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In vitro culture of *Gigaspora decipiens* and *Glomus clarum* in transformed roots of carrot: the influence of temperature and pH

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ABSTRACT. Monoxenic cultures of arbuscular mycorrhizal fungi (AMF) in transformed roots have been used to better understand the symbiosis with these fungi, but few species have been successfully established *in vitro*. The object was to establish monoxenic cultures of *Gigaspora decipiens* and *Glomus clarum* and to verify the effects of temperature and pH on spore formation. Cultures were established from superficially disinfested spores that were germinated on an agar and water. After germination, the spores were transferred to Petri dishes containing transformed carrot roots. After 4-6 days formed newly formed spores and structures typical. The effects of temperature (22, 25, 28 or 32°C) and pH (4.0, 4.5, 5.5 or 6.5) on the production of spores were assessed over three months, resulting in sigmoidal growth curves. The spore increased from 22°C, reaching higher values 28oC and 32oC there was a reduction in the production. The highest spore production of G. decipiens occurred in a pH of 6.5, whereas for G. clarum was pH of 4.0. The cultivation of other species is still necessary to elucidate particular aspects of the symbiosis that so far are unclear, including the effects of environmental factors on the production of spores of different AMF species.

Keywords: arbuscular mycorrhizal fungi, monoxenic culture, *in vitro* culture, monoxenic inoculum, transformed roots.

Cultura *in vitro* de *Gigaspora decipiens* e *Glomus clarum* em raízes transformadas de cenoura: influência da temperatura e pH

RESUMO. Culturas monoxênicas de fungos micorrízicos arbusculares (FMAs) em raízes transformadas têm sido utilizadas para melhor compreensão desses fungos, porém, poucas espécies foram estabelecidas *in vitro* com sucesso. O objetivo do trabalho foi estabelecer culturas monoxênicas de *Gigaspora decipiens* e *Glomus clarum* e verificar o efeito da temperatura e pH na produção de esporos. As culturas foram obtidas de esporos desinfestados e germinados em Agar-água. Após a germinação foram transferidos para placas de Petri contendo raízes de cenoura transformadas, em meio mínimo. Após 4 a 6 dias formou-se novos esporos e estruturas típicas. O efeito da temperatura (22, 25, 28 e 32ºC) e pH (4,0; 4,5; 5,5 e 6,5) na produção de esporos foram analisados por 3 meses, obtendo-se curvas sigmoidais. Os esporos aumentaram a partir de 22ºC, atingindo valores maiores em 28ºC e em 32ºC reduziu-se a produção. A maior produção de esporos de *G. decipiens* ocorreu em pH 6,5, enquanto *G. clarum* em pH 4,0. O cultivo de outras espécies faz-se necessário visando o conhecimento de aspectos particulares da simbiose até hoje não elucidados, incluindo-se os efeitos dos fatores ambientais sobre a produção dos esporos das diversas espécies de FMAs.

Palavras-chave: fungos micorrízicos arbusculares, cultura monoxênica, cultura *in vitro*, inoculum monoxênico, raízes transformadas.

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligatory symbionts that colonize the roots of approximately 80% of terrestrial plants, improving their nutrition, growth and disease tolerance (SMITH; READ, 2008; ELSEN et al., 2008). The difficulties in obtaining axenic cultures of these fungi have not been fully overcome. Cultivation techniques based on Agrobacterium rhizogenes-transformed roots and untransformed roots provide the establishment of the AMF in vitro (BÉCARD; FORTIN, 1988; ADHOLEYA et al., 2005; IJDO et al., 2011). Once obtained, monoxenic cultures of AMF enable continuous observations of fungal colonies, and the organization of the mycelium as well as the sporulation steps can be monitored. Additionally, this technique allows studies that lead to

better knowledge of mycelium development (BAGO et al., 1998a; MAIA et al., 2010), functional aspects of the symbiosis (DEBIANE et al., 2009), dynamics of sporulation (DECLERCK et al., 2001 and 2004; IJDO et al., 2011; VOETS et al., 2009), spore ontogeny (DE SOUZA et al., 2005; MAIA et al., 2010), reproduction and nutritional requirements over the life cycle of these fungi (LABIDI et al., 2011; IJDO et al., 2011) and production of viable inoculum that is free of contaminants (IJDO et al., 2011; TIWARI; ADHOLEYA, 2002; VOETS et al., 2009). However, only a few species of AMF have been successfully established *in vitro* (TIWARI; ADHOLEYA, 2002; IJDO et al., 2011). From the literature and culture collections, it is estimated that over 100 different strains are maintained *in vitro*. For instance, ginkgo harbors at least 20 species and 30 strains of AMF from the families Glomeraceae and Gigasporaceae (IJDO et al., 2011).

