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ORIGINAL ARTICLE

Effects of arbuscular mycorrhizal fungal inoculations on the growth and polyphenol levels of garden leek (*Allium porrum*)

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Abstract

Arbuscular mycorrizal (AM) fungi may enhance plant growth and polyphenol production, however, there have been limited studies on the relationships between root colonization of different fungal species and polyphenol production on cultivated *Allium porrum* (garden leek). The effects of inoculation of AM fungi spores from *Rhizophagus intraradices*, *Giga -spora margarita*, *Glomus geosporum, Paraglomus occultum*, *Claroideoglomus claroideum*, and *Glomus* species on colonization of garden leek roots and symbiotic changes in polyphenol production and plant growth were evaluated in greenhouse experiments. There were significant differences ($p < 0.05$) in colonization of leek roots by AM fungi species. The greatest level of root colonization was recorded on plants inoculated with *R. intraradices* (73%) and the lowest level on *C. claroideum* (3.2%). Significant differences ($p < 0.05$) in plant height were recorded between AM inoculated plants and the controls. Polyphenol levels differed significantly ($p < 0.05$) between garden leek plants inoculated with AM fungi and the non-inoculated controls. The percentage increases in polyphenol (a derivative of kaempferol) on garden leeks inoculated with *G. geosporum* relative to the untreated controls ranged from 28 to 1123%. Due to symbiosis with different AM species, other polyphenols decreased in some instances (negative values) and increased in others for values of up to 590%. Results showed that AM fungi species exhibited remarkable differences in polyphenol levels in garden leeks. The high polyphenol production by garden leek plants inoculated with *G. geosporum,* and *Glomus* species could be exploited for enhanced resistance of garden leeks to insects and diseases. This research highlights an understudied area, notably the relationships between AM fungal inoculations, root colonizations and polyphenol production in garden leeks. The findings can be utilized to improve pest resistance and the quality of garden leek plants.

Key words: *Allium porrum*, arbuscular mycorrhizal fungi, plant height, polyphenol levels, root colonization, shoot and root weight

Introduction

A variety of different species of arbuscular mycorrhizal (AM) fungi are known to occur naturally in diverse soils (Franke-Snyder *et al*. 2001; Smith and Read 2008; Don-Rodriguez *et al*. 2013). AM symbiosis with plants often increases water and nutrient uptake in different plant species, especially under low water availability, and under deficiencies of less mobile nutrients in the soil, such as phosphorus, zinc, and copper. Therefore, various management strategies have been developed to improve vegetable production by utilizing mycorrhizal symbiosis (Sorensen *et al*. 2003). The increased availability of water and nutrients in mycorrhizal plants is

generally attributed to the extension of the root systems through protruding hyphae of the AM fungi in soils.

In the case of garden leek plants, it has been reported that increased nutrient uptake from AM symbiosis is particularly important due to their shallow root system and limited root depth (Finlay 2004). AM mycorrhizal colonization of plant roots has been shown to induce tolerance to environmental stress. Therefore, the growth of AM fungal hyphae from the roots of garden leeks could have positive effects on plant growth and metabolism due to increased water and nutrient uptake (Nelsen and Safir 1982; Ruiz-Lozano *et al.* 1995). For example, AM mycorrhizal symbioses in garden leek plants have been shown to increase plant growth under low phosphorus with various levels of flavonoid glycosides (Malik *et al.* 2015). AM fungal inoculation of herb plants, such as marjoram, lemon balm and marigold, showed that marjoram plants had the highest level of fungal colonization (82%), while lemon had 62% and marigold 16% (Engel *et al.* 2016). AM fungal inoculations also increased the biomass of marjoram in this study. In evaluating the association of AM fungal inoculations with *Dioscorea* spp. (yam), it was shown that inoculated treatment of yams with *G. etunicatum* increased tuber weights in five yam species by values of 20 to 56% (Lu *et al.* 2015).

