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Full Length Research Paper

Impact of vesicular arbuscular mycorrhiza on root anatomy in *Zea mays* and *Lycopersicon esculentum*

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The absorption of water and nutrient ions from the soil solution is among the primary roles of roots. To reach the xylem for axial transport, the water and minerals must circumvent the exodermis and (or) endodermis, both of which act as barriers to radial apoplastic diffusion. To do this, the water and minerals must enter the symplast of cells located outside of the outermost apoplastic barrier. Mycorrhiza are known to impact root anatomy and ion uptake, but their effect on those cells where symplastic entry must occur is not known. To examine their impact, the surface area of those living cells apoplastically exposed to the soil solution in *Zea mays* and *Lycopersicon esculentum* was examined. For each species, plants inoculated with vesicular arbuscular mycorrhiza and uninoculated plants were examined. The average cell size and number of cells located outside the endodermis (*L. esculentum*) or exodermis (*Z. mays*) was assessed by microscopic observation and the appropriate calculations were performed. The absorptive surface area of *L. esculentum* was not significantly different between inoculated (161 cm^2/cm root length) and uninoculated (163 cm^2/ cm root length) plants. However, there was a statistically significant difference between *Z. mays* inoculated (average 37 cm^2 /cm root length) and uninoculated (average 11 cm^2/cm root length). How this may impact ion uptake pathways is discussed.

Key words: Mycorrhiza, *Lycopersicon esculentum*, *Zea mays*, ion uptake potential.

INTRODUCTION

Plant roots serve two primary purposes. First, they anchor the plant in the soil. Second, they are responsible to the absorption of water and nutrients from the soil solution. This second aspect has received a large amount of study, as it is imperative to the health of the plant. However, it is a difficult topic of study as plant root systems are generally large, convoluted, anatomically variable and hidden from view. Researchers have been obligated to undertake generalist studies of entire root systems (Eltrop and Marschner, 1996) or study small aspects via a reductionist approach (Tu et al., in press). Whole root investigations of ion uptake accounting for cellular detail are rare.

Mycorrhiza are a common association between plant roots and fungi native to the soil (Smith and Read, 1997). The most common morphotype are the vesicular arbuscular mycorrhiza (VAM). VAM appear to have been significant in the entry of plants to the land (Simon et al., 1993), likely serving as absorptive organs before the development of true roots. VAM associations are characterized by the vesicles and arbuscules that are found within the root, and the paucity of extraradicle features excepting the hyphae radiating out to explore the soil solution (Bonfante-Fasolo, 1984).

A large body of evidence suggests that VAM are important to modern plant mineral nutrition. They have frequently been associated with increased mineral nutrient acquisition by the plant partner (Barrow et al., 1977; La Rue et al., 1975; Timmer and Leyden, 1978). That being said, most studies have taken an holistic approach (Chen et al., 2005), in which it cannot be discerned as to whether the fungus absorbing the nutrient in question and passing it to the plant, or the plant is absorbing the nutri-

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Abbreviations: PAPS = potential absorbing plasmalemma surface Area.

ent independent of the fungal partner. Few studies have evidence that the fungus does indeed absorb and pass the mineral nutrient (Rufyikiri et al., 2004). Therefore, the mechanism by which VAM increase nutrient acquisition is not established.

The impact of VAM on plant root anatomy generally takes one of two scopes. There is a body of evidence that considers the root system (and plant) as whole. VAM often reduce the root to shoot ratio through a combination of an increase in shoot growth and/or a decrease in root growth (Gavito et al., 2000). Conversely, the association between the symbionts at the cellular level has been well characterized for years (Gallaud, 1905). Research of intermediate scope that considers VAM cellular impacts on the root system as a whole is lacking.

In the present work, the primary goal was to assess the cellular impact of VAM on the absorptive capabilities of root systems as a whole. This was determined by calculating the potential absorbing plasmalemma surface area (PAPS) in mycorrhizal and non-mycorrhizal root systems (Taylor and Peterson, 1999). The plant species employed were *Lycopersicon esculentum* and *Zea mays* . *L. esculentum* is not exodermal, and thus the cortical cells outside the endodermis both contribute to PAPS and represent a region of interaction with the fungal partner. *Z. mays* is exodermal, and this apoplastic barrier (Peterson, 1987) limits the PAPS and tissue available for fungal interacttion to the epidermis. These two root types were chosen as they account for the two most common anatomies, and it seemed probable that VAM association could alter their absorption-related anatomy in different fashions.

