

Root colonisation by the arbuscular mycorrhizal fungus *Glomus intraradices* alters the quality of strawberry fruits (*Fragaria × ananassa* Duch.) at different nitrogen levels

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Abstract

BACKGROUND: Arbuscular mycorrhizal fungi (AMF) increase the uptake of minerals from the soil, thus improving the growth of the host plant. Nitrogen (N) is a main mineral element for plant growth, as it is an essential component of numerous plant compounds affecting fruit quality. The availability of N to plants also affects the AMF–plant interaction, which suggests that the quality of fruits could be affected by both factors. The objective of this study was to evaluate the influence of three N treatments (3, 6 and 18 mmol L⁻¹) in combination with inoculation with the AMF *Glomus intraradices* on the quality of strawberry fruits. The effects of each factor and their interaction were analysed.

RESULTS: Nitrogen treatment significantly modified the concentrations of minerals and some phenolic compounds, while mycorrhization significantly affected some colour parameters and the concentrations of most phenolic compounds. Significant differences between fruits of mycorrhizal and non-mycorrhizal plants were found for the majority of phenolic compounds and for some minerals in plants treated with 6 mmol L⁻¹ N. The respective values of fruits of mycorrhizal plants were higher.

CONCLUSION: Nitrogen application modified the effect of mycorrhization on strawberry fruit quality.

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Keywords: mycorrhizal; nitrogen; strawberry; quality; fruit

INTRODUCTION

Most land plants benefit from their interaction with symbiotic soil-borne fungi known as arbuscular mycorrhizal fungi (AMF). In this symbiosis the AMF receives carbon from the plant, while the fungus takes up nutrients with its extraradical mycelium and provides them to the host plant.¹

The uptake of nitrogen (N) by the extraradical mycelium has been shown before and this N is available to the host plant,^{2,3} so the AMF improves the N status of the host.^{4,5} Nevertheless, it also has been reported that the N availability in the soil affects the dynamic of plant–AMF association.^{4,6}

Nitrogen is an essential element for plant growth. Owing to its role in the synthesis of proteins, nucleic acids, various coenzymes and many products of secondary plant metabolism,⁷ it is important for strawberry fruit quality. It has been shown that a leaf N concentration below 19 g kg⁻¹ (deficiency) causes chlorosis of strawberry leaves, thus decreasing the leaf area, fruit size and anthocyanin concentration,⁸ whereas an excess of foliar N (~40 g kg⁻¹) promotes vegetative growth, delays fruit maturation and causes a loss of firmness in fruits, thus reducing quality.^{9,10}

Strawberry quality and consumer preference for strawberry fruits are determined by parameters such as size, firmness, levels of soluble sugars and acid concentration, the last of which affects the aromatic compounds that impart flavour and aroma.^{11,12}

Strawberry fruits possess antioxidant activity owing to their high content of anthocyanins, flavonoids, phenolic acids and other compounds.¹³

Recent data suggest that mycorrhization not only has a positive effect on various plant growth parameters but can also affect the quality of crop products. For example, root colonisation by different AMF enhances the essential oil concentration in a number of plants from different plant families such as oregano (*Origanum vulgare*),¹⁴ basil (*Ocimum basilicum* L.),^{15,16} menthol mint (*Mentha*

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arvensis)¹⁷ and coriander (*Coriandrum sativum* L.).¹⁸ In others plants such as alfalfa (*Medicago sativa* L.),^{19–21} barrel medic (*Medicago truncatula*),²² red clover (*Trifolium pratense*)²³ and soybean (*Glycine max* L.),²⁴ increases in flavonoid levels after mycorrhization have been reported.

There are several reports on strawberry plants concerning inoculation with AMF and its effects on plant growth. It has been shown that AMF root colonisation stimulates plant growth,²⁵ modifies the production of runners,²⁶ enhances photosynthesis²⁷ and increases the number of fruits.²⁸ However, to the best of our knowledge, there are currently no data on how AMF root colonisation in combination with different N levels affects strawberry fruit quality parameters such as colour, soluble sugars, acids, minerals and phenolic compounds.

MATERIALS AND METHODS

The experiment was conducted in a 'shade'-type greenhouse with 30% shade at the Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia, Michoacán, Mexico. Maximum and minimum temperatures in the greenhouse varied between 28 and 32 °C and between 8 and 18 °C respectively.

Plants of the strawberry cultivar 'Aromas' were used that had previously been grown in a sterilised (95 °C water/steam, 40 min) substrate of coconut fibre/perlite (1:3 v/v) under greenhouse conditions. Before the experiment was established, the absence of AMF in the roots was verified by the ink and vinegar technique,²⁹ modifying the duration of immersion in KOH and ink/vinegar solution (7 and 5 min respectively). Before planting, roots were disinfected by submerging them for 20 s in 20 g L⁻¹ sodium hypochlorite solution and rinsing them in water.

