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Turgor Changes in *Morchella esculenta* during Translocation and Sclerotial Formation

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AMIR, R., STEUDLE, E., LEVANON, D., HADAR, Y., AND CHET, I. 1995. Turgor changes in *Morchella esculenta* during translocation and sclerotial formation. *Experimental Mycology* 19, 129–136. Turgor pressure was measured during six stages of growth and pseudosclerotial formation in *Morchella esculenta* indirectly (by thermocouple psychrometer) and directly (by cell pressure probe). The fungus was grown on a split plate, enabling separation between mycelium growing on defined medium (water potential −0.5 MPa) and sclerotia which formed on glucose noble agar (water potential −2.1 MPa). Under these conditions, nutrients were translocated from the mycelium to the developing sclerotia. Direct turgor potential measurements showed that the gradient between the mycelium and the sclerotia increases during sclerotial development (reaching a maximum of 0.53 MPa), thereby suggesting that translocation is a turgor-driven mass flow. During sclerotial development, the turgor potential in the peripheral tips of the sclerotial hyphae must be high enough to bring about the growth of the numerous hyphae, which comprise the sclerotium, and simultaneously low enough in the primary hyphae, which carry the stream of nutrients, to attract translocation from the mycelium. Since sclerotial hyphae are too small for direct measurement by cell pressure probe, a psychrometer was used, revealing high turgor in the sclerotial tissue (1.2 MPa) during sclerotial development. Direct measurement in the primary hyphae at this time gave a value of 0.7 MPa. Taken together, these measurements indicate the presence of a turgor gradient inside the sclerotial tissue, from the primary hyphae to the peripheral cells. The present study is the first to make use of a cell pressure probe to measure turgor gradients in a fungus during translocation followed by sclerotial morphogenesis. © 1995 Academic Press, Inc.

INDEX DESCRIPTORS: turgor; *Morchella;* sclerotia; cell pressure probe; translocation; water/osmotic potential.

One of the critical requirements for the formation of fruiting bodies from sclerotia of *Morchella esculenta* Pers. is the separation of sclerotia from the mycelium (Ower *et al.*, 1986). This condition is met when the fungus is grown by the split-plate method, due to the differences in nutrient content and water potential between the media on either side of the plate: sclerotia formed on the side containing Noble agar amended with glucose (GNA),[1] mycelium on the side containing defined medium (DM). Inoculum was placed on the GNA side of the plate and the hyphae grew towards the DM side (Amir *et al.*, 1992).

Measurements of [^{14}C]3-*O*-methyl glucose and [^{14}C]D-glucose translocation during growth and sclerotial formation (Amir *et al.*, 1994, 1995) have shown the existence of a source-sink relationship between mycelium and sclerotia. Translocation takes place through the primary hyphae, which connect the mycelium to the sclerotia and undergo morphological adaptations to carry this stream (Amir *et al.*, 1993). The direction of translocation was seen to change three times: (a) During the extension of the colony, translocation was towards the front of the mycelium on the DM side, as well as to secondary hyphae which continued to develop on the GNA side. (b) When the mycelium reached the edge of the plate on the DM side, reverse translocation was observed, as a result of sclerotial formation (i.e., from the younger parts of the colony to the older parts). (c) At

[1] Abbreviations used: GNA, glucose noble agar; DM, defined medium; PDA, potato dextrose agar.

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maturation, the direction of translocation reversed again, from sclerotia to mycelium. Measurements of the rate at which biomass accumulated in the sclerotia and the rate of movement of radiolabeled material to the sclerotia suggested that the mechanism of translocation consists of turgor-driven mass flow (Amir *et al.*, 1994).

