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Effect of osmotic potential on the growth and survival of *Sclerotium rolfsii* Sacc.

Ayed F[1,2,3*], Jabnoun-Khiareddine H[3], Aydi Ben Abdallah R[3] and Daami-Remadi M[3]

[1] Technical Centre of Organic Agriculture, 4042 Chott-Meriam, Sousse, Tunisia
[2] National Agronomic Institute of Tunisia, 1082 Tunis, University of Carthage, Tunisia
[3]UR13AGR09-Integrated Horticultural Production in the Tunisian Centre-East, Regional Research Centre on Horticulture and Organic Agriculture, University of Sousse, 4042, Chott-Meriem, Tunisia

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Abstract

Water availability is an important constraint with significant influence on fungal growth. With regards to soil systems, the osmotic potential is the most important factor that determined the water flow and availability for physiological processes. In this study, the effect of osmotic potential on mycelial growth, sclerotial production and germination of Tunisian isolates of *Sclerotium rolfsii* was studied on culture media osmotically adjusted with potassium chloride from -0.1 MPa to -5.0 MPa. Radial growth and fungal biomass were optimum at osmotic potentials ranging from -0.1 to -1.9 MPa depending on isolates used. Sclerotial initiation started on the 6th day of incubation at -0.1 MPa for all tested isolates and was observed after 9–12 days of incubation at the range of -1.1 to -5.0 MPa. Sclerotia became whitish after 15–18 days of incubation and dark brown after 18–21 days; but no sclerotia were produced at -3.9 and -5 MPa for all isolates. After 21 days, optimal sclerotial production and dry weight (the weight of 100 sclerotia) were recorded at -0.1 and -1.1 MPa. The optimum osmotic potential range for sclerotial germination varied between -0.1 and -1.9 MPa depending on isolates tested and reached 100% after 48 hrs. The decrease of osmotic potential led to lowered sclerotial germination where the lowest germination occurred at -3.9 MPa and -5.0 MPa. Thus, the current investigation demonstrated that the osmotic potential is a key limiting factor for *S. rolfsii* growth and survival.

Key words – Germination – mycelial growth – sclerotial production – southern blight – water potential

Introduction

Sclerotium rolfsii Sacc., the causative agent of southern blight disease on various plants, is a soil-borne pathogen of considerable importance with a wide host range and a worldwide distribution under a variety of environmental conditions (Aycock 1966, Anahosur 2001). Following germination of sclerotia in soil, infection is initiated by direct penetration of developing hyphae and is often preceded by saprophytic growth (Allison 1952, Punja 1985, Kator et al. 2015). Infection process and host tissue invasion are facilitated by the ability of this fungus to secrete oxalic acid

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Corresponding Author: Fakher Ayed – e-mail – ayedfakher@yahoo.fr

and pectinolytic enzymes during its pathogenesis (Bateman & Beer 1965, Punja et al. 1985, Ferrar & Walker 1993). These compounds may act to sequester calcium from cell walls, hydrolyze pectin compounds, lower the pH, and suppress host defensive responses (Bateman & Beer 1965, Kritzman et al. 1977, Punja et al. 1985, Ferrar & Walker 1993).

Southern blight symptoms are expressed as dark brown lesions on stems during any plant growth stage followed by drooping and wilting of the lower leaves and eventually wilting of the whole plant. Wilted plants show white cottony fungal thread girdling the basal part of the stem and moving below the soil line and superficial roots (Punja 1985, Kator et al. 2015). This fungus is responsible for heavy economic losses in many plant species (Fery & Dukes 2002, Anand & Harikesh 2004, Banyal et al. 2008, McCarter & Kays 1984).

Water potential is considered as one of the major environmental factors that affect growth and ecology of various soilborne plant pathogenic fungi (Woods & Duniway 1986, Khattabi et al. 2004, Ayed et al. 2018a, b). It is the sum of four main components: osmotic, matric, gravitational and pressure potential (Cook & Duniway 1980). Osmotic and matric potentials are the major factors that affect the soil biological activity, with optimal and minimal water potentials required for the growth of all microorganisms (Cook & Papendick 1972, Griffin 1981). Fungi have to maintain their internal water potential lower than that of the total water potential of the surrounding environment to maintain turgidity of their cells for optimum mycelial growth (Cook 1973, Eamus & Jennings 1986). Therefore, the internal water potential of the fungus is determined by the surrounding environment, with exposure to changes in the availability of water within the solid matrix itself (matric potential) and the presence of dissolved substances (osmotic potential) (Cook 1973, Carlile et al. 2001).