The inoculum was prepared with spores of *Glomus intraradices* cultivated in liquid medium (3.5 × 10⁶ spores L⁻¹, 90% viability; Premier Tech Biotechnologies Company, Quebec, Canada), which was diluted with fitagel (Sigma P-8169, Saint Louis, MO, USA) solution at 50 g L⁻¹ to obtain a final concentration of about 5 × 10⁴ spores L⁻¹. The viability of spores was determined according to the method of An and Hendrix.³⁰

Eighteen days after setting up the experiment, each plant received 2 mL of inoculum applied directly to the recently formed roots. One month later, after staining,²⁹ the percentage of root colonisation was determined by the gridline intersect method.³¹

The experiment was organised as a full factorial, completely randomised design with two factors: inoculation (two levels: mycorrhizal and non-mycorrhizal plants) and N concentration in the nutrient solution (three levels: 3, 6 and 18 mmol L⁻¹).

The six treatments were replicated four times, producing 24 experimental units with ten plants each. Every second day, all plants were irrigated up to substrate saturation. Nitrogen was supplied as NO₃⁻ and the cation/anion ratio was kept constant by varying the concentration of SO₄²⁻. When N was below 18 mmol L⁻¹, the cation concentrations were maintained as follows: K⁺, 3; Ca²⁺, 3.5; Mg²⁺, 1.5 mmol L⁻¹. They were increased in the 18 mmol L⁻¹ N treatment: K⁺, 6.5; Ca²⁺, 7.5; Mg²⁺, 3.25 mmol L⁻¹. In all nutrient solutions the concentration of phosphorus (P) was 0.3 mmol L⁻¹. The other nutrients in the solutions were: H₃BO₃, 20; CuSO₄ · 5H₂O, 0.5; Fe-EDTA (Ethylenediaminetetraacetic acid iron (III) sodium salt), 15; MnSO₄ · H₂O, 12; (NH₄)₆Mo₇O₂₄ · 4H₂O, 0.05; ZnSO₄ · 7H₂O, 3 μmol L⁻¹. The pH was adjusted to 5.5 at every application date.

Mature fruits of each experimental unit were collected between 140 and 160 days after setting up the experiment. At sampling

time the fruits were separated into two equal batches. One batch was used for the determination of fruit fresh weight, diameter, length and Brix grade (total solids). The last measurement was done at 25 °C using a refractometer (ATAGO CO., LTD) (N-1α). The other batch was frozen in liquid nitrogen and stored at -20 °C. Prior to chemical and colour analyses, these samples were ground to a fine powder (Retsch MM200 mill, Thomas Scientific, New Jersey, United States) in liquid N₂ and then freeze-dried.

Titrate acidity is a measure of organic acids in a sample and is determined by adding enough alkali of known molarity to the sample to neutralise all acids present. For the measurement of titrate acidity, 0.1 g of freeze-dried fruit was mixed with 5 mL of distilled water and shaken, then 0.05 mol L⁻¹ NaOH was added up to a pH of 8.1. The results are expressed as % citric acid.

For macro- and micro-nutrient determination, 10 mL of distilled water was added to 0.2 g of ground sample. The mixture was sonicated (FS30H, Fisher Scientific, Pittsburgh, United State) and then centrifuged (2744 × g', 10 min). The supernatant was filtered through a 0.45 μm membrane (Millipore, Thebarton, South Australia). For macronutrient measurement, 9 mL of 0.5 mol L⁻¹ HCl and 200 μL of lanthanum oxide were added to 1 mL of the filtrate. For micronutrient determination, 200 μL of concentrated HCl was added to 9 mL of the filtrate. All samples were shaken on a vortex for 5 min and their mineral contents were quantified by atomic absorption (Solar 939, ATI Unicam, Basingstoke, U.K.).

Soluble sugars were extracted by the method of Gomez *et al.*,³² with some modifications. All extractions were carried out at 4 °C. Briefly, 4 mL of methanol/water (1:1 v/v) and 1 mL of chloroform were added to 15 mg of lyophilised sample. The mixture was shaken on a vortex for 2 min and then on a horizontal agitator (Libline 4638, Melrose Park, Illinois) at medium speed for 30 min. After centrifugation (1585 × g', 30 min), two liquid phases separated by the plant powder were obtained. A 2.8 mL volume of the methanol/water supernatant was recovered and dried in a vacuum evaporator (Labconco 7810000 Speed-Vac, Kansas City, Missouri). The resulting pellet was stored at -20 °C overnight. Next day it was redissolved in 2 mL of distilled water by shaking on a vortex for 20 min. The aqueous extract was then poured into a tube with 0.015 g of polyvinylpyrrolidone (Sigma P6755) to remove residual phenols by crosslinking. After shaking on a vortex for 20 min, the tube was centrifuged (1585 × g', 90 min). The supernatant was recovered using a 1 mL insulin syringe and stored at -20 °C for the direct measurement of glucose and the indirect measurement of fructose and sucrose by the enzymatic method³³ with a photometer (Multiskan Ascent 354, Thermolabsystem, Finlandia imported by Labtech, Mexico) at 340 nm, using a calibration curve in the range 0–0.2 g L⁻¹ glucose (Baker 1916-01, Xalostoc, Edo. México). To verify the correct measurement of soluble sugars, controls of fructose (Sigma F0127) and sucrose (Sigma S7903) were used. Before measurement the extract was diluted with distilled water (1:20 v/v).