METHOD

Organisms and cultural conditions

Seeds of *L. esculentum* (American Seed) and *Z. mays* (Carolina Seed Company) were planted in autoclaved potting media in six inch pots. The soil of ten plants was mixed with Mycor^{TM} Nursery / Media Mix (Plant Health Care, Inc), containing spores of *Entrophospora columbiana* and*Glomus intraradices*, while the soil of another ten plants was not inoculated. The plants were grown in an EGC growth chamber 24 - 14^{O}C (day-night), day length 14 h, and 70% relative humidity. The plants were watered every other day.

Macroscopic measurements

For ten weeks post germination, the plant heights were measured to ascertain shoot growth. At the end of the ten weeks, the plants were carefully extracted from the potting media. Above ground tissue dry weight was determined, along with root fresh weight for each plant.

Total root length was determined by a relative mass method, and the roots were stored in 80% ethanol.

Mycorrhizal density

The method of trypan blue staining was adapted from Phillips and Hayman (1970) . To measure VAM association, roots from plants ten weeks post germination were cleared in 5% KOH at 80^{O}C for 2 h, and then acidified in 1% HCl for 60 min. Next the cleared roots were stained in a solution of Trypan blue (MP Biomedicals Inc.). The roots were then de-stained in a mixture of 500 ml glycerol, 450 ml water and 5 ml HCl for 24 h, allowing the fungus to be revealed with microscopic examination. Due to their ease of scoring, vesicles were counted to assess fungal density. The frequency of vesicles was correlated with the length of root examined.

For each of the ten plants in the inoculated and non-inoculated groups, five-one centimeter lengths of both primary and five-one centimeter lengths of secondary or higher order roots were examined.

Microscopic measurements

The PAPS was determined by calculating the total plasmalemma surface area of the cells that were alive and with access to the soil solution via a permeable apoplast. These cells include the epidermal cells and the outer tangential face of the exodermal passage cells of *Z. mays*. In *L. esculentum*, the absorptive cells include the epidermal, cortical cells and the outer tangential face of endodermal passage cells. To determine the plasmalemma surface area of the epidermal or cortical cells, the average diameter and length of each was determined. Each cell was assumed to be a regular cylinder with flat ends, and the plasmalemma surface area was assumed to be equal to the cell surface area. The PAPS contribution of an average cell was then multiplied by the average number of cells in a cross-section. Using the cell length data, the contribution of epidermal and/or cortical cells per unit length of root could be determined. The passage cell contribution was determined by multiplying the average width of the outer tangential face of a passage cell in cross-section by the average number of passage cells. This value was multiplied by 1 mm to determine the contribution per millimeter of root length. Kamula et al. (1994) has more details on these calculations.

Epidermal cells

The tangential and radial plasmalemma surface area for all cortical cells in a 1 mm length of root, S_1 (mm^2), was determined by equation 1. Each cell was treated as if it were 1 mm long (to allow the heights of the stacked cells to be combined), and the slight overestimation this introduces (by ignoring the transverse walls of each cell) was disregarded.

$$1) \quad S_1 = n(h * 2\ r_c)$$

Where h = 1 mm (assumed cell height), r_c = average radius of an epidermal cell in cross-section (mm) and n = the average number of epidermal cells in a cross-section.

The transverse plasmalemmae surface area contribution for a 1 mm length of root, S_2 (mm^2), was determined by equation 2.

$$2) \quad S_2 = n(\ r_c^{\ 2}) * 2h * h_c^{\ -1}$$

Where h_c = average epidermal cell height (mm), and the remainder of the abbreviations were as in equation 1. Note that the 2 accounts for the two membranes at the ends of the cell.

Finally, the total contribution of the epidermal cells was determined by adding S_1 to S_2.

Cortical cells

These were measured and calculated in the same manner as the epidermal cells.

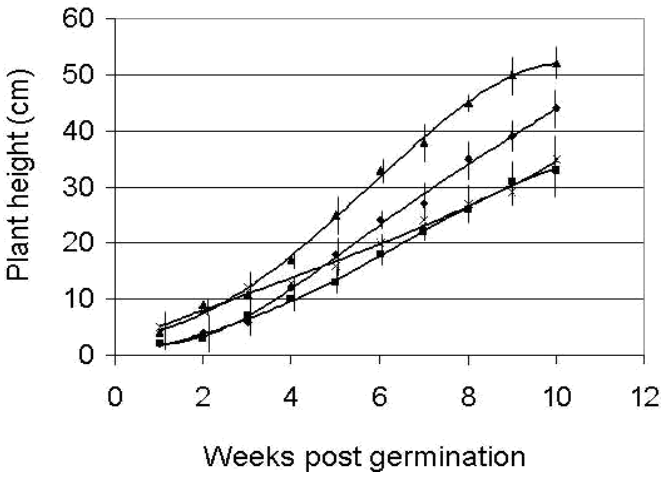

Figure 1. Average plant height (cm) for ten weeks post germination. (*Lycopersicon esculentum* inoculated = x,*Lycopersicon esculentum* uninoculated = , *Zea mays* inoculated = , *Zea mays* uninoculated =); (n = 10).

