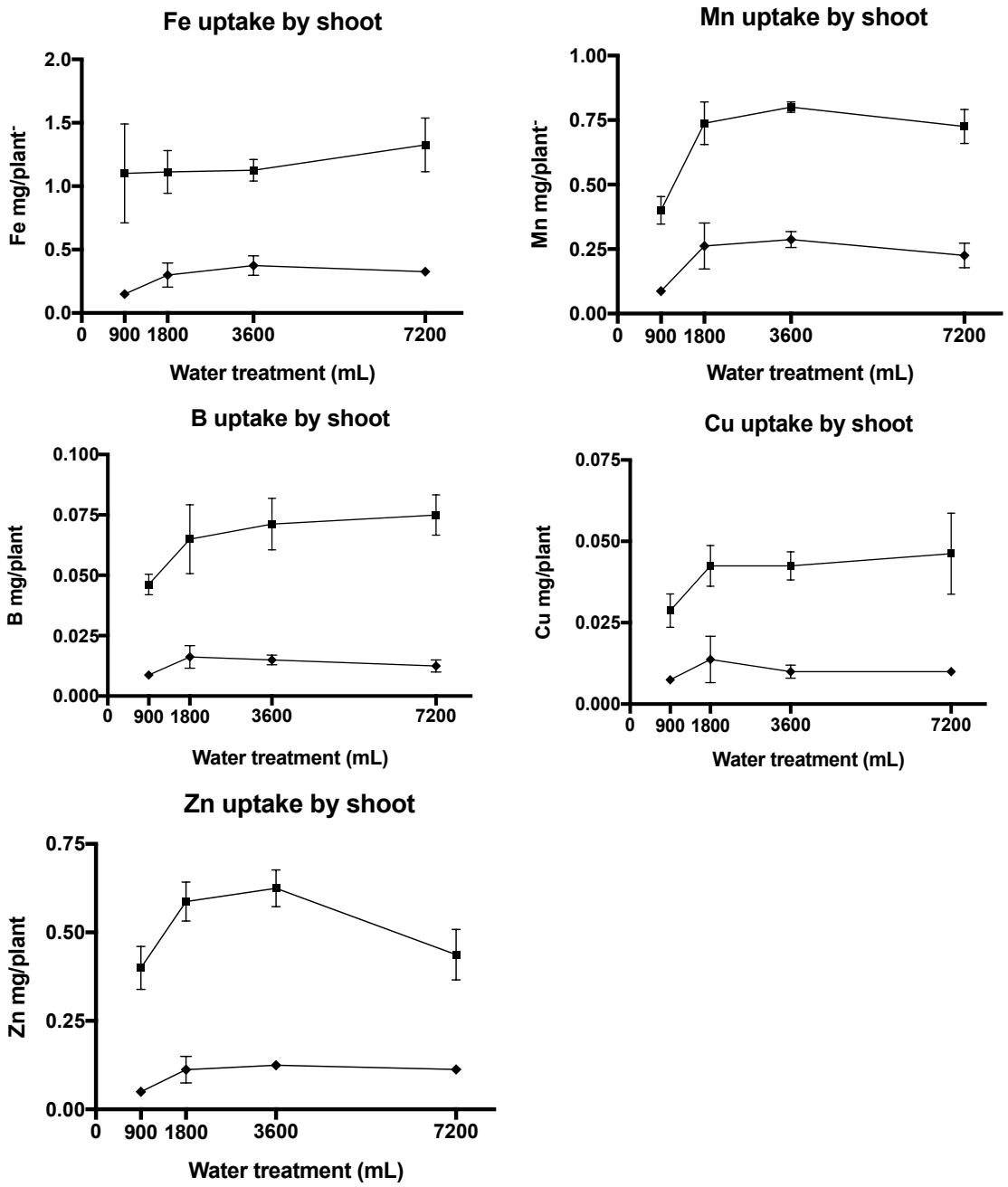

Figure 3. Average of micronutrients Fe, Mn, B, Cu, and Zn uptake (mg/plant) by shoots of corn seedlings grown in Guam cobbly clay soil, either inoculated (■) or not inoculated (♦) with *Glomus aggregatum* and provided one of four volumes of water: W1=7200 mL, W2=3600 mL, W3=1800 mL, and W4=900 mL during the 3-week experiment. Crossbars represent standard deviations of means of four replications.

Table 1. Chemical characteristics of Guam cobbly clay soil used in the experiment.

Parameter	Unit	Value
pH		7.0
Organic Matter	g kg^{-1}	6.4
P	mg kg^{-1}	25
K	mg kg^{-1}	56
Ca	mg kg^{-1}	5678
Mg	mg kg^{-1}	123
Mn	mg kg^{-1}	5.6
Fe	mg kg^{-1}	63
Zn	mg kg^{-1}	0.9
Cu	mg kg^{-1}	0.7

Table 2. Analysis of variance for shoot biomass, root biomass, leaf width, and SPAD chlorophyll reading of corn seedlings. Numbers are P values with F statistics in parentheses. Plants were grown in Guam cobbly clay soil, inoculated or not inoculated with *Glomus aggregatum*, and provided one of four water treatments.