An understanding of the effect of water potential on pathogen and disease development is critical for more efficient control of soilborne plant diseases (Olaya et al. 1996). In previous studies, the decline of the osmotic potential led to decreases in sclerotial production in other fungi where sclerotia production in *Macrophomina phaseolina* and *Sclerotinia minor* ceases thereby limiting their mycelial growth (Shokes et al. 1977, Imolehin et al. 1980). In the current investigation, we aim to examine the effect of different osmotic potentials on the mycelial growth, biomass production, sclerotial formation, survival and germination of three selected Tunisian *S. rolfsii* isolates. Such investigations would improve our understanding of population dynamics of this re-emergent pathogen in soil, and help to implement effective disease control.

Materials & Methods

Pathogen culture and sclerotial production

Three *S. rolfsii* isolates were used in the current investigation. They were previously isolated from artichoke plants showing typical southern blight symptoms (Sr1) and from potato tubers exhibiting atypical soft rots (Sr2 and Sr3). Identification was performed based on previously published descriptions of *Sclerotium rolfsii* (Mordue 1988) as detailed in Ayed et al. (2018a). Pathogenicity of the three isolates was confirmed by inoculation of healthy potato (*Solanum tuberosum*) cv. 'Spunta' tubers with an agar plug colonized by the pathogens (Daami-Remadi et al. 2010).

Isolates were held in the phytopathology laboratory at the Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia. Prior to use, they were grown for one week at 30 °C in the dark on Potato Dextrose Agar (PDA) medium.

Osmotic potential

The effect of osmotic potential on *S. rolfsii* growth was determined using the method of Douglas & Deacon (1994) with some modifications. Prior to autoclaving, potassium chloride (KCl) was added to PDA or Potato Dextrose Broth (PDB) media at different concentrations to produce osmotic potentials varying from -0.1 MPa to -5.0 MPa (-0.1, -1.1, -1.9, -3.0, -3.9, and -5.0 MPa). Mycelial plugs (6 mm in diameter), cut from the margin of 7-day-old cultures, were placed in the

centre of Petri plates (90 mm in diameter) containing PDA medium supplemented with KCl. Similarly, 15 ml of PDB adjusted at different osmotic potentials were poured into 150 ml-flasks and aseptically inoculated with mycelial plugs (6 mm in diameter). Petri plates and flasks were incubated at 30 °C in darkness (Ayed et al. 2018a).

Mycelial growth and sclerotial production

To examine the effect of tested osmotic potentials on the radial mycelial growth of the three selected *S. rolfsii* isolates, mycelial plugs (6 mm in diameter), cut from the margin of 7-day-old growing colonies, were placed in the centre of Petri plates (90 mm in diameter) containing osmotically adjusted PDA medium amended with streptomycin sulfate (300 mg/L w/v). The cultures were incubated in the dark at 30 °C (Ayed et al. 2018a). The diameters of the developing colonies were measured over a three-day test period (after 24, 48 and 72 hrs of inoculation) and the radial growth rate (mm/day) was determined.

The same cultures were further incubated at the same temperature for 21 days. After this incubation period, sclerotial formation and production were determined. The sclerotial development (first initials, white sclerotia and dark mature sclerotia) was visually noted at three-day intervals (Maurya et al. 2010).

For the monitoring of sclerotial production, mature sclerotia were removed with a sharp scalpel, placed in fine mesh nylon bags and washed thoroughly with sterile distilled water (SDW) to remove agar debris. They were counted and the average number of mature sclerotia produced per plate was determined. After counting, sclerotia were placed on pre-dried and pre-weighed Whatman No. 1 filter paper and incubated at 70 °C for 48 hrs. The dry weight of 100 sclerotia formed per plate was determined.

