Nitrogen availability drives the effect of Glomus intraradices on the growth of strawberry (Fragaria x ananassa Duch.) plants

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Abstract

BACKGROUND: On the one hand, the critical nitrogen (N) content curve allows the minimal N content necessary for maximum growth rate at any stage of crop development to be predicted. On the other hand, arbuscular mycorrhizal fungi (AMF) transfer N from the soil to the plants and its growth and activity depends on the availability of soil N. Our objective was to investigate how the availability of N in the soil affects growth and the accumulation of N in inoculated strawberry plants. Root colonisation, dry matter accumulation and the critical N% curve were studied during growth of inoculated and non-inoculated strawberry plants grown at several N levels.

RESULTS: (1) The increase in the availability of N augmented root colonisation by AMF. (2) The effect of AMF on plant growth depended on N availability and the plant developmental status. (3) The critical N% curves were fitted by the following equations: %N = 2.81 × (DM)^0.21 (r² = 0.81) and %N = 2.89 × (DM)^0.32 (r² = 0.80) for inoculated and non-inoculated plants, respectively.

CONCLUSION: N availability was a key factor for root colonisation by AMF and for its contribution to plant growth. The patterns of the critical N% curves suggest that AMF modified the photosynthetic N use efficiency.

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Keywords: nitrogen; arbuscular mycorrhizal fungi; strawberry plants; nitrogen accumulation

INTRODUCTION

It is well known that N is necessary for plant growth, because this element is essential for the synthesis of proteins, nucleic acids, co-enzymes and many products and by-products of secondary metabolism.1 In the last few decades it has been stated that N accumulation and plant dry matter are related.2,3 This relation shows that the N content decreases when plant dry matter increases, even when the availability of N in the soil is non-limiting for plant growth. This so-called N dilution effect has been linked to the remobilisation of N from senescent organs to plant parts with growth activity. Using this N dilution effect, a critical biomass N% curve has been developed, which allows the prediction of the minimal N% in the plant dry matter necessary for reach the maximum growth rate at any stage of the plant development.4 The critical biomass N% curve has demonstrated usefulness in physiological, ecophysiological and agronomical studies.5–7

Arbuscular mycorrhizal fungi (AMF) are symbiotically associated with the roots of more than 90% of all terrestrial plants.5 In recent years some studies have reported that nitrate can be mobilised from soil and transferred to the plant by the external hyphae of AMF, improving the inflow of N to the mycorrhizal plant.9–11 In addition, the direct uptake and transport of nitrate by extraradical mycorrhizal mycelium have been confirmed using 15N.12,13

However, these studies were carried out under plant growth N-limiting conditions and it is well known that growth and activity of AMF are influenced by the availability of N in the soil.12,13 On strawberry plants, AMF have demonstrated a positive effect on the production of dry matter, as well as of flowers, fruit and runners; the rate of photosynthesis; and the production of amino acids.14–17 Nevertheless, most of these studies have been made using in vitro plants and information about the effect of this symbiosis and N accumulation on strawberry plants in the productive stage under non-limiting N conditions, is not yet available.

The aim of the present work was to evaluate the growth of productive strawberry plants inoculated with AMF grown at different
N levels, and to determine if the fungus affects N accumulation during plant growth, using the critical biomass %N curve as a heuristic tool. This curve was recently established in strawberry plants; however, the effect of AMF has never been considered.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse ‘shade’ type with 30% of shade, at the Universidad Michoacana de San Nicolás de Hidalgo in Morelia, Michoacán, Mexico. The maximum and the minimum temperature in the greenhouse varied between 28 °C and 32 °C and between 8 °C and 18 °C, respectively. In these conditions strawberry plants, cultivar ‘Aromas’, were established in black polyethylene bags (3 L), containing sterilised (40 min, 95 °C water steam) substrate (coconut fibre : perlite; proportion 1 : 3 v/v).