Different mechanisms of translocation have been described for various fungi. More than one mechanism of translocation might be operating simultaneously in the same fungus, with one dominating for a certain type of translocation and under certain conditions. Olsson and Jennings (1991a,b) showed that translocation is brought about by simple diffusion, in three different molds. Cowan *et al.* (1972), calculating the isotope translocation in sporangiophores of *Phycomyces*, also assumed that translocation occurs via diffusion. Allen *et al.* (1980) and De Lucas *et al.* (1993) suggested another mechanism, whereby intracellular transport and translocation occur as a result of cytoskeleton activity. Turgor-driven mass flow is a third possible mechanism. To prove its existence, a turgor gradient between source and sink must be shown. This mechanism was demonstrated by Eamus and Jennings (1984) and Thompson *et al.* (1985), who showed the existence of turgor gradients in special structures, e.g., mycelial mats, rhizomorphs, and cords in *Armillaria mellea, Phallus impudicus, Phanerochete velutina,* and *Serpula lacrimans,* under both laboratory and field conditions. To date there has been little evidence of this mechanism in the mycelium or separated hyphae, apart from the work of Robertson and Rizvi (1968), who showed evidence of turgor gradients via the translocation of isotopes in *Neurospora crassa.* They estimated turgor gradients of 0.5 MPa between the tip and the basal region. In addition, Girvin and Thain (1987) suggested, from measurements of translocation of dry weight in the same fungus, that translocation occurs via nonspecific mass flow. This evidence was derived only from indirect methods of measurement (psychrometry, incipient plasmolysis technique, or calculating the rate of transport of isotopes).

Measurement of turgor in the hyphae is important for another reason: turgor is the driving force behind hyphal growth and extension. However, measurements for this purpose have also been indirect, by psychrometry (Eamus and Jennings, 1984, 1986a; Thompson *et al.*, 1985), osmometry (Adebayo *et al.*, 1971; Luard and Griffin, 1981; Woods and Duniway, 1986; Kaminskyj *et al.*, 1992), or incipient plasmolysis (Money, 1990; Kaminskyj *et al.*, 1992).

The first direct measurement of turgor in fungi was performed by Money (1990) and Money and Harold (1992) on *Achlya,* using a cell pressure probe originally developed for plants by Husken *et al.* (1978). Money (1990) found that turgor was the same throughout the length of each individual hypha and its branches.

The work reported here describes a quantitative study of turgor gradients during growth and sclerotial formation in *Morchella.* This is the first study to measure turgor gradients directly during translocation, suggesting that translocation consists of turgor-driven mass flow. Efforts were also made to measure turgor in sclerotial hyphae during their development. High turgor, at this stage, is important to hyphal extension and essential to sclerotial formation (Amir *et al.*, 1992).

MATERIALS AND METHODS

Experimental Setup

M. esculenta was grown on a split plate containing 10 ml GNA, i.e., 2% (w/v) Noble agar (Difco) amended with glucose (90 g liter^{-1}), on one side, with a water potential of −2.1 MPa, and 10 ml DM, with a water potential of −0.5 MPa, on the other (Amir *et al.*, 1994). Dishes were covered with autoclaved cellophane. Disks (4 mm in diameter) from a 5-day culture of *M. esculenta* grown on potato dextrose agar (PDA) medium were transferred to the edge of the plates, on the GNA side. After this inoculation, the plates were incubated at 27°C. Hyphae from five plates were sampled at each stage of development (Amir *et al.*, 1994) to determine their

fresh and dry weights (FW and DW, respectively), and water content in the tissue was calculated.

To determine turgor by the cell pressure probe, method plates were prepared as described above, with one difference. Media of the same composition—GNA or DM (3 ml) were poured on top of the cellophane but at a higher agar concentration (30 g liter^{-1} vs 20 g liter^{-1}). The agar at higher concentration is stiffer, thus supporting mechanically the hyphae in the media, allowing the capillary to penetrate without excessive movement of the hypha. This minimized leakage and pressure drops during measurements.

As a control, the fungus was grown on DM medium in a regular plate. The plate was covered with cellophane and DM with concentrated agar was placed on it. The fungus was inoculated in the center of the plate.