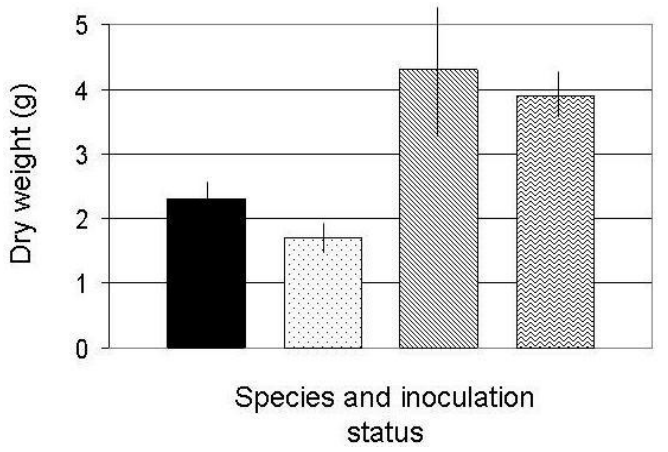

Figure 2. Average dry weight (g) of above ground biomass at ten weeks post germination (*Zea mays* uninoculated = solid bar, *Zea mays* inoculated = dotted bar, *Lycopersicon esculentum* uninoculated = diagonal line bar, *Lycopersicon esculentum* inoculated = wavy line bar); (n = 10).

Passage cells (exodermal or endodermal)

The contribution to PAPS of the passage cells per 1 mm of root length, P (mm^2), was determined by equation 3.

$$3) \qquad P = n_p * w_p * h$$

Where n_p = the average number of passage cells in cross-section, w_p = the average width of the outer tangential face of a passage cell, and h = 1 mm.

For each of the ten plants in the inoculated and non-inoculated groups, five-one centimeter lengths of both primary and secondary or higher order roots were examined. All statistical analysis was performed using an ANOVA.

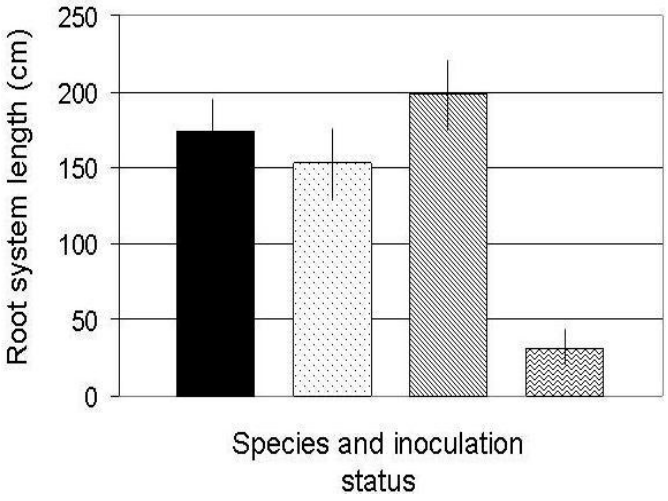

Figure 3. Average root system length at ten weeks post germination. (*Zea mays* uninoculated = solid bar, *Zea mays* inoculated = dotted bar, *Lycopersicon esculentum* uninoculated = diagonal line bar,*Lycopersicon esculentum* inoculated = wavy line bar); (n = 10).

RESULTS

Macroscopic measures

In both *L. esculentum* and *Z. mays*, the uninoculated plants grew significantly taller than did the inoculated plants (Figure 1). The difference was significant (P = 0.05) in *L. esculentum* by 4 weeks post germination and in *Z. mays* by 5 weeks post germination. This translated to a significant difference (P = 0.05) in dry weight for *Z. mays*, but not for *L. esculentum* (Figure 2). Conversely, the non-inoculated *Z. mays* root systems were not significantly different in length from the inoculated root systems, while *L. esculentum* uninoculated root systems proved to be significantly longer (P = 0.05) than their inoculated counterparts (Figure 3).