Before planting, the roots were disinfected by submerging them for 20 s in a 20 g L⁻¹ sodium hypochlorite solution and rinsing them in water. The absence of AMF in the roots was verified by the ink (Sheaffer® black) and vinegar technique, modifying the duration of immersion in KOH and ink/vinegar solution (7 and 5 min, respectively).

The inoculum was prepared with spores of *Glomus intraradices* grown in liquid medium (3.5 × 10⁶ spores L⁻¹, 90% viability; Premier Tech Biotechnologies Company, Quebec, Canada), which was diluted with fitagel (P-8169; Sigma, Saint Louis, MO, USA) solution at 5 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. Eighteen days after setting up the experiment, each plant received 2 mL of inoculum applied directly to the recently formed roots.

The experiment was organised as a full factorial completely randomised design with two factors: (1) inoculation with AMF with two levels: inoculated and non-inoculated plants, and (2) N concentrations in the nutrient solution with six levels: 0.0, 0.03, 0.3, 3.0, 6.0 and 18.0 mmol L⁻¹. The 12 treatments were replicated four times, producing 48 experimental units with 10 plants each. Every second day, during 25 days after setting up the experiment, all plants were irrigated up to substrate saturation with deionised water (with around 0.3 L per plant). After this date a complete nutrient solution was used. In this solution N 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⁺, 3.0; Ca²⁺, 3.5; and Mg²⁺, 1.5 mmol L⁻¹, and they were increased in the treatment of 18 mmol L⁻¹ at: K⁺, 6.5; Ca²⁺, 7.5 and Mg²⁺, 3.25 mmol L⁻¹. In all nutrient solutions the concentration of phosphorus was 0.3 mmol L⁻¹. The other nutrients in the solution were (μmol L⁻¹): 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. The pH was adjusted to 5.5 at every application date.

The percentage of root colonisation and the plant growth were evaluated by determining leaf dry matter, whole plant dry matter (105 °C, 24 h, Oven Felisa; FELI S.A.de C.V., Jalisco, México; Analytic Balance, Mettler Toledo, AT200; Intl Inc, México D.F.) and leaf area (LI-COR, Li-3100; LI-COR Biosciences, Lincoln, NE USA) at 49 and 159 days after setting up the experiment. The critical N curves were calculated considering the dry matter production of leaves and its N content (Dumas method, Autoanalyzer Carlo Erba, Flash EA 1112; CE Instruments, United Kingdom) at 49, 87, 128 and 159 days after the experiment had been set up. In all cases all N treatments were considered and one plant per experimental unit was sampled at each sampling date. The critical N curves were calculated by the equation: \( \%N = a \times (\text{DM})^{b} \), where \( \%N \) is the percentage of N in the leaf dry matter, \( a \) is the %N for leaf biomass, equivalent to 1 g plant⁻¹; DM is the weight of the leaf dry matter, in g plant⁻¹; and \( b \) indicates the rate of decline of leaf %N during plant development. This equation was fitted on the critical %N points estimated by the Justes method, for inoculated and non-inoculated plants: with the experimental data of the each sampling date, the relationship between %N and DM was described as a bi-linear relationship, comprising: (1) an oblique line representing a joint increase in DM and %N; and (2) a vertical line corresponding to an increase in %N without an increase in DM. To discriminate the inclusion of experimental points on each line, the equality of means of DM was tested using Student’s bi-lateral t-test with at 5% probability level. The critical %N point is the ordinate at the intersection of both lines.

The results are presented as means of four replicates; statistical analyses were performed using SYSTAT for Windows Version 9.01 (Systat Software version 9.01; Cranes Software International, Systat Software Inc. Washington St.). The values were submitted to the ANOVA test and when statistically significant differences were found the pair-wise comparison mean tests least significant difference (LSD) was applied.

RESULTS

The percentage of root colonisation of inoculated plants was higher than non-inoculated plants, at both sampling dates. In all cases, root colonisation augmented when N concentration in the nutrient solution was increased. In inoculated plants this percentage increased from 39% to 83% and from 26% to 75%, at 49 and 159 days after the experimental set-up, respectively. In contrast, in non-inoculated plants increased from 12% to 37% and from 4% to 26%, for the same sampling dates, respectively (Fig. 1).

