

Effect of Water Potential on Mycelial Growth and Production of Sclerotia of *Sclerotium cepivorum*

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*Sclerotium cepivorum*의 군사생장 및 균핵 생성에 대한 수분압의 영향

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ABSTRACT: The effect of water potential (Ψ_w) and temperature on mycelial growth and sclerotial production of *Sclerotium cepivorum* was determined in potato dextrose agar (PDA) and potato dextrose broth (PDB) adjusted to different Ψ_w with NaCl, KCl, sucrose or polyethylene glycol (PEG) at 15, 20 and 25°C. The growth of mycelium was not significantly affected by Ψ_w values between -1,970 and -2,240 J/Kg, but severely decreased lower than -2,240 J/Kg. Dry weight was slightly increased at Ψ_w values between -450 and -2,240 J/Kg. The reduction of dry weight was slower than the reduction of mycelial growth as the Ψ_w decreased. The mycelial growth and dry weight were more severely influenced on PEG amended media than on other osmotica amended media. About 50% reduction of mycelial growth and dry weight was occurred about -1,000 and -2,240 J/Kg, respectively. The production of sclerotia was more severely affected than the mycelial growth as the Ψ_w decreased. The 50% reduction of sclerotial production occurred between -450 and -810 J/Kg. Sclerotium was not produced lower than -2,240 J/Kg. Mycelial growth and sclerotial production was better at 25°C as the Ψ_w decreased than at 20°C which is optimal temperature in the unamended media. The influence of Ψ_w on mycelial growth and sclerotial production of *S. cepivorum* adjusted with NaCl, KCl, sucrose or polyethylene glycol showed similar patterns.

Key words: *Sclerotium cepivorum*, garlic, water potential, temperature.

• White rot of garlic caused by *Sclerotium cepivorum* causes severe damage in major garlic production areas in Korea (4, 5). This pathogen is soilborne in the form of sclerotia, which are the primary source of infection. The sclerotia are formed at the surface of bulbs and able to survive in soil for a long period of time. This fungus is also seedborne and the infected bulbs can serve as a source of inoculum. These are efficient methods for the dispersal of the pathogen (5).

Disease development is generally considered to be closely related to soil water and temperature interactions (6, 14, 17, 18), and fungi differ in ability to get available water from soil. Water potential (Ψ_w) considered to be an important factor in the growth and ac-

tivity of plant pathogenic fungi (6, 9, 10, 19). Fungal response to the Ψ_w may vary with different temperatures (6, 10, 14), that is to say the optimal Ψ_w may vary with temperatures.

Numerous reports deal with the effect of Ψ_w on the growth of various plant pathogens, especially soilborne pathogens (7, 10, 11, 13). But the effect of Ψ_w on the mycelial growth, production of sclerotia of the *S. cepivorum* and on the development of white rot in garlic is lacking.

The purpose of this study was to determine the effect of Ψ_w and temperature on the mycelial growth and production of sclerotia of *S. cepivorum*.

MATERIALS AND METHODS

Isolates. The isolate of *S. cepivorum* was obtained

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from the diseased bulb of garlic growing in the field of Muahn, Chonnam. The isolate was maintained in potato dextrose agar (PDA) at 10°C and transferred to PDA as needed.

Effect of water potential on mycelial growth of *S. cepivorum*. The effect of Ψ_w on radial growth of *S. cepivorum* was measured on PDA adjusted to different osmotic water potentials (Ψ_s) with NaCl, KCl or sucrose at 15, 20 and 25°C.

Mycelial disks (5 mm in diameter) from the margin of 5-day-old colonies growing on PDA were transferred to the center of PDA plates adjusted to different Ψ_s . There were three replications in each treatment.

The Ψ_s of the media was determined with a HR33-T dew point microvoltmeter and C-52 sample chamber (Wescor Inc. Logan, UT, U.S.A.) which has the precision of $2\% \pm 10$ J/Kg. Agar samples were placed in the chambers and equilibrated at 15, 20 and 25°C until constant dew-point readings were obtained (1, 2). Radius of the colony was measured after 5 days of incubation at different temperatures. Ψ_s was expressed in J/Kg and one J/Kg is equivalent to 0.01 bar.

Growth of the fungus on the medium amended with polyethylene glycol 6,000 (PEG) having matric (Ψ_m) effect than osmotic (Ψ_s), was measured as the radius of colony and dry weight. The radial growth was measured in the potato dextrose broth (PDB) adjusted to different Ψ_w with PEG. Fifteen ml of PDB amended with PEG was poured into petri dishes and a filter paper (Advantec No.2) was placed on the top of the broth. A mycelial disk was inoculated on the filter paper which has a thin film of PDB and PEG.