Total phenols and the anthocyanins cyanidin-3-glucoside and pelargonidin-3-glucoside were extracted by the method of Markakis,³⁴ with some modifications. Briefly, 5 mL of methanol/HCl (1:5 v/v) was added to 0.1 g of lyophilised sample. The mixture was sonicated for 300 s and then centrifuged (2744 × g', 10 min). The supernatant was filtered (0.45 μm membrane, Millipore) and the filtrate obtained was used for the measurement of total phenol and anthocyanin concentrations. Colour parameters and the absorbance at 500 nm were also measured in the same filtrate.

Total phenol concentration was quantified by the Folin–Ciocalteu method,³⁵ with minor modifications. The volumes of sample, Folin–Ciocalteu's phenol reagent and sodium carbonate were reduced to one-tenth of those used in the original method, giving a final volume of 20 mL. The measurement was made at 765 nm (Spectrophotometer – Cintra 10e, GBC, Dandenong, Victoria, Australia), using a linear calibration curve of caffeic acid (0–250 mg L⁻¹) to calculate the total phenol concentration.

Strawberry fruit colour was determined by measuring the absorbance at 500 nm³⁶ with a spectrophotometer (Shanghai, Analytical Instrument LTD, China) (HP-8452A, Cheadle Heath, Stockport Cheshire, UK). Additionally, colour was measured using a photometer (Licor-2000, DR Lange, Dusseldorf, Germany) in terms of L^* , a^* and b^* values, where L^* defines lightness (from white = 100 to black = 0), a^* defines red/greenness (from -60 to +60) and b^* defines blue/yellowness (from -60 to +60). From the a^* and b^* values the following colour parameters were also calculated: colour evolution (a^*/b^*), shade ($\tan^{-1}(b^*/a^*)$), ranging from 0° (red) to 90° (yellow) to 270° (blue) and chromaticity ($C^* = (a^{*2} + b^{*2})^{1/2}$), indicating the vividness of colour and ranging from 0 (discoloured) to 60 (powerful).

Phenolic acids and flavonols were extracted by acid hydrolysis.³⁷ Briefly, 7.5 mL of 5.33 g L⁻¹ ascorbic acid solution, 12.5 mL of methanol (liquid chromatography/mass spectrometry grade) and 5 mL of 6 mol L⁻¹ HCl were added to 0.25 g of sample. The mixture was sonicated for 2 min, the air in the mixture was replaced with gaseous N₂ (1–1.5 min) and the mixture was shaken on a horizontal agitator (35 °C) for 16 h. The cold sample was filtered (0.45 µm membrane, Millipore), concentrated in a rotavapor (35 °C) and redissolved in 1 mL of methanol. This solution was filtered (0.45 µm membrane, Millipore) and 10 µL of the filtrate was used for the measurement of phenolic acids and flavonols.

Phenolic compounds (anthocyanins, phenolic acids and flavonols) were quantified by reverse phase high-performance liquid chromatography (RP-HPLC)³⁸ using an Agilent 1090 Aminoquant HPLC system (Waldbröt, Germany). Each 10 µL sample was injected for separation on two narrow-bore HP-ODS Hypersil RP-18 columns (Shandon, U.K) (5 µm, 200 mm × 2.1 mm and 5 µm, 100 mm × 2.1 mm) linked in series. A linear gradient of 5 g L⁻¹ formic acid (pH 2.3) and methanol at a flow rate of 0.2 mL min⁻¹ was used. The column temperature was 40 °C and detection was achieved at 320 nm for all compounds. The standards used and the concentration ranges of their calibration curves were as follows: callistephin (Extrasynthese 0907S, Lyon, France), 1–200 mg L⁻¹; kuromanin (Extrasynthese 0915S), 1–200 mg L⁻¹; gallic acid monohydrate (Roth 7300, Karlsruhe, Germany), 10.9–545 mg L⁻¹; *p*-coumaric acid (Roth 9908), 26.2–1308 mg L⁻¹ *p*-coumaric acid; ferulic acid (Roth 9936), 9.8–490 mg L⁻¹; ellagic acid (Sigma E2250), 8.1–81.4 mg L⁻¹; quercetin dehydrate (Extrasynthese 1135S), 8.7–436 mg L⁻¹; kaempferol (Fluka 60010, Saint Louis, MO, USA), 8.5–426 mg L⁻¹; catechin (Roth 6200), 9.2–460 mg L⁻¹.

The results are presented as means of four replicates (each replicate consists of fruits from all plants in one experimental unit). Statistical analyses were performed using SYSTAT for Windows, Version 9.01 (Systat Software versión 9.01, Cranes Software International, LTD). The effects of each single factor (N concentration and inoculation) and their interaction (N concentration × inoculation) were evaluated using two-way analysis of variance (ANOVA). Multiple comparisons were made using Tukey's test. Differences at $P < 0.05$ were considered significant.