In addition to the effect of symbiotic associations of AM fungi on plant growth, the relationships between AM mycorrhizal colonization of roots could enhance resistance of plants to insects and diseases (Sundaresan *et al.* 1993). In previous studies, AM symbiosis in roots of vegetable plants has been shown to enhance resistance of roots to diseases, nematodes, and insects (Baum *et al.* 2015). Thus, inoculations of plants with AM fungi were noted to improve plant health, which was attributed to the production of polyphenol and flavonoid compounds. Flavonoids, flavonoid glycosides, and polyphenols with similar beneficial attributes to plant health have been previously documented in artichoke leaves and flowers (Ceccarelli *et al.* 2010). In addition, increased resistance to *Phytophthora* and other diseases have been noted in plants with AM symbioses (Liu *et al.* 2007; Pozo and Azcon-Aguilar 2007; Watanaiojanaporn *et al.* 2011). The high resistance to diseases in mycorrhizal plants is linked to increased levels of polyphenols, particularly phytoalexins and phytoflavonoids (Ceccarelli *et al.* 2010). Additionally, environmental stimuli such as feeding activities of insects or pathogens may also stimulate the production of polyphenols as resistance mechanisms of plants to insects or pests (Sundaresan *et al.* 1993). In many field and potted plants in which root crops were inoculated with AM fungi, positive effects of AM fungi associations on plant growth and polyphenol levels (secondary metabolite) were documented (Douds and Reider 2003).

The production of secondary metabolites by agricultural plants when inoculated with AM fungi under different stress conditions has been documented (Engel *et al.* 2016), however, the relationships between inoculations of different AM species to cultivated garden leek plant colonization and polyphenol levels have received comparatively little attention. Therefore, the objective of this research was to determine the effect of inoculations of cultivated garden leek plants with different AM fungal species on plant growth (plant height, shoot and root weight), root colonization, and changes in polyphenol levels.

Materials and Methods

Plant growth

Seeds of garden leek (*Allium porrum* L.), cultivar 'Colossal' were purchased from Johnny Seeds Company (Winslow, ME). Approximately, 150 seeds were planted in a 15.3 cm diameter pot filled with vermiculite moistened with adequate water. The seeds were covered with a 1.3 cm layer of additional moist vermiculite and pots were placed in a growth chamber for seed germination. The growth chamber was maintained at 14 h photoperiod; daytime temperature was set at 25°C and nighttime temperature at 18°C. Twenty-eight days after planting, the seedlings were transferred from pots to 66 ml plastic cones, 2.5 cm diameter by 16 cm depth (Stuewe & Sons, Tangent, OR). The cones were filled with standard potting mix with vermiculite, acid washed sand, and washed surface sieved farm soil at ratios of 1.5 : 1.5 : 1. The mixture was autoclaved (for 60 min at 121°C) and cooled prior to use. A single seedling was transferred into each cone. Each potted plant was an experimental unit and eight treatments (seven AM fungal inoculation treatments plus one un-inoculated control plant) × 12 leek plants for each treatment, resulted in a total of 96 plants used in the experiment. The experiment was repeated two times under the same experimental conditions. The non-inoculated plants were designated as the untreated controls. Treatments (potted plants) were arranged in a completely randomized design with 12 replications.

Inoculations of plants with different species of mycorrhizae fungi

The AM fungal isolates were obtained from the laboratory collection of Dr. David Douds (USDA--ARS, ERRC, Wyndmoor, PA, USA). The *R. intradices*, *G. rosea* and *G. margarita* isolates had accession numbers of DAOM 181602, DAOM 194757 and PA201, respectively. The isolates of *G. geosporum, P. occultum, C. claroideum* and *Glomus* sp. were iso-

lated from Rodale Institute Farming Systems trial in Kuztown, PA (Franke-Synder *et al.* 2001). The AM fungal spores for this study were collected from approximately 6-month-old Bahia grass cultures (grown in 66 ml plastic cones, 2.5 cm diameter by 16 cm depth). The pots were filled with standard potting mix as described above (Douds 2009). AM fungal spores were extracted by the wet sieving and sucrose centrifugation method (Jenkins 1964). The number of spores used for inoculation of garden leek varied depending on the type of mycorrhizal species, but was based on required numbers adequate for plant colonization as determined in previous studies (Franke-Snyder *et al.* 2001; Douds 2009; Olanya *et al.* 2016).