Spores are the most important propagules for most AMF. However, even today, little is known about the sporulation dynamics of some species, with research focusing more on spore germination (DECLERCK et al., 2001, 2004). Knowledge of the process of spore production and the impact of environmental factors on sporulation is still limited (DECLERCK et al., 2001). Currently, it is known that spore production in monoxenic cultures of AMF follows a sigmoidal dynamic, composed of the three classical stages (lag, log and stationary) of development (DECLERCK et al., 2001, 2004; VOETS et al., 2009). Spore germination and the establishment of monoxenic cultures of mycorrhizal fungi may be affected by several factors, such as the presence of root exudates or volatiles, flavonoids, substrate composition, humidity, light, CO_2, nutrient availability, presence of contaminants, temperature, and pH (DECLERCK et al., 2004; IJDO et al., 2011; MAIA; YANO-MELO 2001; MAIA et al., 2010). The pH of the substrate influences the germination of AMF, the development of hyphae, the percentage of root colonization, the formation of intraradical structures, and the density of spores (GORANSSON et al., 2008, MAIA et al., 2010; PANWAR et al., 2011; VARGA; KYTÖVIITA 2010). AMF species require different pH ranges for development, with the optimal conditions for germination and mycelial growth depending on the species, but the most favorable range for most species is between pH 6.0 and 7.0 (MOREIRA; SIQUEIRA, 2006; POSTMA et al., 2007). A negative correlation was established between spore density and pH (PANWAR et al., 2011). The germination and development of the hyphae of *Glomus mosseae* (MOREIRA; SIQUEIRA, 2006) and *Glomus intraradices* (MOSSE, 1988) were inhibited in medium with low pH values.

Temperature is another factor that influences spore germination, establishment of the AMF, root colonization (BARRETT et al., 2011; MAIA et al., 2010; WU; NING ZOU, 2010) and spore density (PANWAR et al., 2011). The ideal temperature for germination of *A. laevis* is 20°C, while the optimum for the growth of hyphae ranged from 15 to 25°C (TOMMERUP, 1983). Temperatures between 18 and 25°C are ideal for germination of spores of *Glomus versiforme*, whereas temperatures above 35°C or below 15°C are detrimental (SIQUEIRA; HUBBELL, 1985). In two of the four fields studied in the Bundelkhand region in central India, with temperatures ranging from 15.1 to 29.4°C, the density of AMF spores increased with increasing temperatures (PANWAR et al., 2011). On the other hand, high temperatures (35 and 40°C) had no effect on the development of mycorrhizal plants (ZHU et al., 2010).

To scale up AMF inoculum production, it is necessary to identify and select the species with the maximum potential spore production (DECLERCK et al., 2001, IJDO et al., 2011). In addition, studies on the environmental conditions that increase or stimulate AMF sporulation in monoxenic cultures are needed. Here, we aimed to establish *in vitro* culture of *Gigaspora decipiens* and *Glomus clarum* to study the effects of temperature and pH on spore production in these species.

Material and methods

Transformed carrot (*Daucus carota* L. cultivar Nantes) roots were obtained according to the methodology described by Bécard and Fortin (1988). *Agrobacterium rhizogenes* strain R1601 was obtained from the Plant Tissue Culture Laboratory /BIOAGRO/UFV and contains the plasmid pR and the cosmids pTVK 291 and 1500. Once induced, the carrot roots were transferred every 30 days to a freshly prepared minimal medium (M), pH 5.5, and incubated at 28°C (BÉCARD; FORTIN, 1988).

The experiments followed the biosafety standards established by CTNBio (National Technical Commission on Biosafety, Brazil), with the selective disposal by incineration of all materials involved in transgenic experiments.