Table 1. Fresh weight, diameter and length of fruits of strawberry plants inoculated with *Glomus intraradices* and fertilised with different nitrogen concentrations in irrigation water

Factor	Fresh weight (g per fruit)	Diameter (cm)	Length (cm)
Nitrogen concentration (mmol L ⁻¹)			
3	14.19a	2.98a	3.30a
6	13.14a	2.93a	3.22a
18	13.36a	2.93a	3.26a
Inoculation			
M	13.39a	2.93a	3.22a
NM	13.73a	2.96a	3.26a
Interaction (nitrogen concentration × inoculation)			
3 × M	14.44a	3.01a	3.23a
3 × NM	13.94a	2.96a	3.27a
6 × M	12.47a	2.88a	3.19a
6 × NM	13.80a	2.98a	3.25a
18 × M	13.27a	2.90a	3.27a
18 × NM	13.46a	2.96a	3.25a

Each value represents the mean of four replicates. Two-way ANOVA was applied for each parameter; when statistical differences were found, a Tukey test ($P < 0.05$) was conducted independently for nitrogen concentration (3, 6 and 18 mmol L⁻¹), inoculation (mycorrhizal (M) and non-mycorrhizal (NM)) and nitrogen concentration × inoculation (3 × M, 3 × NM, 6 × M, 6 × NM, 18 × M and 18 × NM). For each factor, means with the same letter in a column do not differ significantly.

RESULTS

Tables 1–5 show the results for the effects of the two factors and their interaction on the variables evaluated. At the end of the experiment the extent of AMF colonisation ranged from 65 to 80%. None of the treatments affected the fresh weight, diameter and length of fruits (Table 1).

In terms of colour, different N concentrations resulted in statistically significant effects only on fruit lightness and absorbance at 500 nm (Table 2). Lightness was significantly higher and absorbance was significantly lower in fruits of plants fertilised with 3 mmol L⁻¹ N than in fruits of plants treated with 6 mmol L⁻¹ N, but both values did not differ from those in fruits of plants fertilised with 18 mmol L⁻¹ N. Mycorrhization resulted in statistically significant effects on all colour parameters except colour evolution and shade. Fruits of mycorrhizal plants showed a 2.0% increase in lightness and 14.3, 12.9, 13.9 and 21.2% decreases in red/greenness, blue/yellowness, chromaticity and absorbance respectively compared with fruits of non-mycorrhizal plants. Increasing N concentration in the irrigation solution did not lead to statistically significant differences in colour parameters between fruits within each mycorrhizal treatment. Nor were there significant differences between fruits of mycorrhizal and non-mycorrhizal plants fertilised with the same N concentration (Table 2).

Titrate acidity, glucose, fructose and Brix grade were lowest in fruits of plants fertilised with 3 mmol L⁻¹ N (Table 3). Their titrate acidity, glucose and fructose values were significantly lower than those in fruits of plants treated with 6 mmol L⁻¹ N, while their Brix grade was significantly lower than that in fruits of plants treated with 18 mmol L⁻¹ N. Mycorrhization modified only fructose concentration, with fruits of mycorrhizal plants containing 8.5% less fructose than those of non-mycorrhizal plants. When the applied N was increased, a significant difference in titrate acidity between mycorrhizal and non-mycorrhizal plants treated with the

Table 2. Lightness (L^*), red/greenness (a^*), blue/yellowness (b^*), colour evolution (a^*/b^*), shade ($\tan^{-1}(b^*/a^*)$), chromaticity ($C^* = (a^2 + b^2)^{1/2}$) and absorbance at 500 nm of fruits of strawberry plants inoculated with *Glomus intraradices* and fertilised with different nitrogen concentrations in irrigation water

Factor	Lightness	Red/greenness	Blue/yellowness	Colour evolution	Shade	Chromaticity	Absorbance
Nitrogen concentration (mmol L ⁻¹)							
3	84.93a	27.69a	17.16a	1.62a	31.77a	32.59a	0.54b
6	83.31b	31.07a	19.79a	1.57a	32.47a	36.85a	0.64a
18	84.31a	29.50a	18.18a	1.63a	31.67a	34.67a	0.57ab
Inoculation							
M	84.99a	27.45b	17.26b	1.59a	32.21a	32.44b	0.52b
NM	83.37b	31.38a	19.49a	1.62a	31.72a	36.95a	0.63a
Interaction (nitrogen concentration × inoculation)							
3 × M	85.72a	25.98a	16.52a	1.57a	32.47a	30.80a	0.48b
3 × NM	84.14ab	29.40a	17.80a	1.66a	31.07a	34.38a	0.59ab
6 × M	83.80ab	29.40a	18.68a	1.57a	32.56a	34.85a	0.60ab
6 × NM	82.82b	32.74a	20.90a	1.56a	32.38a	38.85a	0.68a
18 × M	85.46ac	26.98a	16.58a	1.63a	31.59a	31.69a	0.50b
18 × NM	83.16bc	32.02a	19.78a	1.62a	31.72a	37.65a	0.63ab

Each value represents the mean of four replicates. Two-way ANOVA was applied for each parameter; when statistical differences were found, a Tukey test ($P < 0.05$) was conducted independently for nitrogen concentration (3, 6 and 18 mmol L⁻¹), inoculation (mycorrhizal (M) and non-mycorrhizal (NM)) and nitrogen concentration × inoculation (3 × M, 3 × NM, 6 × M, 6 × NM, 18 × M and 18 × NM). For each factor, means with different letters in a column differ significantly.