The required concentrations of PEG 6,000 were calculated from the formula developed by Michel and Kaufmann (16).

To measure the dry weight, 20 ml of PDB in 100 ml flask was amended with NaCl, KCl, sucrose or PEG. A mycelial disk was inoculated and incubated for 10 days in the shaking incubator (100 rpm) at $20 \pm 2^\circ\text{C}$. Mycelial mats were washed several times in the glass microfibre filter paper (Whatman GF/A) with distilled water to remove the medium and osmoticum, dried at 50°C for 24 hours, and then weighed.

There was 3 replications in each treatment. Data was analyzed by two-way factorial analysis of variance.

Effect of water potential on production of sclerotia. The effect of Ψ_s on the production of sclerotia of *S. cepivorum* was determined by culturing *S. cepivorum* in PDA plates adjusted to the different Ψ_w with

NaCl, KCl or sucrose at 15, 20 and 25°C. After 15 days of incubation the number of sclerotia in 1 cm² of each plates was counted three times per replication with a colony counter.

There was 3 replications in each treatment. Data was analyzed by analysis of variance.

RESULTS

Effect of water potential on mycelial growth of *S. cepivorum*. Radial growth curves of *S. cepivorum* in PDA adjusted to different Ψ_s with various osmotaica showed a similar pattern (Fig. 1). The radial growth was not significantly affected by Ψ_s values between -1,970 and -2,240 J/Kg, but decreased rapidly lower than -2,240 J/Kg. About 50% reduction was occurred

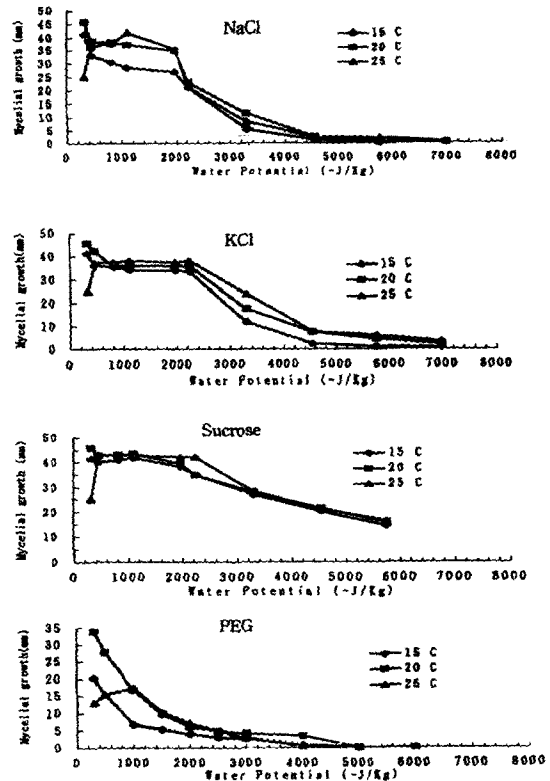


Fig. 1. Mycelial growth of *Sclerotium cepivorum* on potato dextrose agar adjusted to different water potentials with NaCl, KCl, sucrose or polyethylene glycol 6,000 at 15, 20 and 25°C. Radius of colony was measured after 5 days of incubation. The values are means of 3 replications per treatment. LSD values at 1% level using a two-way factorial analysis of variance were 2.4, 2.9, 3.5 and 2.3 for NaCl, KCl, sucrose and PEG, respectively.

at -2,240, -2,800, and -4,550 J/Kg in PDA adjusted with NaCl, KCl and sucrose, respectively. The radial growth was not occurred below -6,980 and -7,000 J/Kg in NaCl and KCl amended PDA, respectively. The radial growth on PDA adjusted with sucrose was more slowly reduced than that of NaCl or KCl amended PDA as Ψ_w values decreased. Sucrose was not used lower than -5,750 J/Kg.

The radial growth on PDA adjusted to different Ψ_w with PEG was more severely affected than that of NaCl, KCl or sucrose amended PDA as Ψ_w values decreased. About 50% reduction compared to unamended PDA was occurred at -1,000 J/Kg.

The fungus grows well at 20°C in the unamended PDA. However, radial growth was better at 25°C as Ψ_w values decreased than at 20°C. The radial growth curves show that mycelial growth was better at high temperature as Ψ_w values decreased. There was a highly significant temperature and Ψ_w interaction according to two-way factorial analysis of variance.

The colony grew radially at low Ψ_w , but lower than -3,300 J/Kg showed a little irregular growth and black pigment was accumulated in the media and the color of colony was slightly different.