The leaf area of inoculated plants was significantly higher than non-inoculated plants with 3.0 mmol L⁻¹ N at 49 days and with 3.0, 6.0 and 18.0 mmol L⁻¹ N at the last sampling date (Fig. 2).

Significant differences on the leaf dry weight were found between inoculated and non-inoculated plants at all N treatments. At 49 days this variable was higher in non-inoculated plants with 0.0, 0.03 and 0.3 and lower with 3.0 mmol L⁻¹ N. However, at 159 days inoculated plants showed the higher leaf dry weight with 3.0, 6.0 and 18.0 mmol L⁻¹ N (Fig. 3).

At day 49 the whole plant dry weight showed a similar pattern to the leaf dry weight, with a higher dry weight of the non-inoculated plants with 0.0, 0.03 and 0.3 mmol L⁻¹ N and a greater dry weight of the inoculated plants with 3.0 mmol L⁻¹ N. At day 159 non-statistically significant differences could be observed (Fig. 4).

The critical N curves of inoculated and non-inoculated plants as well as the equations describing them are shown in Fig. 5. The results showed that the \( a \) value is very close for both curves (2.81 and 2.89, respectively); however, the \( b \) value is higher in non-inoculated plants than inoculated plants: 0.32 and 0.21, respectively.

DISCUSSION

In our study non-inoculated plants showed roots colonisation in all N treatments and sampling dates. Considering that the substrate was sterilised and that the plants were disinfected before planting, this result could be associated with the contamination by plant manipulation in the greenhouse or to the wild AMF colonisation. However, in all cases inoculated plants showed a greater
percentage of root colonisation by AMF than non-inoculated plants. In this context, this work evaluates the effect of inoculation and not of the mycorrhization of the plants. In inoculated and non-inoculated plants the augmentation of the root colonisation was associated with the increase on N concentration in the nutrient solution. This result can be explained as an indirect effect of N treatments: the increase of N availability stimulated the growth of plant leaf area, enhancing carbon fixation by photosynthesis and carbohydrate availability to plant and fungus development.16,21,22 These results suggest that the effect of N availability on AMF root colonisation varies with the growth conditions.

At day 49, the effect of N treatments was more evident on the growth of the leaves (leaf dry weight and leaf area) than of the whole plant. When plants were irrigated with 0.0, 0.03 and 0.3 mmol L\(^{-1}\) N the leaf and plant growth was presumably limited by the low N availability. It is well known that the AMF in the roots of inoculated plants acts as additional carbon sink, reducing carbohydrate availability for tissue development.23 Moreover, it has been well documented that during the early days after plant establishment, the growth of strawberry plants depends mainly on root reserves.24

At upper N concentration in the irrigation solution, leaves growth (leaf dry weight and leaf area) was enhanced, increasing the plant photosynthetic capacity and consequently the availability of photosynthates for tissue growth and, in the case of inoculated plants, for fungus development.22 This was more evident at the treatment with 3.0 mmol L\(^{-1}\) N, where inoculation showed a significant effect on leaf development, which could be due to the increase of the N uptake capacity of roots associated with *G. intraradices*.25,26 When N concentration was higher, the leaf growth of inoculated plants reached an asymptotic pattern, while leaves of non-inoculated plants continued growing.

At whole plant level (considering leaves, stems and roots), biomass accumulation was not associated with the increase of N concentration in the irrigation solution; however, as registered on leaf biomass, with the treatment at 3.0 mmol L\(^{-1}\) N a significant contribution of AMF on biomass production was also found. At 159 days, leaf area, leaf dry weight and whole plant dry weight of both inoculated and non-inoculated plants fit the typical growth response to N supply.27 Nevertheless, in inoculated plants these variables increased at the higher N concentrations (3.0, 6.0 and 18.0 mmol L\(^{-1}\) N) in the irrigation solution, which could be related to the enhanced capacity of strawberry roots to take up N and therefore to increase leaf area, CO\(_2\) capture, and the production of photo-assimilates, and consequently increase whole plant growth.16,26,28