Psychrometric Techniques

Water and osmotic potentials were measured by means of a Wescor HR-337 microvoltmeter coupled to a C-52 chamber (Logan, UT) in dewpoint mode, according to Thompson *et al.* (1985). Samples were removed from the cellophane with tweezers and immediately sealed in the sample chamber. The same tissue was used to determine both water and osmotic potentials. Turgor was calculated by difference. After equilibration and measurement of water potential, the tissue samples, with additional mycelium, were placed in 1.5-ml Eppendorf tubes and rapidly frozen in liquid nitrogen, before being hand-ground in the tube. The osmotic potential was measured from the liquid thus obtained.

Pressure-Probe Measurement

The cell pressure probe consisted of a glass microcapillary, which was inserted into the hyphae, a pressure chamber with a pressure transducer, and a motor-driven metal rod, which was used to change the volume of the pressure chamber (Steudle, 1993). The whole device was filled with silicone oil (AS 4, Wecker-Chemie, Ottobrunn, Germany) to form an inert meniscus. Rubber washers created pressure-tight seals around the micropipette. The cell pressure probe was mounted on a micromanipulator (Leitz, Wetzlar, Germany), at right angles to the hyphae. The microcapillary glass had an external diameter of 1 mm, drawn on an upright puller to a narrow tip of 0.5–1 μm.

When this tip was inserted into a hyphal cell, cell sap entered the tip, and a meniscus formed between the silicone oil in the equipment and the cell solution. Turgor pressures were measured after puncturing cells and adjusting the meniscus to a position close to the hyphae, so that no oil was expressed into the hyphae and no cytoplasm was present in the micropipette tip. Thirty to forty measurements of different primary hyphae (width 14–16 μm) were performed, on each side and at each stage of development. Measurements were also taken in branching hyphae, albeit less of them, because their instability and small size (<8 μm) made penetration very difficult. In 10–15 of each set of measurements, turgor was recorded for 5 min or more (Fig. 1). In the other hyphae, only the initial turgor was measured. Because the significance of the deviation was so small, these latter measurements were considered representative.

RESULTS

Water Content during Fungal Growth

Six major stages have been identified from the beginning of hyphal growth to sclerotial maturation (Amir *et al.*, 1993). During Stage I, the hyphae grow on the GNA side (120 h),

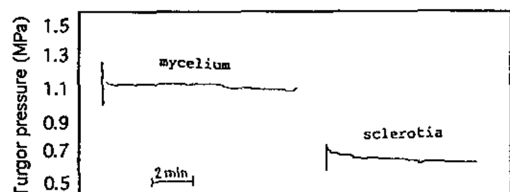

FIG. 1. Turgor potential in hyphae of *M. esculenta*, as measured with the cell pressure probe. Initial spikes result from the pressure exerted by the silicone oil. The upper line refers to turgor examined on the DM side and the lower to turgor on the GNA side during sclerotial development.

spreading to the DM side at Stage II (192 h). Stages III–VI represent the formation and development of sclerotia on the GNA side, as follows: Stage III, initiation (216 h); Stage IV, development of white sclerotia (264 h); Stage V, pigmentation (288 h); Stage VI, sclerotial maturation (360 h).

The water content of the fungal tissue was measured during growth and sclerotial development, on both sides of the plate (Fig. 2a). On the mycelial side, the water content was 17–31% higher than on the sclerotial side. Water content