Dry weight curves after 10 days of incubation in PDB also showed similar patterns (Fig. 2), and the dry weight was slightly increased at Ψ_w values between -450 and -2,240 J/Kg. The reduction of dry weight was slower in PDB than the reduction of radial growth on PDA as Ψ_w values decreased. About 50% reduction was occurred at -4,550, -4,550, -5,800 and -2,400 J/Kg in PDB adjusted with NaCl, KCl, sucrose and PEG, respectively. The dry weight of mycelia decreased most slowly in PDB amended with sucrose and the stimulation was also the greatest. The dry weight was most severely affected in PEG amended PDB as Ψ_w

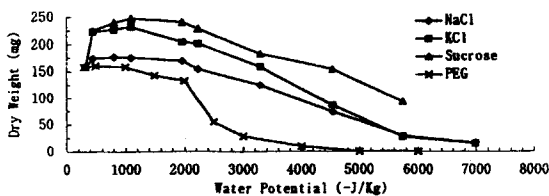


Fig. 2. Dry weight of *Sclerotium cepivorum* after 10 days of incubation in potato dextrose broth adjusted to different water potentials with NaCl, KCl, sucrose or PEG 6,000 at 22°C. The values are means of 3 replications. Highly significant according to analysis of variance.

values decreased and mycelial growth was not detected lower than -5,000 J/Kg. Mycelia grew in agglomerated form like a ball and the size of agglomeration was smaller but the number was more in PEG amended PDB as Ψ_w values decreased. There was a highly significant according to analysis of variance.

Effect of water potential on production of sclerotia. Production of sclerotia of *S. cepivorum* after 15 days of incubation in PDA was more severely decreased as Ψ_w values decreased than mycelial growth and dry weight. About 50% reduction of sclerotia production occurred at the range of -450 and -810 J/Kg. Sclerotia were not formed lower than -2,240 J/Kg. Production of sclerotia in PDB adjusted with NaCl or sucrose was more severely affected than that of KCl amended with PDB. The sclerotia produced in the sucrose amended with PDA were larger than those of NaCl or KCl amended agar.

Production of sclerotia was best at 25°C as the Ψ_w decreased in NaCl or KCl adjusted PDB, but at 20°C in sucrose adjusted PDB. There was a highly sig-

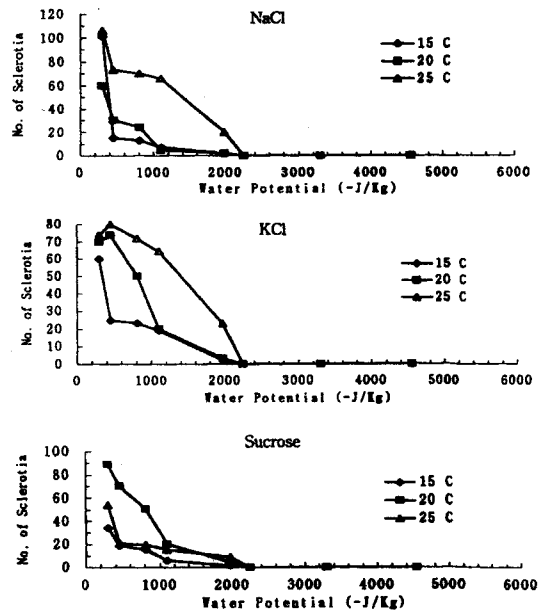


Fig. 3. Number of sclerotia produced on PDA adjusted to different water potentials with NaCl, KCl, sucrose at 15, 20 and 25°C. Number of sclerotia was counted after 10 days of incubation. The values are means of 3 replications per treatment in each test. LSD values at 1% level using a two-way factorial analysis of variance were 15.3, 15.0 and 9.2 for NaCl, KCl and sucrose, respectively.

nificant temperature and Ψ_w interaction on sclerotial production according to two-way factorial analysis of variance.

DISCUSSION

The influence of Ψ_w on fungi may vary with the nature of the media and osmotica (3, 9, 10, 15). And the amount of stimulation and the optimal Ψ_w depends on the fungus, osmoticum, temperature and other factors in the environment (17). In this study, radial growth and dry weight were also depended on the osmotica and temperatures. Radial growth and dry weight was greatest when the Ψ_w was adjusted with sucrose. This is considered as the result of sucrose utilization as a nutrient source suggested by Cook *et al.* (6, 15, 19). But the radial growth and the fungal dry weight showed similar patterns in various osmotica. This result is primarily due to the difference of Ψ_w . The mycelial growth was not significantly affected by Ψ_w values between -1,970 and -2,270 J/Kg, but mycelia couldn't grow as low as -6,980 J/Kg. The dry weight of mycelia was decreased more slowly than the radial growth as the Ψ_w decreased.