Mycorrhizal density

For both *L. esculentum* and *Z. mays*, the mycorrhizal density was significantly different between the inoculated and uninoculated groups (Figure 4). Inoculated *L. esculetum* roots averaged 21.3 vesicles per cm root length, while inoculated *Z. mays* roots averaged 7.2 vesicles per cm root length. This was significantly greater (P = 0.05) than either of the uninoculated groups, which averaged less than one vesicle per cm root length.

Microscopic measurements

PAPS analysis produced surprising results (Figure 5). For *L. esculentum*, the PAPS per cm of root length in the uninoculated plants was 161 cm^2, while the inoculated plant PAPS was 163 cm^2 (not significantly different at P = 0.05). This reveals that the PAPS per unit length of root

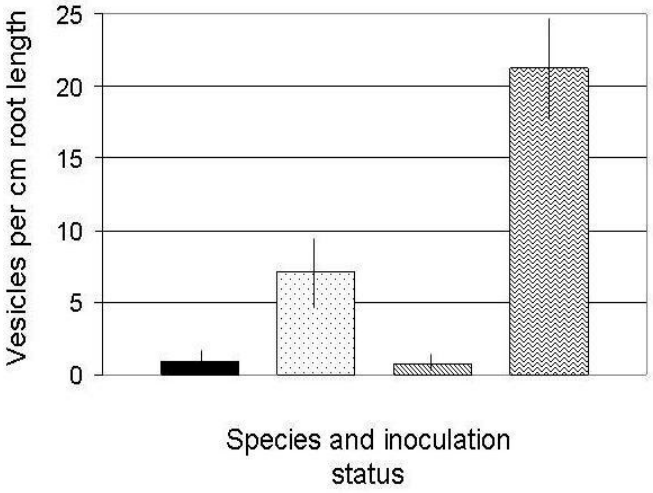

Figure 4. Vesicles per cm root length for inoculated and uninoculated *Lycopersicon esculentum* and *Zea mays* at ten weeks post germination (n =10).

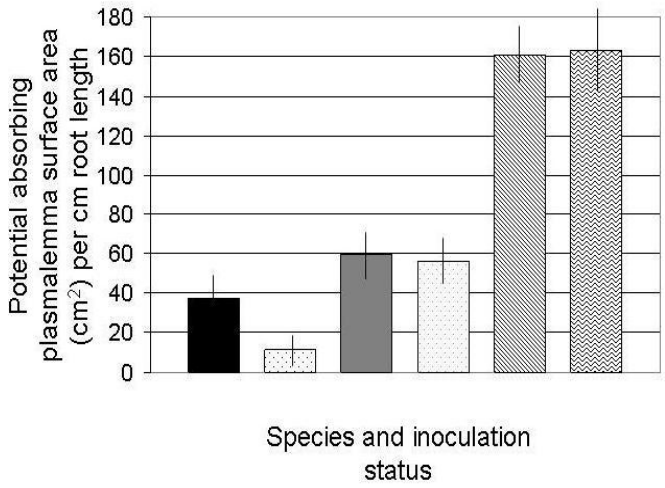

Figure 5. The potential absorbing plasmalemma surface area at ten weeks post germination. (*Zea mays* epidermis uninoculated = solid black bar, *Zea mays* epidermis inoculated = dotted black bar, *Zea mays* cortex uninoculated = solid gray bar, *Zea mays* cortex inoculated = dotted gray bar, *Lycopersicon esculentum* uninoculated= diagonal line bar, *Lycopersicon esculentum* inoculated = wavyline bar); (n = 10).

were essentially identical.

In *Z. mays*, the epidermal PAPS, representing the only absorptive area, was significantly greater (P = 0.05) in the inoculated plants (37.3 cm^2 per cm root length) compared to the non-inoculated plants (11.2 cm^2 per cm root length); (Figure 5). Conversely, the cortical PAPS, which was measured as an internal control due to these cells being blocked from the soil solution by an apoplastic barrier and thus incapable of a direct role in nutrient ion absorption, was not significantly different between the inoculated (59.8 cm^2 per cm root length) and uninocula-ted plants (56.4 cm^2 per cm root length).

DISCUSSION

The findings of this work were very interesting. The macroscopic measures revealed that the above ground portions of the uninoculated plants grew significantly larger (Figure 1), and, in the case of *Z. mays* to a greater dry weight (Figure 2), than did the plants that were VAM inoculated. This is contradictory to the majority of the literature that states that mycorrhizal plants tend to grow both more rapidly and to a greater overall height (Wright et al., 2005).