The spore numbers used for various treatments were as follows: 1 – *R. intraradices* (~350 spores); 2 – *G. rosea* (~100 spores); 3 – *G. margarita* (~75 spores); 4 – *G. geosporum* (~150 spores); 5 – *P. occultum* (~500 spores); 6 – *C. claroideum* (~150 spores); 7 – *Glomus* species (~150 spores); and the 8 – non-AM control garden leek treatment had no spore application (Douds 2002; Douds and Reider 2003). The AM fungal spores were added in 2 ml suspension in water to each replicate of a treatment (Douds 2009). In the treated and control plants, 10 ml of water was added daily to maintain adequate moisture levels necessary for plant growth. Nutrient solution was added to plants at 5 day intervals until harvest at 8 weeks after transplanting (Hoagland and Arnon 1939). The plants were kept in the growth chamber for the duration of the experiment and the growth chamber conditions were maintained at a 14 h photoperiod with a day temperature of 25°C and night temperature of 18°C (Olanya *et al.* 2015).

Shoot and root weights of garden leeks and mycorrhizae colonization of plant roots

At harvest, each plant was cut below the base of the bulb to separate the shoot (top) from the root. Root and shoot length and fresh weights of each treatment and replicated plant were individually measured. The root samples were divided into two portions in which half was oven dried and the other half stained. The fresh roots were stained with trypan blue solution (ThermoFisher Scientific, USA) for 30 min (Phillips and Hayman 1970) to determine colonization of roots by mycorrhizae, using the gridline intersect method (McGonigle *et al.* 1990). Samples of root and shoot were dried in an oven at 80°C for 1 week. The dry to fresh weight ratios were used to determine the theoretical dry weight of the stained samples based on their fresh weight.

Polyphenol extraction from the shoots

Samples for shoots of replicated plants were used for extraction of polyphenols. Shoot samples in each treatment were frozen and subsequently pulverized in liquid nitrogen. All harvested shoots were stored at –80°C until used. The polyphenols and flavonoids were extracted in 80% methanol using the methods previously described (Malik *et al.* 2015). The extracts in methanol were spun in a refrigerated eppendorf micro-centrifuge at 25,000 rpm for 30 min. The extracts were stored at –80°C until utilized for HPLC--MS analysis.

Chromatographic analysis

The chromatographic separation of the methanol extract was performed with a Nano-Acquity (Waters, Milford, MA) ultrahigh performance liquid chromatographer (UHPLC) equipped with an Acquity UPLC BEH C18, 1.7 μm (1 × 100 mm) column (Waters) maintained at 40°C and running at 60 μl · min^{-1}. The UHPLC--UV chromatogram was obtained by attaching it to the UHPLC instrument an Acquity TUV detector (Waters) set to scan at 280 nm. The solvent gradient started with water to acetonitrile 95 : 5 (0.1% formic acid) for 2 min and ramped linearly to water-acetonitrile 60 : 40 (0.1% formic acid) at a final time of 14 min, maintained at that solvent composition for 2 min and followed with column wash of water-acetonitrile 20 : 80 (0.1% formic acid) and returning to the initial condition at 20 min. A 10 min stabilization time was allowed between injections. Samples for the treated and control experiments were combined by mixing 10 μl of each of them, and 10 μl of a kaempferol solution (internal standard, 5 μg · ml^{-1}). The solvent was removed under nitrogen, followed by resuspension in 50 μl of water-methanol 90 : 10. Three injections of 4 μl were made for each sample for determination of the concentration change according to the peak high determined by MassLynx v.4.1 software. The same chromatographic conditions were used for the mass spectrometry analysis (Malik *et al.* 2005; Perez-Gregorio *et al.* 2010; Calderon-Montano *et al.* 2011).