Spores of *Gigaspora decipiens* (Hall and Abbott) and *Glomus clarum* (Nicolson and Schenck) were multiplied in *Brachiaria decumbens* (Stapf and Prain) plants grown in pots containing a sand and clay (2:1,

v v^{-1}) substrate, pH 5.0 to 5.5, and maintained in a greenhouse for 3 months. AMF spores were extracted by wet sieving the soil and sucrose gradient centrifugation (GERDEMANN; NICOLSON, 1963; FURLAN et al., 1980), selected under a stereomicroscope and placed in a glass "vacutainer" tube. The AMF spores were subsequently surface-sterilized according to Bécard and Piché (1992) in a solution of 0.05% Tween (v v^{-1}) for 1 min., a solution of Chloramine T 2% (w v^{-1}) for 10 min., then in a solution of streptomycin (0.02% w v^{-1}) and gentamicin (0.01% w v^{-1}) for 10 min., three times. All disinfection procedures were repeated twice under aseptic conditions. Subsequently, the spores were transferred to a Petri dish containing agar-water, pH 6.0, incubated at 28°C and monitored daily under a stereomicroscope for germination.

To obtain the monoxenic cultures, an apical segment of transformed carrot root (5-7 cm) was transferred to a Petri dish (90 mm diameter) containing M medium, pH 5.5, solidified with 0.4% Phytagel® (SIGMA CHEMICAL COMPANY, USA). Three cubes (0.5 x 0.5 x 0.5 cm) of water-agar containing spores germinated on the roots were placed upside down. The plates were incubated in the dark at 28°C and monitored for AMF structure formation on a weekly basis. After 60 days, the roots were stained with trypan blue (PHILLIPS; HAYMAN, 1970; GIOVANNETTI; MOSSE 1980).

Spore production of *G. decipiens* and *G. clarum* was evaluated at temperatures of 22, 25, 28 and 32°C. A segment (5-7 cm) of apical transformed carrot root was transferred to Petri dishes containing medium M, pH 5.5, plus 0.4% Phytagel®. Then, three cubes (0.5 x 0.5 x 0.5 cm) of medium M containing 2-month-old fungal inoculum (spores, hyphae and colonized root) were placed on the plates and incubated in the dark at the above-mentioned temperatures. Spore production was evaluated only in plates where all three cubes showed fungal growth. The spores formed were counted weekly under a stereomicroscope over a 3-month period.

The effect of pH (4.0, 4.5, 5.5 and 6.5) on the production of spores was evaluated using culture medium M, incubation at 28°C, and the same procedure as previously described.

The experiments were conducted in a factorial split-plot in time, in which the temperatures, pH values and fungi were the main plots, and the time periods of cultivation were represented as sub-plots in a randomized design with 10 repetitions. The data obtained were transformed in logarithm (X + 1) for analysis of variance and subsequently submitted to regression analysis. Tukey's test at 5% probability was applied to the regression coefficients.

Results and discussion

Infection of carrot roots with *A. rhizogenes* resulted in callus formation in the sections after 3 weeks and differentiation of typical transformed hairy-roots in the 4th week. After subculturing on MS-based medium with antibiotics, the transformed roots were successfully freed from bacteria.

All structures that characterize the typical genus *Gigaspora* were observed in monoxenic culture of *G. decipiens*, with only minor differences in the germination process and smaller spores compared to those already described for the species (MAIA; YANO-MELO 2001; DE SOUZA et al., 2005). Spores of the two AMF began to emit germ tubes starting on the 4th to 5th days in water-agar (Figures 1A and 2A); germination was irregular and extended until the 25th day. The spores of *G. decipiens* produced more than one germ tube from each subtending hypha (Figure 1A), while *G. clarum* produced a single germ tube from the same structure (Figure 2A). After transferring germinated spores to plates containing transformed roots in medium

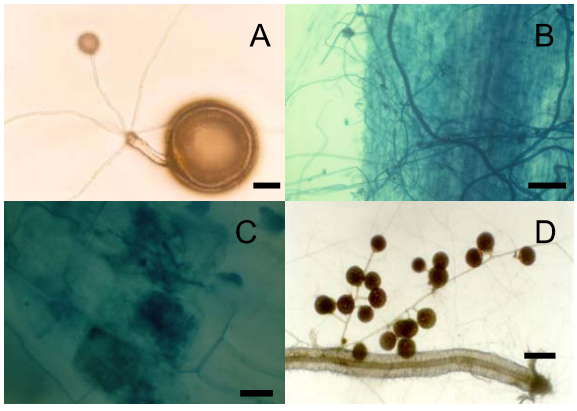


Figure 1. *In vitro* of *Gigaspora decipiens* spore germination and development in transformed carrot roots. A. Spore germination, showing detail of the germ tube. B. External hyphae and internal to the root. C. Mycorrhiza. D. Groups of spores produced *in vitro* in the intermediate or terminal position. Scales: 50 µm (A), 0,01 mm (B), 2 µm (C), 100 µm (D).