For all parameters noted, ten replicate plates were used per individual treatment (per isolate and per tested osmotic potential).

Mycelium dry weight

To study the effect of the tested osmotic potentials on mycelium dry weight, 15 ml of PDB medium adjusted at different osmotic potential values ranging between -0.1 and -5 MPa (-0.1, -1.1, -1.9, -3.0, -3.9, and -5.0 MPa) were poured into 150 ml-flasks. The medium was aseptically inoculated with mycelial plugs (6 mm in diameter) cut from 7-day-old *S. rolfsii* colonies. Flasks were maintained without agitation in the dark at 30 °C (Ayed et al. 2018a). After 5 days of incubation, the growing mycelial mats were filtered through Whatman No 1 filter paper, washed thoroughly with SDW, dried at 60 °C for 3 days and weighed immediately using an analytical electrical precision balance. Ten replicate flasks were used per individual treatment (per isolate and per tested osmotic potential).

Sclerotial germination

Ten sclerotia were placed onto Petri plates (90 mm in diameter) containing PDA medium adjusted at different osmotic potentials (from -0.1 MPa to -5 MPa) and incubated in the dark at 30 °C (Ayed et al. 2018a). Germination was determined after 24, 48 and 72 hrs of incubation by counting sclerotia showing any outgrowing hyphae when observed under a binocular microscope. A sclerotium was considered as germinated when outgrowing hyphae were equal to or greater than its diameter. Ten replicate plates, each containing 10 sclerotia per plate, were used per individual treatment and the percentage of germinated sclerotia per plate was recorded (Ayed et al. 2018a, b).

Statistical analysis

Statistical analyses were performed following a completely randomized factorial design where fungal treatments (*S. rolfsii* isolates) and the tested osmotic potentials were the two fixed factors. Ten replicates were used per individual treatment and means were separated using Fisher's protected LSD or Duncan's Multiple Range test ($P \leq 0.05$). These analyses were carried out using

SPSS software version 20. All the experiments were repeated twice and for each test, the mean data is presented in the current study.

Results

Effect on radial mycelial growth

The radial mycelial growth rate for *S. rolfsii*, noted after 3 days of incubation in the dark at 30 °C on PDA medium, varied significantly ($P \leq 0.05$) depending on the tested osmotic potentials and isolates used. A significant interaction was also noted between these two factors. As shown in Table 1, all isolates of *S. rolfsii* were able to grow over a range of osmotic potentials from -0.1 to -5.0 MPa. Optimal growth occurred at -0.1 and -1.1 MPa for Sr1 isolate with an average growth rate of 23.18 and 22.65 mm/day, respectively; at -0.1 MPa for Sr2 (23.77 mm/day) and at -1.1 and -1.9 MPa for Sr3 (23.48 and 22.87 mm/day, respectively). The rate of *S. rolfsii* mycelial growth decreased significantly ($P \leq 0.05$) with a decline in osmotic potential below -3.0 MPa. For all osmotically adjusted media tested, Sr3 isolate grew significantly ($P \leq 0.05$) faster than Sr1 and Sr2 isolates.

Table 1 Effect of various osmotic potentials of PDA medium on the mycelial growth of three *Sclerotium rolfsii* isolates recorded after 3 days of incubation at 30 °C.

Osmotic potential (-MPa)	Radial growth (mm/day)			Mean radial growth per osmotic potential (mm/day)[1]
	Sr1	Sr2	Sr3	
0.1	23.2 a	23.8 a	22.1 b	23 a
1.1	22.7 ab	22.7 b	23.5 a	22.9 a
1.9	22.2 b	21.5 c	22.9 ab	22.2 b
3.0	18.5 c	18.3 d	19.4 c	18.8 c
3.9	15.8 d	16.1 e	17.4 d	16.5 d
5.0	11.1 e	11.7 f	11.8 e	11.5 e
Mean radial growth per isolate(mm/day)[2]	18.9 b	19 b	19.5 a	-

[1]Mean radial growth per osmotic potential for the three isolates combined.

[2]Mean radial growth per isolate for all osmotic potentials combined.