Mass spectrometry analysis

The mass spectrometry analysis was accomplished by connecting the effluent of the UHPLC instrument to a Synapt G1 quadruple-time of flight mass spectrometer (Waters) operating in the W mode (resolving power of 18,000) and with an electro-spray ionization (ESI) probe operated in the positive mode and controlled by MassLynx v.4.1 software (Waters). The instrument parameters were 2.9 kV capillary voltage, 4 V extractor voltage, 300 l · h^{-1} desolvation gas (N_2) flow, and 120°C and 150°C source and desolvation temperatures, respectively (Calderon-Montano *et al.* 2011). The MS/MS of the protonated precursor ions $[M + H]^+$ were obtained by collision induced dissociation with argon gas at 0.9 ml · min^{-1} with the collision energy ramped

between 10 to 25 eV. The TOF analyzer was calibrated to produce an error below 5 ppm using the exact mass of the product ions of [Glu[1]]-fibrinopeptide B (Waters). The $[M + H]^+$ of the parent ion mass was determined using a raffinose (Waters) as locking mass. For the MS/MS spectra, the $[M + H]^+$ determined as described was used to calibrate the spectra (Calderon-Montano *et al.* 2011).

Data analysis

Data on plant height, dry shoot weight, root weight, and root colonization were compiled for each replicate and treatment. The effect of mycorrhizae colonization on plant height, dry shoot weight, dry root weight and root colonization were analyzed by analysis of variance (Proc GLM) of the Statistical Analysis System (SAS Institute Inc., Version 9.3, Cary, NC). Means and associated standard errors for the above variables were computed by least significance difference (LSD) statistics of Statistical Analysis Systems. The peak heights for various polyphenols and flavonoids at various retention times were subjected to pairwise comparison analysis for differences, or lack of them, between and among treatments (SAS Institute Inc., Version 9.3, Cary, NC).

Results

AM fungi inoculations of garden leek plants at seedling stage resulted in significant ($p < 0.05$) differences in the plant height of garden leeks when compared to the non-inoculated (control) plants (Table 1). The average plant height generally ranged from 17.8 cm in leeks inoculated with *G. geosporum* to 25 cm in plants inoculated with *R. intraradices*. The mean height of the un-inoculated (control) plants was 18 cm (Table 1). The height of garden leek plants inoculated with *G. geosporum*, *C. claroideum*, and *Glomus* sp. did not differ significantly ($p > 0.05$) from the non-mycorrhizae control garden leeks.

Inoculation of garden leek plants with *R. intraradices* and *G. rosea* resulted in increased dry weights of shoots compared to un-inoculated controls (Table 1). Thus, the dry shoot weights of garden leek plants inoculated with *R. intraradices* and *G. rosea* were more than 50% greater than leek plants inoculated with other mycorrhizae species, as well as the control plants (Table 1). Similarly, the dry root weights of garden leek plants ranged from 0.13 to 0.25 g. Plants inoculated with *G. rosea* and *P. occulatum* had significantly ($p < 0.05$) higher root weights than the non-inoculated controls (Table 1).

Although different spore numbers were used in the inoculation of leek plants based on spore density required to achieve adequate colonization, there were no significant ($p > 0.05$) differences in the correlation coefficient between mycorrhizae spore density used in inoculations, shoot and root growth as well as root colonization (%). The root colonization of garden leek plants differed significantly ($p < 0.05$) among the AM fungal species. Root colonization of all inoculated treatments also differed significantly ($p < 0.05$) from the non-mycorrhizal control plants (Table 1). The percentage of root colonization in garden leeks ranged from 0 (no colonization in non-mycorrhizae control) to 72% in plants inoculated with *R. intraradices* (Table 1).