Table 3. Titratable acidity, soluble sugar concentrations and Brix grade of fruits of strawberry plants inoculated with *Glomus intraradices* and fertilised with different nitrogen concentrations in irrigation water

Factor	Titratable acidity (%) citric acid	Soluble sugars (g kg ⁻¹ DM)			Brix grade
		Glucose	Fructose	Sucrose	
Nitrogen concentration (mmol L ⁻¹)					
3	1.28b	136.63b	148.96b	94.16a	4.93b
6	1.36a	153.21a	167.54a	62.68a	5.39ab
18	1.30b	140.50ab	149.35b	83.07a	6.09a
Inoculation					
M	1.30a	139.85a	148.99b	80.45a	5.96a
NM	1.33a	147.04a	161.58a	79.45a	5.99a
Interaction (nitrogen concentration × inoculation)					
3 × M	1.21c	132.02c	141.96c	113.10a	5.05bc
3 × NM	1.35ab	141.24bc	155.96bc	75.21a	4.81c
6 × M	1.38a	146.34bc	158.85bc	45.83a	5.30abc
6 × NM	1.34ab	160.07ab	176.24ab	79.54a	5.48abc
18 × M	1.31ab	141.20bc	146.16c	82.43a	6.17a
18 × NM	1.28bc	139.81bc	152.54bc	83.71a	6.00ab

Each value represents the mean of four replicates. Two-way ANOVA was applied for each parameter; when statistical differences were found, a Tukey test ($P < 0.05$) was conducted independently for nitrogen concentration (3, 6 and 18 mmol L⁻¹), inoculation (mycorrhizal (M) and non-mycorrhizal (NM)) and nitrogen concentration × inoculation (3 × M, 3 × NM, 6 × M, 6 × NM, 18 × M and 18 × NM). For each factor, means with different letters in a column differ significantly.

same N concentration was observed only in the treatment with 3 mmol L⁻¹ N.

Some nutrient concentrations were significantly different between fruits of plants treated with 3 and 18 mmol L⁻¹ N (Table 4). Fruits from the treatment with 3 mmol L⁻¹ N contained 9.4, 13.3, 61.0 and 48.0% more K, Mg, Fe and Zn respectively and 11.3% less

Ca than fruits of plants fertilised with 18 mmol L⁻¹ N. The Mn concentration in fruits of plants fertilised with 3 mmol L⁻¹ N was significantly higher than that in fruits of plants treated with 6 mmol L⁻¹ N. Fruits of mycorrhizal plants had higher K and Cu concentrations but lower Mn concentration than fruits of non-mycorrhizal plants. Mycorrhization significantly modified the Ca, Mg, Fe, Cu, Zn and Mn concentrations in fruits when the N applied was changed from 3 to 18 mmol L⁻¹, and the K concentration in fruits when N changed from 3 to 6 mmol L⁻¹. With the exception of Ca, the concentrations of all elements studied were higher in fruits of plants fertilised with 3 mmol L⁻¹ N. Significant differences between fruits of mycorrhizal and non-mycorrhizal plants of the same N treatment were found for Cu, Zn and Mn concentrations. Fruits of mycorrhizal plants had 38.0 and 39.3% more Cu and Zn respectively in the 6 mmol L⁻¹ N treatment and 39.6% less Mn in the 18 mmol L⁻¹ N treatment than their non-mycorrhizal counterparts.

Nitrogen treatment significantly affected the concentrations of total phenols, gallic acid, ferulic acid, ellagic acid, cyanidin-3-glucoside, quercetin and kaempferol in fruits (Table 5). Fruits of plants fertilised with 3 mmol L⁻¹ N had 20.5, 31.2 and 11.4% lower concentrations of total phenols, gallic acid and cyanidin-3-glucoside respectively and 21.0, 50.0 and 61.5% higher concentrations of ellagic acid, quercetin and kaempferol respectively than fruits of plants treated with 18 mmol L⁻¹ N. Fruits of plants fertilised with 6 mmol L⁻¹ N had a significantly higher concentration of ferulic acid than fruits of plants treated with 3 and 18 mmol L⁻¹ N. Mycorrhization significantly modified the concentrations of all phenolic compounds except pelargonidin-3-glucoside and catechin. Fruits of mycorrhizal plants had 20.0, 15.0, 50.0 and 28.6% higher concentrations of *p*-coumaric acid, cyanidin-3-glucoside, quercetin and kaempferol respectively and 29.0, 50.0 and 11.0% lower concentrations of gallic acid, ferulic acid and ellagic acid respectively than fruits of non-mycorrhizal plants.

Fruits of mycorrhizal plants fertilised with 6 mmol L⁻¹ N had a lower gallic acid concentration than fruits of mycorrhizal plants

Table 4. Macro- and micronutrient concentrations of fruits of strawberry plants inoculated with *Glomus intraradices* and fertilised with different nitrogen concentrations in irrigation water