M, we observed the growth and branching of the hyphae from the germ tube. The hyphae grew toward the roots, associated externally and internally with the roots and formed typical arbuscular structures of mycorrhiza (Figures 1B and C, 2B) and vesicles (Figure 2B). In spores of *G. margarita*, several hyphae germinate from the wall of the spore, near the subtending hyphae (KARANDASHOV et al., 1999; MAIA et al., 2010). The multiple germ tubes in *Gigaspora* species may be formed in response to stimulatory substances in the environment, conditions of the substrate, genetic

information of the spore, and the high number of nuclei close to the wall (MAIA; YANO-MELO 2001; DE SOUZA et al., 2005). During hyphal growth, new spores were formed after 4-6 days of contact between the fungus and the root (Figures 1A and 2A). The spores of *G. decipiens* were formed in a terminal or intercalary position in sporogenous hyphae, in groups or alone (Figure 1D), whereas the spores of *G. clarum* were always present in the intercalary position in the sporogenous hyphae, in groups or alone (Figures 2A and C). The mean diameters of mature spores of *G. clarum* and *G. decipiens* were 50-100 µm and 70-180 µm, respectively, which were smaller than the original spores extracted from soil.

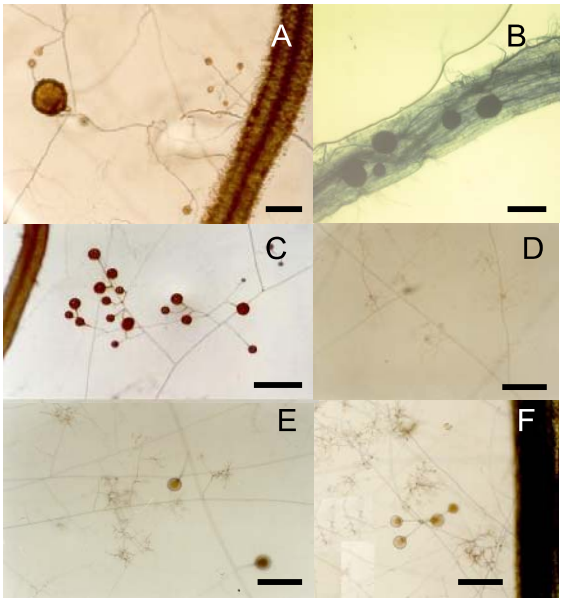


Figure 2. *In vitro Glomus clarum* spore germination and development in transformed carrot roots. A. Spores germinated spores formed with the new *in vitro* and detail of the germ tube. B. Presence of external and internal hyphae and vesicles to the root. C. Spores produced *in vitro* in a mid-term group or individual position. D-F. Development of extraradical mycelium in the middle, accompanied by the formation of numerous structures called mycorrhiza-like structures (ALS) or absorving branched structures (BAS), these structures are easily observed when crowded. Scales: 100 µm (A and B), 200 µm (C and D), 300 µm (E and F).

The multiple germination recorded in spores has been presented as a possible strategy to perpetuate the species, whereas AMF, as obligatory biotrophic species, need to join a compatible host to survive (MAIA et al., 2010).

In addition to the typical structures of arbuscular mycorrhiza, helper cells were also observed and "mycorrhiza-like structures" (ALS) or "branched absorbing structures" (BAS) were observed. Typical auxiliary cells were formed by *G. decipiens* after hyphal branching that occurred 3-4 days after the germinated spores came into contact with the roots, showing arrangement of cells with numerous spike-like globular projections (Figures 3A and B). The typical auxiliary cells of *G. decipiens* had a similar pattern to those described for *G. margarita* (KARANDASHOV et al., 1999) and *Scutellospora reticulata* (DECLERCK et al., 2004). The characteristics of *G. clarum* in relation to the emergence of the germ tube and the process of spore formation were similar to those described by Maia et al. (2010).