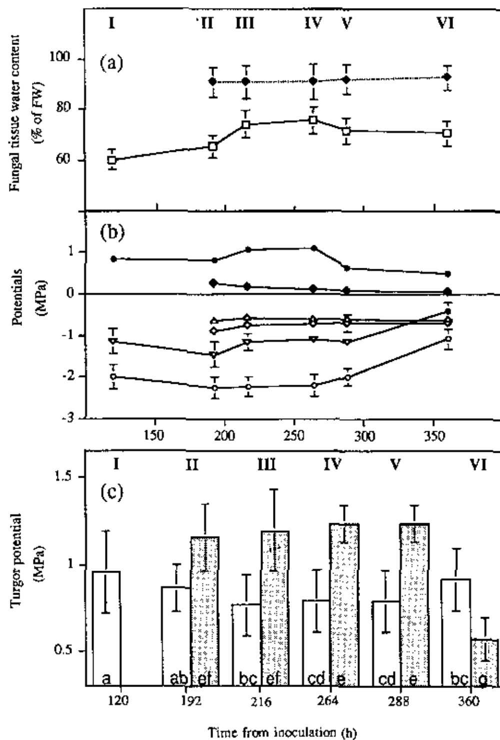

FIG. 2. Changes during the six stages of growth and sclerotial development in *M. esculenta.* (a) Water content in fungal tissue: (□—□) sclerotial side; (♦···♦) mycelial side. (b) Measurements of osmotic and water potentials by thermocouple psychrometer, and the calculated turgor on both sides of the split plate: (▽) water, (○) osmotic, (●) turgor potentials on the sclerotial side; (△) water, (◇) osmotic, (♦) and turgor potentials on the mycelial side. Results are means of five replicates ± SD. (c) Turgor measurements in primary hyphae on both sides of the split plate, as determined by cell pressure probe: (□) sclerotial side; (■) mycelial side. Data followed by identical letters do not differ significantly ($P = 0.05$). The results are means of 30 to 40 measurements ± SD.

in the fungal tissue on the sclerotial side increased from 60 to 76% during sclerotial formation (264 h) and decreased to 70% at maturation. On the mycelial side, water content was nearly constant during the six stages of growth.

Turgor Measurement by Psychrometer

The thermocouple psychrometer measurements gave an unexpected result: the turgor potential on the mycelial side was lower than on the sclerotial side (Fig. 2b). The osmotic potential on the sclerotial side was low (−2.3 MPa) and fairly stable during Stages I–V. At Stage VI both osmotic and water potentials increased. On the mycelial side, turgor potential declined gradually.

The results obtained from the sclerotial side (Fig. 2b) showed that during sclerotial development (Stages III–IV), the calculated turgor is higher (1.2 MPa) than during Stages I and II (0.8 MPa) and Stages V and VI (0.4 MPa). These turgor potential values represent the situation in the sclerotial hyphae, since during sclerotial development the latter comprise most of the biomass on the GNA side.

Turgor Measurement by Cell Pressure Probe

Direct turgor measurement showed that an increase in turgor gradient (difference between the mycelial and sclerotial sides) of up to 0.53 MPa occurs during sclerotial development (Fig. 2c). At maturation (Stage VI), the gradient reversed, descending from the sclerotia to the mycelium. During Stage II, two sinks were active, in the front 2 mm of the colony on the DM side the turgor potential was 0.66 ± 0.16 MPa (average of 15 hyphae) and in the primary hyphae on the GNA side, 0.86 ± 0.13 MPa (data not shown). The hyphal turgor potential generated in the mycelium on the DM side was 1.15 MPa, higher than in the above-mentioned sinks, thereby allowing translocation to both sides simultaneously (Table 1). The developing mycelium on the DM side created a stronger sink than that on the GNA side.

At the initials stage (Stage III), colony extension ceased on the mycelial side while initials were forming on the GNA side. Consequently,

TABLE 1
Six Stages of Growth and Sclerotial Development in *M. esculenta* Grown on a Split Plate and the Direction of the Turgor Gradient

Stages	GNA side-sclerotia	DM side-mycelium	Direction of turgor gradient
Stage I	Sparse hyphal growth	No growth	→
Stage II	Continued with short hyphal branching	Mycelial growth	→ ←
Stage III	Sclerotial initiation	Cessation of colony extension Additional mycelial branching	← →
Stage IV	Sclerotial formation	No visible changes	←
Stage V	Sclerotial pigmantion	No visible changes	←
Stage VI	Sclerotial maturation	No visible changes	→

translocation occurred mainly toward the GNA side. The high, even overlapping, standard deviation (SD) observed in turgor values of both sides (Fig. 2c) may imply that translocation toward the sclerotial side is taking place simultaneously with translocation to the mycelial side, due to additional branching on the mycelial side. During sclerotial development (Stages IV and V), the gradient between the two sides became steeper (1.24 vs 0.88 MPa; Fig. 2c). The turgor in the primary hyphae (1.15 MPa; Fig. 2c) on the mycelial side was higher than that in the secondary branches of the mycelium where turgor was as low as 0.46 ± 0.2 MPa (average of 10 hyphae). This situation indicates that active loading was taking place from the secondary hyphae to the primary hyphae.