The changes in polyphenol levels in shoots of leek plants inoculated with AM fungal species, and expres-

Table 1. The physiological characteristics and root colonization of garden leeks inoculated with different arbuscular mycorrhizal (AM) fungal species and garden leeks grown without AM fungus (control)

Treatment[a]	Plant height[b] [cm]	Dry shoot weight[c] [g]	Dry root weight [g]	Root colonization[d] [%]
Rhizophagus intraradices	25.0 ± 2.1 a	0.23 ± 0.03 a	0.15 ± 0.23 b	72.5 ± 6.1 a
Gigaspora rosea	24.8 ± 1.3 a	0.25 ± 0.2 a	0.25 ± 0.02 a	65.1 ± 4.5 ab
Gigaspora margarita	20.7 ± 0.8 b	0.13 ± 0.01 b	0.18 ± 0.03	57.3 ± 5.0 b
Glomus geosporum	17.8 ± 0.6 c	0.12 ±0.01 b	0.20 ± 0.02 ab	20.0 ± 10.3 d
Paraglomus occultum	23.0 ± 1.1 b	0.19 ± 0.01 b	0.22 ± 0.01 ab	34.3 ± 6.9 c
Claroideoglomus claroideum	18.4 ± 0.8 c	0.10 ± 0.01 b	0.15 ± 0.02 b	3.2 ± 1.7 e
Glomus sp.	18.6 ± 0.9 c	0.10 ± 0.00 b	0.13 ± 0.02 b	7.1 ± 4.7 e
Non-mycorrhizal control	18.0 ± 1.0 c	0.13 ± 0.00 b	0.19 ± 0.02 b	0 ± 0.1 e

[a]garden leeks were inoculated with different mycorrhizae species, ranging from 75 to 500 spores per 2 ml, [b]plant height was quantified at harvest. Data represent the means and associated standard errors from replicated samples. Means with the same letters are not significantly different ($p > 0.05$), [c]dry shoot weight was quantified at harvest. Data represents the means and associated standard errors from replicated samples. Means with the same letters are not significantly different ($p > 0.05$), [d]root colonization by different AM fungal species was quantified at harvest after staining samples with tryphan blue. Data represent the means and associated standard errors from replicated samples. Means with the same letters are not significantly different ($p > 0.05$)

Table 2. Effect of inoculation of garden leek with different AM fungal species on changes (%) in polyphenol levels relative to the control (non-mycorrhizal garden leek). Data was compiled by retention time of HPLC profile of garden leek (*Allium porrum*)

Peak numbers	Retention time	*Rhizophagus intraradices*	*Gigaspora rosea*	*Gigaspora margarita*	*Glomus geosporum*	*Paraglomus occultum*	*Claroideoglomus claroideum*	*Glomus* sp.
1	6.76	….[a]	….[a]	….[a]	….[a]	….[a]	….[a]	….[a]
2	6.89	535	657	310	1123	––[b]	345	783
3	7.90	_[c]	….[a]	….[a]	….[a]	_[c]	….[a]	….[a]
4	8.09	_[c]	….[a]	….[a]	….[a]	….[a]	….[a]	….[a]
5	8.40	178	235	106	590	182	462	506
6	8.53	68	213	117	335	126	320	348
7	9.77	–30	27	–14	28	16	97	60
8	10.01	3	14	–12	63	–40	32	39
9	10.85	–50	–55	–26	–37	–43	–11	6
10	11.00	–28	–36	–53	–24	–4	57	7
11	11.15	–67	–42	–27	–33	–48	79	16
12	11.58	48	–29	–32	–29	–22	49	27
13	11.93	–7	–32	–23	NS	–30	99	30

[a]indicates that the mycorrhizal sample produced a polyphenol peak, but no peak in the non-mycorrhizal control sample. The polyphenol data with positive values (indicate increases in polyphenol levels) and data with negative numbers (indicate decreases in polyphenol levels) and were significant ($p < 0.05$) at different retention times, [b]indicates that the mycorrhizal sample did not produce a polyphenol peak in treatments, while the peak was present in the non-mycorrhizal control sample. The polyphenol data with positive values (indicate increases in polyphenol levels) and data with negative numbers (indicate decreases in polyphenol levels) and were significant ($p < 0.05$) at different retention times, [c]indicates that the polyphenol peak was not present in either mycorrhizal nor the non-mycorrhizal samples.