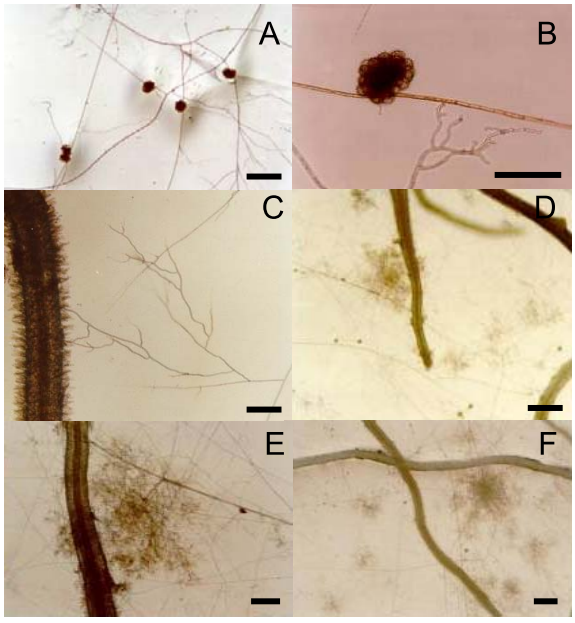


Figure 3. Specialized structures formed by *G. decipiens in vitro*. A. Auxiliary cells through M medium. B. Details of auxiliary cells presenting globular-shaped spikes on the edges. C. Branching hyphae producing structures called ALS or BAS. D. Development of extraradical mycelium in the middle, accompanied by the formation of ALS or BAS, showing their distribution and arrangement on the M medium and the presence of spores after 60 days of cultivation. E. Detail of the agglomeration of ALS or BAS, with shape similar to intracellular arbuscules. F. Aspect of the amount and distribution of ALS or BAS, formed at irregular intervals. Scales: 300µm (A), 100µm (B e C), 200µm (E), 400µm (D e F).

During the establishment of cultures of *G. clarum* and *G. decipiens* (4-8 weeks), the density of hyphae on the surface of the medium increased considerably (Figures 3C to F and 2D to F). The thicker vegetative hyphae branched at irregular intervals producing thinner vegetative hyphae (Figures 2D and 3C).

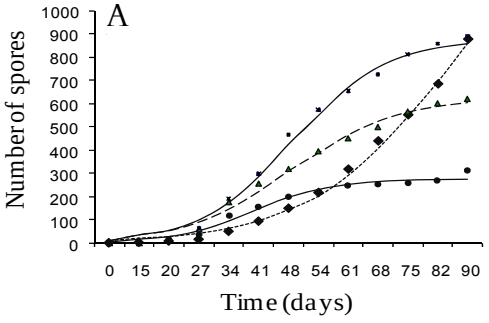
Significant levels of ALS/BAS formation were generated by both fungi, at irregular intervals, associated with groups of spores or isolated, on the surface of culture medium (Figures 3C to F and 2D to F). The formation of these structures increased over time, and after two months of cultivation of *G. decipiens*, forming tangled of hyphae, which were

very similar to the intracellularly produced arbuscules (Figure 3D to F). *In vitro* cultures of *G. clarum* also showed high production of ALS/BAS, but not at the same frequency and distribution found for *G. decipiens* (Figures 2D to F). The forming numerous highly branched dichotomous structures named ALS or BAS, similar to those observed by Bago et al. (1998a). The development of the extraradical mycelium of AMF in culture medium is usually accompanied by the production of these structures (BÉCARD; FORTIN, 1988; BAGO et al., 1998b, DECLERCK et al., 2004).