In the control, the turgor measured in hyphae grown only on DM (in regular petri dishes) was 1.07 ± 0.26 MPa during growth, whereas in the experiments, the sclerotia on the GNA side generated a higher turgor in the mycelium during Stages IV and V (up to 1.24 MPa). It is evident that, in the latter case, the sclerotia initiated a process that raised the turgor on the DM side.

DISCUSSION

This study deals with turgor changes occurring during growth and sclerotial development in *M. esculenta.* Turgor potential plays a major role in fungal physiology, by regulating translocation (Jennings, 1984, 1987; Eamus and Jennings, 1986b) and by providing the driving force for hyphal extension (Money, 1990; Kaminskyj *et al.,* 1992). The turgor potential of the hyphae that comprise the developing sclerotia on the GNA side must be high enough to create the driving force for hyphal growth and development of those sclerotia. At the same time, it should be low enough to attract mass flow from the mycelium. Here we try to elucidate this apparent contradiction.

The first mention above of turgor is to create gradients which drive mass flow. The higher water content of the mycelium during all stages implies a turgor gradient between the mycelium and sclerotia. Measurement by thermocouple psychrometry failed to demonstrate this. However, this method is liable to error: (i) the method requires sampling the entire tissue, without differentiating between the hyphae in which translocation takes place and the sclerotial hyphae; (ii) the osmotic potential is determined in the cytoplasm together with cell-wall water, as a result of pulping the tissue, leading to higher values than in the cytoplasm alone (Markhart *et al.,* 1981).

Since psychrometry was incapable of measuring turgor gradients during growth and sclerotial development, turgor was measured directly using cell pressure probe developed by Husken *et al.* (1978) for higher plant cells and first applied to fungi by Money (1990). Measurements showed that the gradient between the mycelium and the sclerotia increases during sclerotial development (up to 0.53 MPa). This strongly suggests that translocation is a turgor driven mass flow.

The result is consistent with earlier findings, obtained when translocation was measured using [^{14}C]3-*O*-methyl glucose and [^{14}C]D-

glucose (Amir *et al.*, 1994). There, during the extension of the colony, the two sinks (i.e., hyphal tips on the DM side and additional branching on the GNA side) were active and attracted mass flow. After plate colonization, reverse translocation was caused by sclerotial development, and a significant turgor gradient between the mycelium and sclerotia was created. At maturation, there was higher turgor in the primary hyphae on the sclerotial side than on the mycelial side; and in fact, the direction of translocation of [^{14}C]3-*O*-methyl glucose and [^{14}C]D-glucose reversed again, from sclerotia to mycelium.

On the basis of these results, we propose a model for translocation during sclerotial development. In the mycelium (source), the hyphae absorb nutrients from the medium and synthesize solutes in the cytoplasm of the branch hyphae. These solutes are loaded, through membranes, into the primary hyphae. The increased solute concentration in the latter attracts water from the medium and creates high turgor. Thus, our measurements showed lower turgor pressure in the secondary vs primary hyphae. A similar phenomenon is known in plants, where osmotic potential in mesophyllic cells is lower than that in sieve elements (Geiger *et al.*, 1973). The developing sclerotia, with their vigorous metabolism, attract translocation by creating a strong sink. Thus, turgor pressure generated in the mycelium on the DM side drives flow of the solution to the sclerotia growing on the GNA side. The primary hyphae undergo a morphological change to carry this stream (Amir *et al.*, 1993); i.e., the septa disappear. Once at the sclerotia (sink), the dissolved nutrients are unloaded into numerous sclerotial hyphae, leaving a low level of solutes and low turgor in the primary hyphae. In the sclerotial cells, nutrients are used for growth and for forming polymer reserves, the remainder exuding into the medium (Amir *et al.*, 1994). It should be noted, however, that translocation rate is determined not only by the turgor gradient between different parts of the plate but also by the resistance towards the flow, i.e., by the axial hydraulic resistance of the hyphae.