*LSD (Osmotic potentials × *S. rolfsii* isolates) = 0.6 mm/day at $P \leq 0.05$.

*For each isolate and each mean radial growth (per osmotic potential or per isolate), values followed by the same letter are not significantly different according to Duncan's Multiple Range test ($P \leq 0.05$).

Effect on mycelium dry weight

The different *S. rolfsii* isolates were screened for their response to different osmotic potentials. ANOVA analysis revealed that their mycelium dry weight varied significantly ($P \leq 0.05$) depending on tested potentials and isolates and their interactions.

All tested *S. rolfsii* isolates were able to grow on PDB medium osmotically adjusted at a range of -0.1 to -5.0 MPa (Table 2). For Sr2 and Sr3 isolates, fungal biomass, produced after 5 days of incubation at 30 °C, was optimum at osmotic potentials ranging from -0.1 to -1.9 MPa with an average mycelium dry weight varying between 165.4 and 202.82 mg, respectively. Similarly, the Sr1 isolate grew quickly at -1.1 and -1.9 MPa (170.12 and 177 mg, respectively). Nevertheless, there was a significant decrease, for all *S. rolfsii* isolates, in this growth parameter with the decline in the osmotic potential below -3.0 MPa where fungal biomass was markedly restricted at -5.0 MPa (Table 2).

For all osmotic potentials combined, the isolate Sr3 showed the highest mycelium dry weight (150.11 mg) followed by Sr1 (140.27 mg) and Sr2 (135.44 mg).

Effect on sclerotial formation and production

As shown in Table 3, pathogen sclerotial formation was influenced by osmotic potentials. Sclerotial initiation started on the 6th day of incubation from the branching of hyphae and their anastomosis, followed by their aggregation and network formation at -0.1 MPa for all isolates

tested. Therefore, pinhead-like structures became whitish sclerotia after 15–18 days and dark brown mature sclerotia after 18–21 days. However, sclerotial initiation was observed after 9-12 days of incubation at the range -1.1 to -5.0 MPa but no sclerotia were produced at -3.9 and -5 MPa for all isolates even after 21 days of incubation (Table 3). Moreover, complete inhibition of sclerotial formation for Sr3 isolate occurred at -1.1 and -1.9 MPa.

Table 2 Effect of various osmotic potentials of PDB medium on the dry mycelium weight of three *Sclerotium rolfsii* isolates recorded after 3 days of incubation at 30 °C.

Osmotic potential (-MPa)	Dry mycelium weight (mg)			Mean dry mycelium weight per osmotic potential (mg)[1]
	Sr1	Sr2	Sr3	
0.1	150.2 bc	165.4 a	202.8 a	172.8 a
1.1	170.1 ab	166.7 a	211.4 a	182.7 a
1.9	177 a	175 a	197.4 a	183.1 a
3.0	140.6 c	119.6 c	170.8 b	143.7 b
3.9	137.8 c	139.7 b	80.3 c	119.3 c
5.0	65.8 d	46.2 d	37.8 d	50 d
Mean dry mycelium weight per isolate (mg)[2]	140.3 b	135.4 b	150.1 a	-

[1]Mean dry mycelium weight per osmotic potential for the three isolates combined.
[2]Mean dry mycelium weight per isolate for all osmotic potentials combined.
*LSD (Osmotic potentials × *S. rolfsii* isolates) = 14.43 mg at $P \leq 0.05$.
*For each isolate and each mean dry mycelium weight (per osmotic potential or per isolate), values followed by the same letter are not significantly different according to Duncan's Multiple Range test ($P \leq 0.05$).

Table 3 Effect of various osmotic potentials of PDA medium on sclerotial development of three *Sclerotium rolfsii* isolates noted during 21 days of incubation at 30 °C.