Factor	Macronutrients (g kg ⁻¹ DM)				Micronutrients (mg kg ⁻¹ DM)			
	K	Na	Ca	Mg	Cu	Fe	Zn	Mn
Nitrogen concentration (mmol L ⁻¹)								
3	190.94a	2.09a	15.09b	15.04a	2.5a	6.6a	11.1a	9.9a
6	174.63b	1.69a	16.25ab	14.03ab	2.5a	4.6b	10.1a	7.1b
18	174.55b	2.24a	16.79a	13.28b	2.7a	4.1b	7.5b	9.3a
Inoculation								
M	185.53a	1.93a	15.86a	13.75a	2.8a	4.8a	9.8a	7.7b
NM	177.38b	2.09a	16.24a	14.49a	2.3b	5.4a	9.3a	9.9a
Interaction (nitrogen concentration × inoculation)								
3 × M	194.90a	1.20a	14.14b	14.65a	3.1a	6.3ab	10.7abc	9.2b
3 × NM	185.49ab	2.19a	16.05ab	15.43a	1.8b	6.9a	11.5ab	10.6ab
6 × M	178.33b	1.49a	16.98a	14.03ac	2.9ad	5.2abc	11.7a	6.8c
6 × NM	170.40b	1.89a	15.52ab	14.04ac	2.1be	4.0bc	8.4bcd	7.4c
18 × M	183.38ab	2.30a	16.44a	12.56bc	2.5cde	2.9c	7.0d	7.0c
18 × NM	175.72b	2.19a	17.15a	13.99ac	2.9ac	5.3abc	8.0cd	11.6a

Each value represents the mean of four replicates. Two-way ANOVA was applied for each parameter; when statistical differences were found, a Tukey test ($P < 0.05$) was conducted independently for nitrogen concentration (3, 6 and 18 mmol L⁻¹), inoculation (mycorrhizal (M) and non-mycorrhizal (NM)) and nitrogen concentration × inoculation (3 × M, 3 × NM, 6 × M, 6 × NM, 18 × M and 18 × NM). For each factor, means with different letters in a column differ significantly.

Table 5. Total phenol and phenolic compound concentrations of fruits of strawberry plants inoculated with *Glomus intraradices* and fertilised with different nitrogen concentrations in irrigation water

Factor	Total phenols (g kg ⁻¹ DM)	Phenolic compounds (mg kg ⁻¹ DM)								
		Phenolic acids				Flavonoids				
		Gallic	<i>p</i> -Coumaric	Ferulic	Ellagic	Anthocyanins ^a		Flavonols		
				Cya-3-glu	Pel-3-glu	Quercetin	Kaempferol	Catechin		
Nitrogen concentration (mmol L ⁻¹)										
3	541.63b	11b	99a	1b	753a	248b	3370a	3a	21a	249a
6	503.32b	10b	96a	2a	699ab	275a	3710a	2b	15b	219a
18	682.04a	16a	100a	1b	622b	280a	3545a	2b	13b	368a
Inoculation										
M	571.31a	10b	107a	1b	681b	287a	3692a	3a	18a	322a
NM	580.01a	14a	89b	2a	765a	249b	3391a	2b	14b	236a
Interaction (nitrogen concentration × inoculation)										
3 × M	590.38ab	11bc	109ab	1b	633bc	258bc	3564a	4a	21a	324a
3 × NM	492.87b	11bc	89ab	1b	873a	238b	3176a	3ab	21a	174a
6 × M	506.74b	6c	113a	1b	595c	314a	4198a	3ab	20a	233a
6 × NM	499.89b	14ab	78b	2a	803ab	236b	3222a	2c	10b	206a
18 × M	616.79ab	13ab	99ab	1b	626bc	288ac	3314a	3ac	13ab	408a
18 × NM	747.29a	18a	101ab	1b	618c	272ab	3776a	2bc	12ab	328a

Each value represents the mean of four replicates. Two-way ANOVA was applied for each parameter; when statistical differences were found, a Tukey test ($P < 0.05$) was conducted independently for nitrogen concentration (3, 6 and 18 mmol L⁻¹), inoculation (mycorrhizal (M) and non-mycorrhizal (NM)) and nitrogen concentration × inoculation (3 × M, 3 × NM, 6 × M, 6 × NM, 18 × M and 18 × NM). For each factor, means with different letters in a column differ significantly.

^a Cya-3-glu, cyanidin-3-glucoside; Pel-3-glu, pelargonidin-3-glucoside.

treated with 18 mmol L⁻¹ N and a higher cyanidin-3-glucoside concentration than fruits of mycorrhizal plants treated with 3 mmol L⁻¹ N, the difference being significant in both cases. Significant differences between fruits of mycorrhizal and non-mycorrhizal plants of the same N treatment were found for

all phenolic compounds except pelargonidin-3-glucoside and catechin. Fruits of mycorrhizal plants had higher *p*-coumaric acid, cyanidin-3-glucoside, quercetin and kaempferol concentrations and lower gallic acid, ferulic acid and ellagic acid concentrations than fruits of non-mycorrhizal plants when fertilised with

6 mmol L⁻¹ N, and a lower ellagic acid concentration when fertilised with 3 mmol L⁻¹ N (Table 5).

DISCUSSION

In fruit production, parameters such as fruit fresh weight, diameter and length are important for fruit quality. In a study of the effect of N application on peaches, Crisosto *et al.*³⁹ observed that different N levels did not affect the size of peach fruits,³⁹ indicating that the N levels tested were not a determining factor for this parameter. In our study, neither N treatment nor mycorrhization had an effect on these parameters of strawberry fruits.

Colour is another important determinant of fruit quality. Shade and chromaticity are two parameters used to quantify purity, while red intensity is used for the description of colour.^{40,41} The values of these variables determined in the present study are within the ranges observed previously in strawberry fruits.⁴² In our experiment, some colour parameters were modified by N treatment. Fruits of plants fertilised with 6 mmol L⁻¹ N had lower lightness and higher absorbance at 500 nm than fruits of plants treated with 3 mmol L⁻¹ N.