The other role of turgor examined in the present study is its function in hyphal branching and development in the sclerotia. During sclerotial development, there is extensive branching and formation of new hyphal tips (*Morchella* sclerotia are classified as pseudosclerotia—see Volk and Leonard, 1990; Amir *et al.*, 1993). These elements comprise the larger part of the entire sclerotia. Since attempts to measure turgor by cell pressure probe failed, due to the small size of the hyphae, indirect measurements by psychrometer were employed. They indicated a higher turgor pressure during Stages III–V of development (1.2 MPa) than earlier or later (0.8 and 0.4 MPa, respectively). The overwhelming predominance of the sclerotial cells over the primary hyphae justified the use of psychrometry. In a previous study (Amir *et al.*, 1992), application of this same technique showed a positive correlation between the turgor in the sclerotia and their quantity. Turgor values confirmed the implication of water content in the tissue at these stages, showing a higher water content (76%) during sclerotial development than at earlier or later stages (60–70%, respectively).

According to direct measurements, during sclerotial development on the GNA side the turgor was 0.7 MPa in the primary hyphae, while in the sclerotia it was 1.2 MPa, as established by indirect measurement. Thus, it was confirmed that a low turgor value in the primary hyphae attracts translocation from the mycelium, while high turgor in the sclerotial hyphae enables them to grow and develop. This is made possible by the compartmentation inside the sclerotia between the primary hyphae, the sclerotial hyphae and the periphery of the latter (where growth takes place). Solutes translocated from the mycelium are unloaded into the sclerotial hyphae and maintain a low concentration in the primary hyphae. In the sclerotial hyphae, solutes are metabolized at a high rate. However, in the peripheral cells of the developing sclerotia, a high concentration of solutes is maintained, as a result of nutrient absorption from the medium and sclerotial cells, nutrients which are only partly utilized for growth and reserves. This high concentration attracts additional water from the central sclerotial area, thus maintain-

ing high turgor at the periphery. Jennings (1984) and Thompson *et al.* (1985) suggested that hyphal tips are protected against the changes in potential that take place in other parts of the hyphae, via the direct absorption of nutrients from the medium enabling them to maintain high turgor pressure.

In the present experiments, the direction of growth during Stages I and II was from the medium with low water potential (the GNA side, with −2.1 MPa) to the medium with high water potential (the DM side, with −0.5 MPa). After the plate was completely colonized, the site of extensive hyphal proliferation changed from one region of the colony (DM) to the other (GNA), as a result of reverse translocation (Stages III–V). During these stages, the higher turgor was found in the mycelium. Eamus and Jennings (1986a) used a central barrier in the petri dishes to study the growth of *P. impudicus* in both directions. Although the growth rate hardly changed as it moved from low to high water potential, when the fungus grew from high to low potential it only grew for a very short distance before stopping, with turgor in the hyphal tip very close to zero. The difference between their study and the present one arises from the fact that *P. impudicus* is a fungus that extends on the medium in one direction only, with the hyphal tips at the front of the colony, whereas in *M. esculenta* reverse translocation makes growth on the low potential medium possible. Therefore, although higher turgor is created in the mycelium (DM), it remains sufficiently high on the GNA side for continued growth.

In conclusion, this study is the first to show turgor pressure gradients in fungi between source and sink by direct measurement. This indicated a turgor-driven mass flow along hyphae and not only along specialized structures, such as rhizomorphs. This explains how solutes absorbed by the mycelium can be rapidly translocated to sclerotia during the morphogenetic process.

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