The arrangement formed by the grouping of ALS/BAS of *G. decipiens* differs from that of *G. intraradices* (BAGO et al., 1998a), in which the vegetative hyphae grew straight and branched at angles of 45°, forming ALS/BAS at regular intervals from 25 to 300 µm. Conversely, the ALS/BAS structures of *G. decipiens* were formed at irregular intervals, occurring in certain areas of the culture medium, as clusters and irregular arrangements, similar to intraradical arbuscules (Figure 3). In the case of *G. clarum*, the ALS/BAS were formed in lower numbers, with dichotomous branching to form small and thin hyphae at irregular intervals, but not so much overlapping as observed in *G. decipiens* (Figure 2). Thus, the arrangement formed by groups of ALS/BAS seems to differ between species grown in culture medium. The ALS/BAS structures are considered a specific response to a greater or lesser interaction of the fungus with the roots or a localized response to unknown stimuli in the culture medium (BÉCARD; FORTIN, 1988; BAGO et al., 1998b). Karandashov et al. (2000) suggested that the development of monoxenic culture leads to changes in the culture medium, such as the decrease in pH to levels corresponding to the apoplastic pH or the accumulation of certain metabolites in roots. These changes may represent the signal for the fungal extraradical mycelium to begin forming typical structures, such as mycorrhiza. The ALS/BAS and arbuscules share many ultrastructural similarities and ALS/BAS may play an important role as a preferred site of nutrient absorption mediated by AMF extramatrical mycelium, providing increases in the nutrient supply to the associated spores (BAGO et al., 1998a). The high number of ALS/BAS structures produced by the highly branched *G. decipiens* hyphae suggests that this fungus can provide the most efficient nutrient absorption from the culture medium because ALS/BAS increase the substrate-fungus contact surface (BAGO et al., 1998a). The formation of these structures in the soil can provide benefits to associated plants, such as increased acquisition of nutrients and water as well as stabilization of the soil structure with improved porosity. In future studies, the dynamics of nutrient uptake by this species of fungus should be compared to other species.

Spore production of *G. decipiens* and *G. clarum* at the tested temperatures exhibited a sigmoidal curve dynamic, with typical lag, log and stationary phases (Figures 4A and B). The dynamics of sporulation of *G. decipiens* and *G. clarum* in monoxenic culture are best described by sigmoidal patterns, which have three distinct classic stages: lag, log and stationary. These dynamics of sporulation also occur in *G. versiforme*, *G. caledonium*, *G. intraradices* and *Scutellospora reticulata* (DECLERCK et al., 2001, 2004).

$\hat{Y}22 = 1326{,}3442/(1+190{,}2377*EXP(-0{,}0651*t))$ $r^2 = 0{,}9689$
$\hat{Y}25 = 620{,}3605/(1+56{,}0584*EXP(-0{,}0838*t))$ $r^2 = 0{,}9242$
$\hat{Y}28 = 876{,}4396/(1+104{,}3624*EXP(-0{,}0956*t))$ $r^2 = 0{,}9393$
$\hat{Y}32 = 274{,}2316/(1+91{,}5047*EXP(-0{,}1133*t))$ $r^2 = 0{,}9157$



$\hat{Y}22 = 597{,}7859/(1+98{,}6778*EXP(-0{,}0785*t))$ $r^2 = 0{,}9747$
$\hat{Y}25 = 835{,}1416/(1+47{,}2775*EXP(-0{,}0748*t))$ $r^2 = 0{,}9673$
$\hat{Y}28 = 847{,}624/(1+62{,}7912*EXP(-0{,}0859*t))$ $r^2 = 0{,}9415$
$\hat{Y}32 = 275{,}0899/(1+63{,}0789*EXP(-0{,}1161*t))$ $r^2 = 0{,}9847$

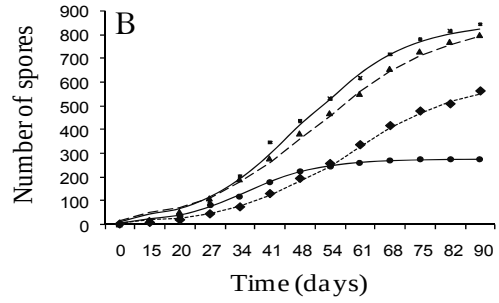


Figure 4. Effect of temperature on *Gigaspora decipiens* (A) and *Glomus clarum* (B) spores formation in transformed roots of carrots on M medium, ph 5.5, in function of the period of time. 22ºC (♦); 25ºC (▲); 28ºC (■) e 32ºC (●).