Reducing Ψ_w increased the growth of many fungi, such as *Botrytis squamosa* (1), *Fusarium oxysporum* (3), *Sclerotinia sclerotiorum* (11), *Sclerotinia minor* (12), *Verticillium dahliae* (13), *Phytophthora* spp. (15) and etc. But the growth of *Ophiobolus graminis* was the greatest in the basal media and progressively decreased in a linear fashion (8). Stimulation of growth may result from nutrient source or ion uptake of the solute, which may lower the Ψ_w of the protoplasm to a value more ideal for cellular processes or may increase turgor and hence acceleration of the growth (7, 15, 17). The results obtained in this study show that radial growth decreased without stimulation as the Ψ_w decreased although significant reduction was not occurred as low as -1,970 J/Kg. But, dry weight was slightly increased at Ψ_w values between -450 and -2,240 J/Kg.

The influence of osmotic and matric water potential has shown significant difference in effects on the growth of soil fungi. The growth of various soilborne plant pathogenic fungi has usually been more affected at low Ψ_m than Ψ_s (3, 8, 9, 17). So, PEG (the primary effect is matric than osmotic) of large molecular weight has been used to adjust Ψ_w in liquid media for microbial studies. The advantage of PEG is the inability to pass through membranes, low toxicity and

very slow microbial degradation (3). This study showed that the effect of PEG was greater than other osmotica. Lag phase which was shown at NaCl, KCl or sucrose amended media was not shown at PEG amended media. Mycelial growth was not occurred below -4,000 J/Kg in PEG amended PDA and dry weight of mycelia was not detected lower than -5,000 J/Kg in PEG amended PDB.

This result indicated that the effect of Ψ_w shouldn't be interpreted solely from Ψ_s , and careful studies should be conducted to establish the effect of Ψ_w on fungi.

In this study, it has been found that good growth of the fungus differed with temperatures and Ψ_w . The fungus generally grew well in a little higher temperature than optimal temperature at Ψ_w values between -810 and -1,970 J/Kg. This result was interpreted as an adaptive mechanism of the organisms needed to meet a common situation, namely, a dry environment when temperature is high or vice versa as Cook (7) suggested.

Sclerotial formation could be influenced by various factors, i. e., chemicals, temperature, moisture, isolates and etc(2). The osmoticum might affect to the development and maturation of sclerotia. The formation of sclerotium was better on KCl amended PDA than that of NaCl or sucrose amended PDA. So, it may not be said that the result of this study relevantly account for the effect of Ψ_w on the production of sclerotia of *S. cepivorum*. But it could be primarily thought as the effect of Ψ_w because sclerotial production curves showed similar patterns. In this study, the number of sclerotia was more rapidly decreased as the Ψ_w decreased than the reduction of mycelial growth and sclerotium was not formed at Ψ_w values lower than -2,240 J/Kg.

Considering all of the results, it may not be possible to explain the effect of Ψ_w in vitro condition, and Ψ_w probably does not directly affect on pathogens in the field. But their pathogenic potential will be decreased and in some cases the disease could be controlled by regulating proper Ψ_w .

요 약

마늘 흑색썩음균핵병인 *Sclerotium cepivorum*에 대한 수분압과 온도의 영향을 알아보기 위하여 PDA와 PDB에 NaCl, KCl, 설탕 및 polyethylene glycol 6,000으로 수분압을 조절한 후 15, 20, 25°C에서 균사생장 및 균핵 생성량을 조사하였다. 균사신장은 -1,970에서 -2,240 J/Kg까지는 크게 영향을 받지 않는으나,

-2,240 J/Kg 이하에서는 균사신장이 급격히 저하되었다. -450에서 -2,240 J/Kg까지의 수분압에서는 기본배지에서 보다 건물중이 약간 증가하였다. 수분압 감소에 따른 건물중의 영향은 균사신장에서 보다는 작았다. PEG를 이용해 수분압을 조절했을 경우 균사신장과 건물중 모두 다른 용질을 사용했을 경우보다 크게 감소했다. 균사신장은 -1,000 J/Kg에서, 건물중은 -2,240 J/Kg에서 약 50%가 각각 감소했다. 수분압 감소에 따른 균핵 형성에 대한 영향은 균사생장에서보다 더욱 컸다. -450에서 -810 J/Kg 사이에서 균핵형성이 50% 이상 줄었으며, -2,240 J/Kg 이하에서는 균핵이 형성되지 못했다. 균사신장과 균핵형성은 기본배지에서는 20℃에서 양호하였으나, 수분압이 감소하면서 25℃에서 더 좋은 경향을 보였다. NaCl, KCl, 설탕 및 PEG에 따라 차이는 있었으나, 균사생장과 균핵 형성은 비슷한 경향의 증감을 보였다.

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