		Growth parameters			
Source	***df***	Shoot biomass (g/plant)	Root biomass (g/plant)	Leaf width (cm)	SPAD chlorophyll reading
Water (W)*	3	<0.01 (12.52)	<0.01 (11.73)	<0.01 (10.19)	0.62 (3.77)
Inoculation (I)	1	<0.001 (406.63)	<0.001 (272.41)	<0.001 (195.20)	<0.001 (234.03)
W × I	3	<0.01 (6.06)	<0.05 (4.09)	0.44 (0.97)	<0.01 (1.68)

*Water treatment (W) was tested against main-plot error while both inoculation (I) and interaction (W × I) were tested against the sub-plot error.

Table 3. Analysis of variance for macronutrient (N, P, K, Mg and Ca) uptake by shoots of corn seedlings. Numbers are P values with F statistics in parentheses. Plants were grown in Guam cobbly clay soil, inoculated or not inoculated with *Glomus aggregatum*, and provided one of four levels of water.

		Macronutrients				
Source	***df***	N	P	K	Mg	Ca
Water (W)*	3	<0.05 (4.19)	<0.05 (6.06)	<0.01 (6.23)	<0.01 (8.03)	<0.01 (6.75)
Inoculation (I)	1	<0.001 (159.51)	<0.001 (437.94)	<0.001 (816.51)	<0.001 (364.49)	<0.001 (116.39)
W × I	3	0.27 (2.30)	0.07 (2.70)	0.37 (0.95)	0.08 (2.44)	0.18 (2.27)

*Water treatment (W) was tested against main-plot error while both inoculation (I) and interaction (W × I) were tested against the sub-plot error.

Table 4. Analysis of variance for micronutrient (Fe, Mn, B, Cu, and Zn) uptake by shoots of corn seedlings. Numbers are P values with F statistics in parentheses. Plants were grown in Guam cobbly clay soil, inoculated or not inoculated with *Glomus aggregatum* and provided one of four levels of water.

		Micronutrients				
Source	***df***	Fe	Mn	B	Cu	Zn
Water (W) *	3	0.75 (0.36)	<0.01 (9.57)	0.22 (1.67)	0.12 (0.89)	<0.05 (7.23)
Inoculation (I)	1	<0.001 (31.16)	<0.001 (169.46)	<0.001 (69.93)	<0.001 (38.63)	<0.001 (436.02)
W × I	3	0.88 (0.37)	0.18 (1.83)	0.65 (0.62)	0.59 (0.91)	0.24 (1.29)

*Water treatment (W) was tested against main-plot error while both inoculation (I) and interaction (W × I) were tested against the sub-plot error.

Table 5. Correlation analysis of plant growth parameters (shoot biomass, root biomass, leaf width, SPAD chlorophyll reading) and uptake of macro- and microelements in leaf tissues.

Growth parameter	Macroelements (mg/plant)					Microelements (mg/plant)				
	N	P	K	Mg	Ca	Fe	Mn	B	Cu	Zn
Shoot biomass (g)	0.8873	0.9521	0.9482	0.9746	0.9551	0.7587	0.9439	0.8959	0.8534	0.9321
Root biomass (g)	0.8258	0.9079	0.9150	0.9452	0.9497	0.7199	0.9112	0.9358	0.7390	0.8730
Leaf width (cm)	0.8569	0.9067	0.9394	0.9251	0.8954	0.8011	0.8957	0.8779	0.8478	0.9030
Chlorophyll (SPAD)	0.852	0.8211	0.8664	0.8104	0.7893	0.7284	0.7616	0.7377	0.7400	0.8377

All correlations are highly significant at $P \leq 0.001$, Pearson correlation.

Discussion

Inoculation with *Glomus aggregatum* improved development of corn seedlings grown in calcareous Guam cobbly clay soil in pot culture. The AM fungus improved shoot and root biomass, leaf width and leaf chlorophyll content under all water treatments. In earlier studies, plant growth improved due to AM colonization in agronomic plants including field corn (*Zea mays* L.) (Islam & Ayanaba 1981), cowpea [*Vigna unguiculata* (L.) Walp.], forage legumes and grasses (Saif 1987). Greater mineral assimilation in AM plants found in our study was also shown in a study with *Sesbania grandiflora* where AM improved plant nutrient uptake of N and P, and growth (Habte & Aziz 1985). It has been reported that assimilation of N, P, K, Ca, and Mg in tropical forage species was improved with AM colonization (Saif 1987). AM colonization increased uptake of N and P in cowpea and increased growth and P uptake in maize (Islam & Ayanaba 1981). Similarly, mycorrhizal wheat plants (*Triticum aestivum* L.) had higher P, Fe, Zn, and Cu contents at tillering stage (Al-Karaki et al. 2004). In a pot culture of horticulture plant, snapdragon (*Antirhinum majus*), mycorrhizal plants had higher nutrient contents (N, P, K, Mg, and Ca) in leaves than nonmycorrhizal plants at all water stress level (Asrar et al. 2012). Positive correlations between nutrient uptake and plant growth suggested that improved uptake of nutrients is a result of AM colonization (Table 5).