The critical N content curve represents the minimal N content required to achieve maximum plant growth rate at any development stage.2 The earlier critical %N points around the curves correspond to the first sampling date, with low dry matter and high %N and the later points likely represent the plants at advanced development stages, with higher dry matter and lower %N. The model fits well the experimental points of inoculated and non-inoculated plants. The a values were 2.81 and 2.89, while the b values were −0.21 and −0.32 for inoculated and non-inoculated plants, respectively. In our work, the critical %N curves showed a typical behaviour: the %N decreased as the dry matter increased.

According to these models, and to the critical %N points of the first sampling date, the %N minimum necessary to achieve the maximum growth rate was higher for non-inoculated than inoculated plants. This is in agreement with the leaf dry matter production at 49 day after the experiment had been set up, where inoculated plants appear to reach the growth critical point at lower N concentration in the irrigation solution (3.0 mmol L\(^{-1}\)). These results point toward a more efficient use of N by inoculated plants.
Effect of nitrogen and *G. intraradices* on strawberry growth

Figure 3. Leaf dry weight of strawberry plants fertilised with different N concentrations in the irrigation solution. (□) Non-inoculated and (■) inoculated plants, (a) 49 and (b) 159 days after the experiment had been set up. *Indicates statistically significant differences ($P < 0.05$) between inoculated and non-inoculated plants fertilised with the same N treatment, by pair-wise comparison mean test LSD, $n = 4$.

Figure 4. Dry weight of strawberry plants fertilised with different N concentrations in the irrigation solution. (□) Non-inoculated and (■) inoculated plants, (a) 49 and (b) 159 days after the experiment had been set up. *Indicates statistically significant differences ($P < 0.05$) between inoculated and non-inoculated plants fertilised with the same N treatment, by pair-wise comparison mean test LSD, $n = 4$.

Figure 5. Critical N content curves of strawberry plants fertilised with six N concentrations in the irrigation solution. (●) Inoculated and (○) non-inoculated plants. The curves were made by the method proposed by Justes et al.6 Dashed line, inoculated plants: %N $= 2.81 \times (DM)^{-0.21}$ ($r^2 = 0.81$). Dotted line, non-inoculated plants: %N $= 2.89 \times (DM)^{-0.32}$ ($r^2 = 0.82$). DM, leaf dry matter, in g plant$^{-1}$.

at an earlier stage of development. Indeed, in a recent paper it has been well documented that, in mycorrhizal plants, photosynthesis may increase due to the carbon sink strength of the symbioses, which increase the removal of triose-P utilisation limitation of photosynthesis, and as a consequence, more carbon is fixed per time and per unit of leaf N, resulting in higher photosynthetic N use efficiency. However, this feature was inverted during growth, because the critical %N points of the non-inoculated plants fitted a lower critical %N curve than inoculated plants. According with the N dilution theory, the lower critical %N curve imply higher N remobilisation from older senescent shaded leaves to the leaves growing in the light. The higher critical %N curve of inoculated plants presume that AM symbioses reduced N remobilisation from older to younger leaves and that in older leaves N-photosynthetic proteins as rubisco and light-harvesting chlorophyll probably kept its activity. Recently, it has been postulated that the sink strength of AM symbioses lead to an increased period of leaf activity or delayed senescence and that the higher photosynthesis prior to the period of senescence may actually lead to a longer photosynthetically active life of leaves. In accordance with this hypothesis our results shows that the leaf area of inoculated plants registered at the six N concentrations in the irrigation solution and at the last sampling date (159 days after the experiment had been set up), was equal or higher than non-inoculated plants, which suggest that leaf senescence was delayed.

The topological arrangement of the plants used in this work and the calculation of the critical N curves only with the leaves, makes the comparison of this result with others reported in literature difficult. However, these results indicate that the N availability in the soil is a key factor for the contribution of *G. intraradices* to the growth of strawberry plants and gives new information about the relation between biomass accumulation and N content along growth in inoculated plants.

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