Osmotic potential (-MPa)	Isolates	Days after incubation		
		Observation of initial sclerotia	Observation of white sclerotia	Observation of dark mature sclerotia
0.1	**Sr1**	6	15	18
	Sr2	6	15	18
	Sr3	6	18	21
1.1	**Sr1**	9	15	21
	Sr2	9	12	18
	Sr3	9	-	-
1.9	**Sr1**	12	15	21
	Sr2	12	18	21
	Sr3	9	-	-
3.0	**Sr1**	9	15	18
	Sr2	9	18	21
	Sr3	9	18	21
3.9	**Sr1**	9	-	-
	Sr2	9	-	-
	Sr3	9	-	-
5.0	**Sr1**	9	-	-
	Sr2	9	-	-
	Sr3	9	-	-

After 21 days of incubation at 30 °C on osmotically adjusted PDA medium, sclerotium yield (total number of mature sclerotia per plate) varied significantly depending on osmotic potentials and tested isolates. A significant interaction ($P \leq 0.05$) was also noted between these two fixed

factors. As given in Table 4, optimal sclerotial production by Sr1 and Sr3 isolates was recorded at -0.1 MPa, estimated at 136 and 84.4 sclerotia/plate respectively, which was significantly reduced when osmotic potential declined below -1.1MPa. For Sr2, the highest sclerotial production was noted on PDA adjusted at -0.1 and -1.1 MPa with an average of sclerotial production estimated at 104.4 and 107.8 sclerotia/plate, respectively. Furthermore, sclerotium yield of Sr1 and Sr2 isolates was greater than that of Sr3 at all osmotic potentials tested (Table 4).

Table 4 Effect of various osmotic potentials of PDA medium on the number of sclerotia formed by three *Sclerotium rolfsii* isolates after 21 days of incubation at 30 °C.

Osmotic potential (-MPa)	Number of sclerotia per plate			Mean number of sclerotia per osmotic potential[1]
	Sr1	Sr2	Sr3	
0.1	136 a	104.4 a	84.4 a	108.3 a
1.1	53.8 bc	107.8 a	0 b	53.9 b
1.9	70.4 b	47 b	0 b	39.1 bc
3.0	13.8 bc	25.2 b	2.2 b	13.7 c
3.9	0 c	3.2 b	0 b	1.1 c
5.0	0 c	0 b	0 b	0 c
Mean number of sclerotia per isolate[2]	45.7 a	47.9 a	14.4 b	-

[1]Mean number of sclerotia per osmotic potential for the three isolates combined.
[2]Mean number of sclerotia per isolate for all osmotic potentials combined.
*LSD (Osmotic potentials × *S. rolfsii* isolates) = 39.61 sclerotia at $P \leq 0.05$.
*For each isolate and each mean number of sclerotia (per osmotic potential or per isolate), values followed by the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$).

The dry weight of 100 sclerotia, recorded after 21 days of incubation on osmotically adjusted PDA medium, was significantly ($P \leq 0.05$) affected by osmotic potentials and isolates tested. A significant interaction ($P \leq 0.05$) between these two factors was also noted. The highest dry weight of 100 sclerotia was noted on Sr1 cultures grown on PDA adjusted at -1.1 MPa (52.62 mg), those of Sr2 cultured at -0.1 and 1.1 MPa (38.03 mg and 48.16 mg, respectively) and those of Sr3 grown at -0.1 MPa (30.12 mg). Nevertheless, this parameter declined significantly and reached its lowest levels for osmotic potentials below -3.0, -1.9, and -1.1 MPa for Sr1, Sr2 and Sr3 isolates, respectively (Table 5).

Table 5 Effect of various osmotic potentials of PDA medium on the dry weight of 100 sclerotia produced by three *Sclerotium rolfsii* isolates after 21 days of incubation at 30 °C.

Osmotic potential (-MPa)	Dry weight of 100 sclerotia (mg)			Mean dry weight of 100 sclerotia per osmotic potential[1]
	Sr1	Sr2	Sr3	
0.1	27.1 b	38 a	30.1 a	31.8 a
1.1	52.6 a	48.2 ab	0 b	33.6 a
1.9	24.7 b	8.8 c	0 b	11.1 bc
3.0	16.2 bc	16.2 bc	9.5 b	14.1 b
3.9	0 c	16.5 bc	0 b	5.4 bc
5.0	0 c	0 c	0 b	0 c
Mean dry weight of 100 sclerotia per isolate[2]	20.1 a	21.3 a	6.6 b	-

[1]Mean dry weight of 100 sclerotia per osmotic potential for the three isolates combined.
[2]Mean dry weight of 100 sclerotia per isolate for all osmotic potentials combined.
*LSD (Osmotic potentials × *S. rolfsii* isolates) = 14.5 mg at $P \leq 0.05$.
*For each isolate and each mean dry weight of 100 sclerotia (per osmotic potential or per isolate), values followed by the same letter are not significantly different according to Duncan's Multiple Range test ($P \leq 0.05$).