Mineral uptake of N, K, Mg, Ca, Mn, and Zn due to treatment with 7200 mL/pot was generally less than for plants treated with 3600 and 1800 mL/pot, indicating that the highest irrigation treatment may have resulted in the limited nutrient absorption by over-watering and leaching of plant nutrients (Fig. 3 and 4). Guam cobbly clay soil used in this experiment is characterized as having a low water-holding capacity and it is known that there may have been N-leaching as a result of excess water limiting amounts of N available to plants (Young, 1988). At water stress plants had less vigorous growth, implying that the amount of water applied in this treatment was not sufficient to achieve maximum plant growth with limited mineral uptakes. The growth increase of AM plants compared to noninoculated plants grown with less water supported earlier research on mycorrhizae where AM fungi improved drought tolerance of many plants. Water-stressed wheat plants inoculated with AM fungi had enhanced leaf area and leaf, stem, and root weights (Ellis et al. 1985). Al-Karaki et al. (2004) also demonstrated that mycorrhizal inoculation reduced effects of water stress of field grown wheat having greater growth and yields. Sylvia et al. (1993) found that mycorrhizal inoculation of field grown maize resulted in increased growth and grain yield which was increased linearly as irrigation levels increased. A study in rose plants (*Rosa hybrida* L., cv. New Dawn) indicated that AM promoted a more intense electron flow and higher photosynthetic activities under drought stress (Pinior et al. 2005). A physiological mechanism of intensified photosynthetic activity in drought-stressed AM corn seedlings might support greener foliage of plants.

Although some studies indicated that mycorrhizal dependency of a given plant could be altered by irrigation level (Asrar et al. 2012; Azcón & Ocampo 1981; Menge et al. 1978), the present study did not show the influence of irrigation regime on mycorrhizal dependency at the early growth stage of corn seedling, probably due to high coefficient of variation of data with small number of replications and the short period of experiment not allowing seedlings to grow further. In a meta-analysis study of previously published data and findings to establish a statistical quantification of the effect of mycorrhizae on water-stress plants, Jayne & Quigley (2014) supported earlier reports that mycorrhizal plants showed better growth response to water deficit than non-mycorrhizal plants did.

Synergistic effects of different mycorrhizae and other soil microorganisms were studied in advancement of sustainable agriculture (Ghorchiani et al. 2018), where they found that root colonization of *Funneliformis mosseae* (=*Glomus mosseae*) and bacterium *Pseudomonas fluorescens* made maize plants more tolerant to water stress in the field experiment. Sharma et al. (2017) studied

that combination and of two mycorrhizal fungi, *Glomus mosseae* and *Acaulospora laevis* and potassium fertilizers and found that combination of two fungi had the highest concentration of total chlorophyll in leaf of *Vigna mungo* under water stress at the vegetative stage. *G. mosseae* was the best single treatment for water stress alleviation and growth improvement. Mena-Violante et al. demonstrated that combination of mycorrhizal fungus species inoculation affected quality of chile ancho fruits. In their study, three mycorrhizal treatments were tested: *G. fasciculatum* alone; a species consortium from the tropical forest in Mexico including *G. constrictum*, *G. geosporum*, *G. fasciculatum*, and *G. totuosum*; a species consortium from the desert in Mexico containing *G. aggregatum*, *G. deserticola*, *G. geosporum*, *G. microaggregatum* and *Sclerocystis coremioides*. Under water-stress, two consortium treatments improved the fresh fruit weights. It was noted that effective host-plant-mycorrhizal combinations need to be determined for practical use in the field to advance use of mycorrhizal fungi as 'bio-fertilizer' in sustainable agriculture.

In conclusion, the study demonstrated that corn seedlings inoculated with *G. aggregatum,* an isolate of mycorrhizae from Hawaii, grown in a tropical calcareous soil had greater uptake of mineral nutrients and biomass than non-mycorrhizal plants under different water treatments. The increase of shoot and root biomass of mycorrhizal plants was directly correlated with the increase of nutrient uptake. Mycorrhizal corn seedlings thrive despite water deficits and sustain vigorous vegetative growth in pot culture.

Guam Cobbly Clay Loam is not the best-suited soil for agricultural crop production (Young, 1988). The cultivating of this soil for crop production is somewhat unavoidable since it is most abundant soil in the northern part of Guam (Demeterio, et al, 1986). In this experiment, the positive effects of AMF colonization of roots of corn clearly enhanced plant growth and development. Similar studies are recommended to further evaluate AMF with other important agricultural crops, including evaluation of other parameters such as yield. More studies with mycorrhizae in agriculture should be further studied on Guam to increase the knowledge and understanding of the benefits of mycorrhizal associations to sustainable agriculture, and its potential to improve crop development, and reduce negative economic and environmental impacts that may derive from conventional agricultural practices.

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