Table 3. Polyphenol derivatives associated with the peak numbers in the chromatogram data obtained from garden leeks inoculated with different AM fungal species

Peak numbers	Retention time[a]	Aglycone	$[M+H]^+_{Cal}$ [b]	$[M+H]^+_{Exp}$ [b]	Pent[d]	Hex[d]	HexA[d]	Malonyl	Ferul.[d]	Coumaroyl
1	6.76	Quercetin	875.2088	875.2090	… [e]	3	…	1	…	…
2	6.89	Kaempferol	1021.2670	1021.2670	…	4	…	1	…	…
3	7.90	Quercetin	1127.2933	1127.2940	…	4	1	…	…	…
4	8.09	Kaempferol	1359.3669	1359.3622	…	5	…	1	1	…
5	8.40	Kaempferol	1197.3140	1197.3163	…	4	…	1	1	…
6	8.53	Kaempferol	1167.3015	1167.3040	…	4	…	1	…	1
7	9.77	Kaempferol	1229.3614	1229.3624	1	5	…	…	…	…
8	10.01	Quercetin	1245.3563	1245.3578	1	5	…	…	…	…
9	10.85	Apigenin	1243.3770	1243.5848	…	6	…	…	…	…
10	11.00	Apigenin	1213.3665	1213.5814	1	5	…	…	…	…
11	11.15	Quercetin	889.1881	889.1840	…	2	1	1	…	…
12	11.68	Unknown	[c]N/D	1197.5889	1	5	…	…	…	…
13	11.93	Unknown	[c]N/D	1033.5569	1	3	…	…	…	…

[a]retention time in minutes, [b]$[M+H]^+_{cal}$ is the calculated exact mass of the protonated molecule and $[M+H]^+_{Exp}$ is the experimental mass, [c]N/D – not determined, [d]mass from the Q-TOF analysis of the polyphenols: Pen – pentose, Hex – hexose, HexA – hexuronic acid. The values refer to the number of peaks detected, [e]no peaks were detected in the chromatogram

sed as percent of the un-inoculated control plants, are given in Table 2. The levels of polyphenol indicated in peak 2 (Kaempferol malonyl derivative) increased tremendously, by 1123%, in plants inoculated with *G. geosporum* compared to controls (Table 2). This increase exceeded any other polyphenol in garden leeks inoculated with any AM fungi species used in this experiment (Table 2). The polyphenol from peak 3, Quercitin hexuronic acid (Table 3) was not present in plants inoculated with *R. intraradices* and *P. occultum* as well as in the un-inoculated control plants, but this peak was present when the plants were inoculated with

G. rosea, G. margarita, G. geosporum, C. claroideum, and *Glomus* sp. (Table 2). Peak 4 (Kaempferol derivative) was not present in un-inoculated leeks or when inoculated with *R. intraradices,* however, Kaempferol derivative was detected in leek plants inoculated with other AM fungal species.

Although increases in different polyphenol levels resulted from inoculation of garden leek plants relative to the non-mycorrhizae plants, decreases in levels of some polyphenol species also resulted from inoculation with certain AM species (Table 2). When compared to un-inoculated controls, no decreases in polyphenol species were detected in garden leek plants inoculated with *Glomus* sp. (Table 2). For leek plants inoculated with *C. claroideum*, only one polyphenol derivative (Apigenin derivative with six hexose molecules) decreased (negative values). Overall, the reported increases in polyphenol (positive numbers) and decreases in polyphenol levels (negative numbers) were significantly different ($p < 0.05$) from the un-colonized leek plants (Table 2). For example, at retention peak 2, the greatest percentage increases (1123%) in polyphenol levels were recorded on garden leek plants inoculated with *G. geosporum,* while the least increase for peak 2 (310%) was recorded in garden leek plants inoculated with *G. margarita* (Table 2). Neither of these inoculants showed the highest colonization rate or the highest shoot growth, but documented the largest percent increases in polyphenol production for peaks 2–6. At the same retention peak, garden leek plants inoculated with *P. occultum* did not have any polyphenol peak detected even though a peak was present in the non-mycorrhizal garden leek plant (Table 2). In contrast to the above findings, for example at retention peak 9, all garden leek plants inoculated with AM fungi had significant ($p < 0.05$) decreases in polyphenol levels (indicated by the negative signs) relative to the garden leek plants with no mycorrhizae inoculations, with the exception of plants inoculated with *Glomus* sp.