For all the osmotic potentials pooled, the dry weight of 100 sclerotia produced by Sr1 and Sr2 isolates, after 21 days of incubation on osmotically adjusted PDA medium, was significantly higher than that of the Sr3 isolate (Table 5).

Effect on sclerotial germination

The germination of *S. rolfsii* sclerotia, after 24 hrs of incubation at 30 °C on PDA medium osmotically adjusted with KCl, varied significantly ($P \leq 0.05$) depending on tested osmotic potentials and pathogen isolates and on their interactions (Table 6). The germination of sclerotia was maximum on PDA osmotically adjusted at -0.1 MPa for Sr1 isolate (88%), over the range -0.1 to -1.9 MPa for Sr2 (90-98%) and -0.1 and -1.1 MPa for Sr3 (94 and 86%, respectively). However, the lowest percentage of sclerotial germination was recorded at -5.0 MPa for Sr1 and Sr2 and at -3.9 MPa and -5.0 MPa for Sr3 (Table 6). Furthermore, total sclerotial germination for all *S. rolfsii* isolates was noted after 48 hrs of incubation (data not shown).

For all osmotic potentials combined, the highest sclerotial germination (76.33%) was recorded for Sr2 isolate, followed by Sr1 (68%) and Sr3 (59%) (Table 6).

Table 6 Effect of various osmotic potentials of PDA medium on the sclerotial germination of three *Sclerotium rolfsii* isolates noted after 24 hrs of incubation at 30 °C.

Osmotic potential (-MPa)	Sclerotial germination (%)			Mean sclerotial germination per osmotic potential[1]
	Sr1	Sr2	Sr3	
0.1	88 a	98 a	94 a	93.3 a
1.1	68 c	90 a	86 a	81.3 b
1.9	78 b	90 a	60 b	76 b
3.0	74 bc	76 b	50 b	66.7 c
3.9	68 c	66 c	32 c	55.3 d
5.0	32 d	38 d	32 c	34 e
Mean sclerotial germination per isolate (%)[2]	68 b	76.3 a	59 c	-

[1]Mean sclerotial germination per osmotic potential for the three isolates combined

[2]Mean sclerotial germination per isolate for all osmotic potential values combined

*LSD (Osmotic potentials × *S. rolfsii* isolates) = 8.14% at $P \leq 0.05$

*For each isolate and each mean of sclerotial germination (per osmotic potential value or per isolate), values followed by the same letter are not significantly different according to Duncan's Multiple Range test ($P \leq 0.05$).

Discussion

Some studies were concentrated on the characterization of *S. rofsii* and others were more focused on the effect of nutritional and environmental conditions on its growth and survival (Ayed et al. 2018a, b, Basamma et al. 2012). Therefore, this study determined the optimum level and range of osmotic potentials permitting optimal mycelial growth, biomass production, sclerotial formation and germination for three selected Tunisian *S. rolfsii* isolates.

All *S. rolfsii* isolates were able to grow over a range of osmotic potentials varying from -0.1 to -5.0 MPa. Radial growth and fungal biomass were optimum at osmotic potentials ranging from -0.1 to -1.9 MPa depending on isolates used. However, the radial and dry mycelial growth decreased with the decline in the osmotic potential on all PDA media osmotically adjusted below -3.0 MPa. The mycelial growth responses of *S. rolfsii* isolates to different osmotic potentials in this study are similar to those previously observed for other fungal species. For example, mycelial growth of *Rhizoctonia solani* on osmotically adjusted media is fastest at -0.4 MPa and ceased between -3.5 and -4.0 MPa (Ritchie et al. 2006). Also, a fifty-percent growth reduction did not occur until the osmotic potential was reduced to <-2.5 MPa (Ferrin & Stanghellini 2006). Similar decreases in mycelial growth in response to the declines in the osmotic potentials are observed in *Coniothyrium minitans*, *Fusarium oxysporum*, *Idriella bolleyi*, *Pythium ultimum*, *Rhizoctonia solani*, and *Verticillium dahliae* (Sterne & McCarver 1978, Brownell & Schneider 1985, Douglas & Deacon