In general, at all temperatures, the production of spores initially grew slowly and gradually up to 27 days of cultivation, with a sharp increase up to 76 days, and thereafter, spore production stabilized. For *G. decipiens* grown at 22°C, a tendency to increase spore production after the 90th day was observed. Also, the production of fungal spores by both AMF gradually increased with temperature from 22 to

28°C, but decreased sharply at 32°C. At this temperature, the root and mycelial growth lessened and spore sizes were smaller. The influence of temperature on spore production was more pronounced at 27 days of incubation. This effect is represented by a quadratic curve obtained from this date until the 90th day of evaluation, for both fungi, according to the models expressed by $Y_{GD} = -4600.60 + 375.8\ T^{-7}, 08T^2$ ($r^2 = 0.7829$) and $Y_{GC} = -5777.26 + 464.23\ T^{-8}, 69T^2$ ($r^2 = 0.9933$). The estimated optimum temperatures for spore production were 26.5 and 26.7°C for *G. decipiens* and *G. clarum*, respectively. By Student's test ($p < 0.05$), the regression coefficients for the number of spores did not differ among the temperatures of 22, 25 and 28°C. Comparing spore production, Tukey's test ($p < 0.05$) revealed that there was no difference between *G. decipiens* and *G. clarum* in relation to production of spores (Figures 4A and B). The production and size of spores of both fungi were influenced by temperature (Figure 4A and B). This effect for mycorrhizal fungi grown *in vitro* has not been yet reported in the literature, although spore germination studies have demonstrated a decrease in the percentage of germination of *G. clarum* as temperature increased, with inhibition at 35°C (LOUIS; LIM 1988). High temperatures affect cell survival, possibly by interfering with their metabolic activity (TOMMERUP; KIDBY, 1980). Decreases in growth, in the percentage of root colonization and in the numbers of arbuscules and vesicles are observed when there is an increase in temperature (VOGELZANG et al., 1993; ZHU et al., 2010). These authors suggested that high temperatures directly affect the ability of the fungi to grow, colonize and establish themselves within the root of the plant, and that there are variations between species of AMF in the face of changes in growth temperature (VOGELZANG et al., 1993).

The production of *G. clarum* spores showed a trend of gradual increase when grown at temperatures higher than 22°C, and these values did not differ from the temperature of 28°C. In *G. decipiens*, there was a gradual increase in the production of spores at a temperature of 22°C, with age growing, and after three months, the number of spores was similar to that produced when the fungus was grown at 28°C. This behavior indicates a possible adaptive response of *G. decipiens* to low temperatures.

Spore production by *G. decipiens* and *G. clarum* at different pH values was also characterized by sigmoidal curves (Figure 5A and B). At all pH values tested, the production of spores initially grew slowly until the 24th day of cultivation, with sharp increases through the 58th or 65th days, followed by a slow decline with a tendency to stabilize. The influence of pH on the production of spores from the 10th to 86th days of cultivation was pronounced.

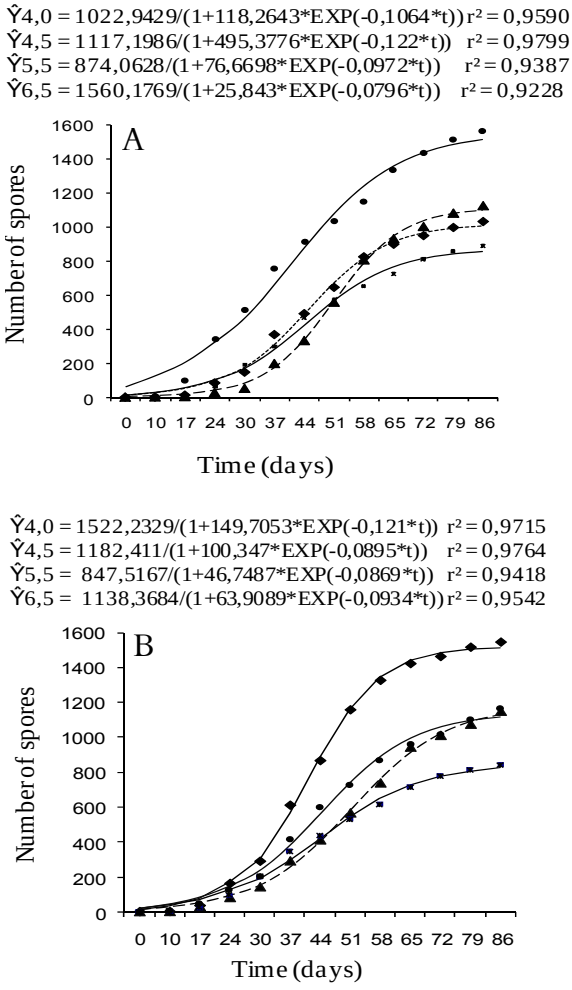


Figure 5. Effect of pH on *Gigaspora decipiens* (A) and *Glomus clarum* (B) spores formation in transformed roots of carrots on M medium, at 28ºC, in function of the period of time. 4,0 (♦); 4,5 (▲); 5,5 (■) e 6,5 (●).