Discussion

Although many research findings have previously evaluated the beneficial attributes of mycorrhizae interactions with plants as they relate specifically to uptake of nutrients such as phosphorus, nitrogen and water (Bolan 1991), our research focused primarily on the relationships of AM fungal inoculations of garden leek to plant growth and polyphenol production and its implications on plant health. In this research, leek plants inoculated with AM fungal species were treated with the same nutrient solution as the un-inoculated control plants, thus the differences were strictly related to the effects of specific symbiosis between leek plants and the type of AM fungi involved. Increased biomass as measured by increased plant height and increased shoot and root dry weights in combinations with specific AM fungi species probably resulted from increased nutrient uptake as reported previously (Engel *et al*. 2016). In that study, AM fungi inoculation led to 1.5 fold increases in the biomass of marjoram and 1.2 times the number of marigold flowers (Engel *et al*. 2016). In a similar study on AM symbiosis between plants and fungi, leek transplants inoculated with *G. intradices* or other AM fungal species had increased colonization, P and Zn uptake and growth of leek plants (Sorensen *et al*. 2003). In their research, the shoot dry weight of leek plants increased from less than 1 g to more than 6 g at 40 days after transplanting. Therefore, our studies showed consistent increases in shoot length and weight of plants inoculated with AMF.

Our findings that AM fungal inoculations resulted in increases in growth of garden leek plants have been supported by the findings of other researchers. In assessing management strategies for AM fungi in field-grown vegetables, it was shown that increased sustainability of vegetable production was attained by using a previous mycorrhiza crop, pre-established mycorrhizae crops and transplants pre-colonized by mycorrhizae (Sorensen *et al*. 2003). In research conducted on AM fungal inoculations of yam (*Dioscorea* spp.), significant ($p < 0.05$) increases of tuber weight in the range of 20% to 56% in five yam species were recorded (Lu *et al*. 2015). Other researchers have also documented that AM fungal inoculations positively influenced the growth of tuber crops such as cassava, potato, and sweet potato (Don-Rodriguez *et al*. 2013). In this study, we hypothesize that garden leek plants may be similarly influenced by the positive attributes of AM fungal interactions following inoculations, perhaps due to increased water and nutrient uptake.

The differences in root colonization recorded on treatments of leek plants inoculated with different AM fungal species imply that AM fungal species may interact differently with garden leek plants. The various levels of colonization of garden leek plants may have contributed to the growth of garden leek plants, while in other cases, little or no responses were observed. Previous reports have documented various levels of colonization of yam plants based on mycorrhizae symbiosis and consequently different tuber weights (Lu *et al*. 2015).

It is not clearly understood why the polyphenol levels from the garden leek plants inoculated with two AM fungal species were totally distinct in this study, and yet the colonization levels in garden leeks from the two inoculation treatments were 20% for *G. geosporum* and 57% for *G. margarita*. However, high polyphenol production from low colonization (20%) or moderate colonization (57%) of plant roots may be a useful trait to select for in garden leek improvement,

since progenies derived from such garden leek plants may exhibit high levels of polyphenols and possible resistance to many insects and diseases. The differential response of such plants could also be subsequently used as sources of resistance (parental lines) of garden leeks with elevated polyphenol levels. Increases in polyphenol and other secondary metabolites have often been associated with resistance to various plant diseases (Liu *et al*. 2007). In a research study on AMF colonization of yams and tuber weight, it was noted that polyphenol derivatives varied for different species of yam (*Dioscorea* sp.) such as tanning 1 and tanning 2 following AM fungal inoculation of yams (Lu *et al*. 2015). Similarly, major polyphenols were detected when AMF effects on growth and colonization of economically important herbs were evaluated (Engel *et al*. 2016). In research on the diversity of communities of AM fungi in conventional and low-input agricultural systems, the percentage of *G. geosporum* colonization of maize (*Zea mays* L.) and soybeans (*Glycine max* L.) was significantly ($p < 0.05$) lower than that of other AM fungi in all systems evaluated in this study, showing variability in the colonization (Franke-Synder *et al*. 2001).