1994, Jones et al. 2011). Moreover, in many zoosporic and non-zoosporic fungi, the osmotic potential at high levels usually leads to several disease developments (MacDonald 1994). Some studies demonstrated that salt stress is more effective in reducing the osmotic potential of the cell sap and results in stronger osmo-regulation than stress induced by a water deficit (Sepaskhah & Boersma 1979, Woods & Duniway 1986, Amir et al. 1992). Mycelial growth under KCl osmotic stress may result from the uptake of potassium ions and its accumulation in microbial cells, which lowers the water potential of the protoplasm to a value more ideal for cellular processes, or may increase turgor and hence the acceleration of growth (Olaya et al. 1996).

Sclerotial initiation started on the 6th day of incubation at -0.1 MPa and on the 9th–12th day at the range -1.1 to -5.0 MPa for all tested *S. rolfsii* isolates. Dark brown sclerotia were observed after 18–21 days but no sclerotia were produced at -3.9 and -5 MPa. After 21 days of incubation, optimal sclerotial production and dry weight were recorded at -0.1 and -1.1 MPa, which were significantly reduced when osmotic potential declined below -1.1MPa for sclerotial yield (weight of 100 sclerotia) and below -3.0, -1.9, and -1.1 MPa for *S. rolfsii* isolates Sr1, Sr2 and Sr3, respectively. These results are in agreement with other studies reporting that sclerotium yield of *R. solani* declines with the decrease in the osmotic potential, and its formation ceased between -1.5 and -3.5 MPa (Ritchie et al. 2006). Furthermore, Abd-Elmagid et al. (2015) found that different levels of osmotic potentials affect the number of sclerotia produced by three *Sclerotinia minor* isolates.

As for sclerotial germination after 24hrs of incubation, the optimum osmotic potential range varied between -0.1 and -1.9 MPa depending on isolates tested and reached 100% after 48 hrs of incubation. However, the lowest percentage of sclerotial germination was recorded at -3.9 MPa and -5.0 MPa. Previous studies reported that the decline in the osmotic potential decreases the germination of *S. rolfsii* sclerotia so that the lowest germination occurs at the lowest osmotic potential level (Saadatmand et al. 2007). Furthermore, Saadatmand et al. (2006) demonstrated that the sclerotial germination of *Verticillium dahliae* increases with the decrease in the osmotic potential and reaches its peak at -0.6 MPa; but a reduction of 50% is achieved at -1.5 MPa. Moreover, germination of *R. solani* sclerotia also declines by decreasing the osmotic potential with a total inhibition occurring over -3.0 to -4.0 MPa osmotic range (Ritchie et al. 2006). The capacity of sclerotia to germinate at low osmotic potentials could be attributable to solute uptake by the sclerotium, which reduces its osmotic potential and thus maintains the germination processes (Cook & Al-Hamdani 1986).

Conclusion

In this study, the decline in the osmotic potential caused similar decreases in *S. rolfsii* mycelial growth, biomass production, sclerotial formation, production and germination. Nevertheless, there were significant differences, based on some growth and survival parameters, in the responses of the tested isolates to the different osmotic potentials, especially at lower osmotic potential levels. The tolerance of *S. rolfsii* to reduced osmotic potentials may be an important factor in the development of the Southern blight disease, which is favored by temperatures and droughts. An understanding of the effect of osmotic potential on disease development is critical to control this soilborne pathogen. Of the cultural practices that reduce damage induced by this fungus, water management seems to be the most effective on root colonization. Therefore, further experiments into the effect of environmental factors on pathogen virulence would provide more information on the behavior of local *S. rolfsii* isolates under field conditions.

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