Changes in chromaticity due to mycorrhization have been reported previously in *Capsicum annum* L. by Mena-Violante *et al.*,⁴³ who found that fruits of mycorrhizal plants had a lower chromaticity than fruits of non-mycorrhizal plants. Interestingly, our study showed a similar effect of mycorrhization on chromaticity, with a lower chromaticity being found in fruits of mycorrhizal plants than in fruits of non-mycorrhizal plants. These data suggest that the effect of mycorrhization on chromaticity is a general one and not fruit-specific. It has been proposed that the colour of strawberry fruits is closely linked with the synthesis and/or expression of pelargonidin-3-glucoside and cyanidin-3-glucoside, two principal anthocyanins.⁴⁴ In our context, this means that the colour changes we observed as a result of mycorrhization are possibly due to changes in the levels of these two anthocyanins.

The flavour of strawberry fruits is determined by the balance of sugars and acids.¹² Glucose, fructose and sucrose are the most important sugars for the sensory quality of strawberry fruits, representing 99% of the total carbohydrate content.⁴⁵ Moreover, citric acid and malic acid are the most important acids in strawberry fruits.⁴⁶ Besides their impact on flavour, acids are important because they affect the gelling properties of pectin. Brix grade is a composite parameter reflecting sugars, acids, salts and others compounds soluble in water and is measured as the total soluble solids present in the fruit.

In our study, titratable acidity and Brix grade varied between 1.21 and 1.38% and between 4.81 and 6.17 respectively in all treatments. These values are within the ranges reported by Perkins-Veazie and Collins⁴⁷ for titratable acidity (0.5–1.87%) and Brix grade (5–12). Glucose and fructose concentrations were higher than sucrose concentration in all cases and the fructose/glucose ratio was about 1 : 1, in agreement with values reported previously for strawberry fruits.⁴²

The level of applied N had a significant effect on titratable acidity, glucose and fructose concentrations and Brix grade. Fruits of plants fertilised with 6 mmol L⁻¹ N were more acidic and their glucose and fructose concentrations were higher in comparison with fruits from other treatments, without significant differences in Brix grade. These data indicate that fruits from the 6 mmol L⁻¹ N treatment had the best quality according to Mitcham.⁴⁸ In our experiment, foliar area was also measured (data not shown). The higher production of sugars in the 6 mmol L⁻¹ N treatment could

be explained by the enhanced foliar area of these plants (35.9 and 25.3% higher than that of plants in the 3 and 18 mmol L⁻¹ N treatments respectively) when fructification started.

Fruits of mycorrhizal plants had a lower fructose concentration than fruits of non-mycorrhizal plants, indicating that mycorrhization reduced the accumulation of this carbon compound in the fruits. This could be explained by the fact that AMF act as carbon sinks (4–20% of the total carbon fixed by the plant).⁴⁹

Around 4% of the dry matter of plants comprises mineral elements, which, owing to their role in enzymatic reactions essential for fruit development and its cold conservation, are important for fruit quality.⁵⁰ In this study we observed that different N levels modified the concentrations of some minerals in the fruits. Fruits of plants fertilised with 3 mmol L⁻¹ N showed higher K, Mg, Fe and Zn levels than fruits of plants treated with 18 mmol L⁻¹ N. These results suggest that the roots of plants fertilised with 3 mmol L⁻¹ N took up higher amounts of these minerals.

It has been shown previously that a low availability of N in the soil affects root growth. Tolley-Henry and Raper⁵¹ suggested that under conditions of low N availability the roots have priority to N compared with other plant organs and therefore root growth is promoted. Rufty *et al.*⁵² demonstrated that a low availability of N in the soil increases the amount of photosynthates addressed to the roots, thus being available for enhanced root growth. In our experiment, root dry weight and volume were also measured (data not shown). The root dry weight of plants fertilised with 3 and 18 mmol L⁻¹ N was 2.0 and 1.7 g per plant respectively, while the root volume of these plants was 22.0 and 14.8 cm³ per plant respectively. These data suggest that a higher soil volume was explored by plants fertilised with 3 mmol L⁻¹ N compared with plants treated with 18 mmol L⁻¹ N, which could explain the higher K, Mg, Fe and Zn levels in fruits of plants of the 3 mmol L⁻¹ N treatment.

To date, no adequate data are available on the effect of mycorrhization on macro- and micronutrients in fruits. In our experiment, mycorrhization significantly modified the concentrations of K, Cu and Mn, with fruits of mycorrhizal plants having higher K and Cu levels and a lower Mn level. Although mycorrhizal root colonisation frequently increases macro- and micronutrient accumulation in the leaves and stalks of plants,^{53,54} Liu *et al.*⁵⁵ found lower Cu, Zn, Mn and Fe concentrations in the shoots of mycorrhizal corn plants. The inconsistent results on nutrient acquisition by mycorrhizal plants have been attributed to changes in the rhizosphere due to increased N levels in the soil, which affect mycorrhizal development.⁵⁶