The increases and decreases in polyphenol levels in garden leek plants inoculated with different AM fungal species demonstrated that mycorrhizae symbiosis with garden leeks may produce various positive and negative results. Similarly, decreases in polyphenol levels (negative numbers) indicate that polyphenol levels may also be significantly ($p < 0.05$) reduced as a consequence of certain AM specific symbiotic relationships. In our research, at least three novel polyphenol derivatives such as: quercitin (a derivative of three hexose and malonic acid), quercitin (a derivative of four hexose and hexuronic), and kaempferol (derived from five hexoses), one malonic acid and one ferulic acid were observed in garden leak plants inoculated with AM fungal species and were not observed in the un-inoculated leak or control plants (Table 3). Similarly, different levels of several polyphenols, which are sugar derivatives of three aglycones such as quercetin, kaempferol, and apigenin, were also detected.

The largest percent increases in polyphenol production as documented in peaks 2–6 could be useful findings since this sort of trait (high polyphenol production) implies resistance to insects and diseases and could be selected for garden leek plants. The presence of polyphenol levels in plants have been shown to impede colonization and infection of plants by plant pathogens (Sundaresan *et al*. 1993; Liu *et al*. 2007; Perez-Gregorio *et al*. 2010). Similarly, selection of AM fungi for growth promotion in citrus was shown to enhance the suppression of *Phytophthora* pathogen in citrus (Watanarojanaporn *et al*. 2011).

Many polyphenol derivatives or compounds such as hexose, hexuronic acid, ferul, malonyl and coumaroyl were also shown to be associated with various aglycones, resulting from AM fungal symbiosis (Smith and Read 2008). AM fungi inoculations were shown to have resulted in increased polyphenol profiles of marjoram, lemon balm, and marigold plants (Engel *et al*. 2016). Therefore, based on mass spectrometry data, the structures derived from our research are similar to compounds previously reported in other published literature (Yao *et al*. 2004).

Although the polyphenol derivatives at various retention times were prepared from extractions of shoots of garden leek plants only, other than polyphenol derivatives aglycone (quercetin, kaempferol, and apigenin), hexuronic acid was detected in peaks of 1 to 13, indicating that they are important structural components of polyphenol compounds detected. In this study, hexose, pentose, malonyl, ferul, and coumaryl were also detected at low frequencies indicating that they are important structural components of the polyphenols of AM fungal symbiosis with garden leek plants.

Conclusions

Significant ($p < 0.05$) root colonization, plant height and polyphenol production were detected in garden leek plants inoculated with spores of AM fungi species. Root colonization ranged from 3 to 70%, and the greatest root colonization was recorded on *R. intraradices* while the lowest level was on Glomus sp. The mycorrhizal symbiosis elicited changes in the levels of various polyphenols and percentage increases or decreases differed significantly ($p < 0.05$) between AM fungi inoculated versus non-inoculated garden leek plants. The polyphenols were separated using HPLC and 13 peaks were identified by comparing the retention times and mass spectra of standard compounds. The significant ($p < 0.05$) polyphenol production by garden leek plants inoculated with *G. geosporum, Glomus* species and other AM fungal species could be exploited by enhancing sources of resistance to insects and diseases and diversity of parental lines in garden leek plants. The relationships between AM fungal inoculations and polyphenol production in cultivated garden leeks and other plants is an understudied area and our research findings can be utilized to improve this understanding as well as the potential quality of garden leek plants.

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