G. decipiens and *G. clarum* showed different behaviors in relation to the pH levels tested. For *G. decipiens*, the greatest spore production occurred at pH 6.5, whereas for *G. clarum*, it occurred at pH 4.0 (Figure 5A and B).

For *G. decipiens*, spore production did not differ in medium at pH 4.0, 4.5 and 5.5, while for *G. clarum*, there were no difference in spore production in medium at pH 4.5, 5.5 and 6.5. Spore production values for *G. decipiens* at pH 6.5 were similar to those for *G. clarum* grown at pH 4.0 (Figure 5A and B). The increased production of spores by *G. decipiens* in a medium with a near-neutral pH (6.5) and *G. clarum* at a more acidic pH (4.0) confirms the

need to establish the optimal pH of the culture medium for the production of spores for each species of AMF and also verified that spore germination requires an optimal pH for each species (VAN AARLE et al., 2002). In certain natural soils, AMF spores occur within a certain range of pH. For example, spores of *G. margarita* and *G. mosseae* were found in soils with a pH greater than 5.5 (SIEVERDING, 1991). In contrast, the presence of acidic zones in the culture medium coincides with an increased production of spores of *Glomus intraradices* (BAGO et al., 1998b). The mycelial growth, spore formation, spore germination and root colonization processes are inhibited by low pH in the substrate (GORANSSON et al., 2008; MAIA et al., 2010; PANWAR et al., 2011; POSTMA et al., 2007; VAN AARLE et al., 2002; VARGA, KYTÖVIITA, 2010).

In studies with acidic conditions, it was observed that certain AMF species are more frequent in this environment than in soils with higher pH (POSTMA et al., 2007; GORANSSON et al., 2008). The growth of plants and the percentage of mycorrhizal root colonization may vary depending on the isolates of AMF and the soil pH, characteristics of adaptive responses of isolates to edaphic conditions (GORANSSON et al., 2008; POSTMA et al., 2007; VARGA, KYTÖVIITA, 2010). Among several AMF species tested in acid soils, *G. clarum* was found to provide larger amounts of shoot and root dry mass in *Panicum virgatum* L., at pH 4.0 (CLARK et al., 1999).

The dynamics of AMF sporulation are poorly known because the methods of spore counting are generally destructive, requiring successive rhizospheric samples for observations of sporulation, which in the long-term often becomes impractical (BAGO et al., 1998b). Monoxenic cultivation of AMF can be used to follow the dynamics of sporulation without causing disturbances in the intimate coexistence of the fungus with its host (DECLERCK et al., 2001; IJDO et al., 2011). The influence of environmental factors on sporulation of AMF could be studied using the technique of *in vitro* monoxenic culture of two AMF. Thus, it was possible to demonstrate that environmental factors may interfere with the dynamics of AMF sporulation and that spore production can be optimized by controlling these environmental factors.

The number of AMF species known to have been successfully cultivated *in vitro*, using the technique transformed roots or not associated with these fungi is limited (IJDO et al., 2011; TIWARI, ADHOLEYA, 2002). Several species of *Glomus* and few species of *Gigaspora* were established in root cultures *in vitro*. This study represents the first report of *in vitro* culture of *G. decipiens* in transformed carrot roots. The cultivation of other species is necessary for elucidation of the ecological, physiological and genetic aspects of mycorrhizal symbiosis that still remain unknown.

Conclusion

The monoxenic cultures of *G. clarum* and *G. decipiens* were successfully established in transformed root of carrot. The best conditions for production of spores of *G. decipiens* in culture medium are pH 6.5 and 28ºC and pH 4.0 and 28ºC for *G. clarum*. The cultivation of other species of AMF is still necessary to elucidate particular aspects of the symbiosis that so far are unclear, including the effects of environmental factors on the production of spores of different AMF species.

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