There are three possible explanations for the observed reduction in growth induced by inoculation, all of which may be related. The first is that mycorrhizal plants grown under 'ideal' conditions (that is, nutrient-rich soil, appropriate temperatures and light exposure, regular watering) as is experienced in growth chambers have been found to grow more slowly than non-mycorrhizal controls (Eissenstat et al., 1993). The second is that both high levels of inoculation (Douds et al., 1988) and inoculation with poorly compatible fungal species (Helgason et al., 2002) can act to reduce plant growth. The third is that the small size of the plants, and thus their limited photosynthetic capabilities, may have proven unable to meet the metabolic needs of the plant and fungus, therefore limiting plant growth (Daft and El-Giahmi, 1978).

The consequences of all of these explanations deserve consideration. Should the first be the dominant factor, the value of mycorrhiza in managed (that is, fertilized and irrigated) agricultural systems must be questioned. If, on the other hand, the latter two have a greater impact, than the value of commercial mycorrhizal inoculum is debatable. Most commercial inoculum, including that employed in this study; contain generalist species that are not necessarily ideal for the plant partner. As well, commercial inoculum produce very high levels of inoculation (Taylor et al., in preparation) and are usually applied to the soil pre-planting, thus subjecting germinating plants to rapid inoculation. Considered in total, the value of commercial inoculum seems highly questionable for managed soils.

These results also call into question the role of mycorrhiza in nature. Clearly they must have some value given their near ubiquitous status. It must be taken into account that our speculation pertains only to ideal conditions that plants in nature would rarely experience for an extended period of time. A plant is typically exposed to conditions in which soil-derived nutrients are lacking, and in those times mycorrhizal associations have been established as beneficial in studies e.g. (Brown and Bethlenfalvay, 1988). However, when soil nutrients are sufficiently plentiful so as to be absorbed in adequate concentration by the plant independent of the fungal partner, the maintenance of the fungal partner is superfluous. During

these times, the fungus could be likened to an insurance policy, into which the plant is paying metabolically such that it is present to provide assistance when environmental conditions prove less amenable.

Despite similar levels of vesicle density (Figure 4), the PAPS data reveals dramatic difference in the response of the plant species to VAM inoculation (Figure 5). The PAPS per centimeter in *L. esculentum* was nearly identical between the inoculated and uninoculated plants. Due to the reduction in the length of the root system, the overall PAPS of the inoculated plants were much smaller – only about 16% of the uninoculated plants. From this, it appears that VAM impacts are not prominent at the cellular level in a manner that impacts membrane availability for nutrient uptake, but instead function at the level of root growth.

Z. mays proved to be dramatically different. The PAPS of the absorptive regions was significantly greater in the inoculated plants. The impact seems likely to be influenced by VAM association, as the neighboring tissues that are not involved in either the association or ion uptake into the symplast were not affected by VAM inoculation. Unlike *L. esculentum*, there was no significant difference in root length between the inoculated and uninoculated plants. The combination produced a PAPS in the inoculated plants that was 290% that of the uninoculated plants.

The work presented here has established that the impact of VAM association ion uptake potential is highly variable. Non- exodermal *L. esculentum* showed little impact on the absorptive tissue at the cellular level, but a macroscopic impact that reduced the root system PAPS of inoculated plants. Conversely, exodermal *Z. mays* expressed no macroscopic impact, but a strong cellular impact that increased the roots system PAPS of inoculated plants. How these impacts influence the pathways of nutrient uptake can be interpreted by variable explanations. It could be argued that the non-exodermal species suffers a growth alteration that increases its dependence on the fungal partner to absorb mineral nutrients, while the exodermal species is modified in a fashion that makes it better able to absorb mineral nutrients independent of the fungal partner.

However, it must be considered that the PAPS region also corresponds to the tissue of interaction between the plant and fungal partner. Given that this is highly reduced in exodermal compared to non-exodermal species, the cellular-level modifications may have been induced to increase the interactive region between the species.

To conclude, VAM inoculation had no impact on the cellular-level PAPS of a non-exodermal species, but a positive impact on the PAPS of an exodermal species. This, along with the other findings of this work, reveals that the impacts of VAM inoculation on plant growth are com-plicated by a variety of factors, and generalizations as to their impact should be done with great caution. It is important that further studies be carried out on additional plant species in more natural settings.

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