Fruits of mycorrhizal plants fertilised with 3 mmol L⁻¹ N had higher concentrations of those minerals than fruits of mycorrhizal plants treated with 18 mmol L⁻¹ N. Similar results of lower, equal or higher acquisition of macro- and/or micronutrients dependent on the level of mineral fertilisation have been reported in lettuce inoculated with *Glomus mosseae*.⁴ The lack of a beneficial effect of mycorrhization in terms of mineral acquisition in our 18 mmol L⁻¹ N treatment could be attributed to a negative effect of this N concentration on the extraradical mycelium development of the AMF. The suppressive effect of high N levels on the formation of extraradical mycelium has been described previously and has been linked with reduced nutrient acquisition in mycorrhizal plants.⁵⁷ Fruits of mycorrhizal plants fertilised with 6 mmol L⁻¹ N had significantly higher Cu and Zn concentrations than fruits of non-mycorrhizal plants fertilised with the same N level. This indicates a positive effect of mycorrhization and N treatment on

the acquisition of Cu and Zn by the roots and their translocation to the fruits.

Our results on the effect of N fertilisation on phenolic compounds in strawberry fruits show that from the 3 mmol L⁻¹ N treatment to the 18 mmol L⁻¹ N treatment the concentrations of ellagic acid, quercetin and kaempferol decreased while the concentrations of total phenols, cyanidin-3-glucoside and gallic acid increased.

A decrease in quercetin and kaempferol concentrations at high N levels has been reported in tomato fruits,⁵⁸ while an increase in ellagic acid concentration at low N levels has been found in strawberry fruits.⁵⁹ In addition, Keller and Hrazdina⁶⁰ reported that the N concentration in the soil had different effects on the total phenol concentration in grapes. The application of high N levels led to low accumulation of flavonols, whereas the proportion of anthocyanins was similar to that at low N levels. Our results could be explained by the effect that N has on the biosynthetic pathways of phenolic compounds. Phenylalanine ammonia-lyase (PAL) is the principal enzyme of the phenylpropanoid pathway.⁶¹ This enzyme catalyses the transformation of the amino acid L-phenylalanine by deamination to *trans*-cinnamic acid, which is the first product necessary for the synthesis of phenolic compounds. Interestingly, it has been reported that at low N levels the enzymatic activity of PAL is increased, liberating N for the amino acid metabolism, and whereas the carbon products are diverted via 4-coumaroyl-CoA into the flavonoid biosynthetic pathway.⁶² In our study, this could be an explanation for the increase in concentrations of some flavonoids (cyanidin-3-glucoside, kaempferol and quercetin) in fruits of plants fertilised with 3 mmol L⁻¹ N.

Mycorrhization modified the levels of most phenolic compounds. The cyanidin-3-glucoside, *p*-coumaric acid, quercetin and kaempferol concentrations were higher and the gallic acid, ferulic acid and ellagic acid concentrations were lower in fruits of mycorrhizal plants than in fruits of non-mycorrhizal plants. To our knowledge, there are no data on the effect of AMF on phenolic compound accumulation in fruits. However, there are reports on changes in the levels of *p*-coumaric acid and ferulic acid in the roots of mycorrhizal onion plants,⁶³ changes in the levels of biochanin A, formononetin, genistein and daidzein in the roots of mycorrhizal alfalfa (*M. sativa* L.)^{19–21} and barrel medic (*M. truncatula*)²² and changes in the level of glyceoline in mycorrhizal soybean (*G. max* L.).²⁴ Most recently, it has been shown that through mycorrhization the levels of phenols can also be altered in plant shoots.²³ Our results extend these observations, showing that mycorrhization can induce changes in phenolic compound levels even in fruits.

An increase in applied N modified the concentrations of some phenolic compounds between fruits of mycorrhizal and non-mycorrhizal plants. Differences were determined in fruits of plants fertilised with 6 mmol L⁻¹ N. These results indicate that N fertilisation modifies the response of the strawberry plant to the AMF *G. intraradices*. This could be attributed to changes in the rhizosphere due to N levels in the soil, which affect mycorrhizal development⁵⁶ and thus the acquisition of other nutrients necessary for the production of phenols. To our knowledge, we have provided the first evidence that, depending on the N level applied, the accumulation of phenolic compounds is altered in fruits of mycorrhizal strawberry plants.

CONCLUSION

Mycorrhization did not modify the weight, diameter or length of strawberry fruits but had a negative effect on most colour

parameters. Moreover, fruits of mycorrhizal plants had higher K and Cu concentrations and showed greater accumulation of most phenolic compounds. The results indicate that the 3 mmol L⁻¹ N treatment had a positive effect on the accumulation of some minerals in strawberry fruits, and fruits of mycorrhizal plants had significantly higher phenolic compound, Cu and Zn concentrations than fruits of non-mycorrhizal plants when they were treated with 6 mmol L⁻¹ N. In recent years, much interest has focused on the intake of phenolic compounds from the human diet and the health benefits due to their antioxidant nature. It is therefore of interest to produce crops rich in flavonols without the need for genetic modification. Although previous studies have identified a link between nutrient deficiency and phenolic compound accumulation in plant tissue, the present study provides evidence that mycorrhization and N application in strawberry plants can be one strategy for increasing phenolic compound concentrations in the fruits. In addition, up-regulation of the flavonoid biosynthetic pathway in strawberry fruits may afford protection against pathogen attack or light-induced damage. Further studies are required to test this theory.

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