RESEARCH ARTICLE

# Screening of Selected Korean Sweetpotato (*Ipomoea batatas*) Varieties for Fusarium Storage Root Rot (*Fusarium solani*) Resistance

**Seung-yong Lee[1,†], Narayan Chandra Paul[1,†], Won Park[1], Gyeong-Dan Yu[1], Jin-Cheon Park[1], Mi-Nam Chung[1], Sang-Sik Nam[1], Seon-Kyeong Han[1], Hyeong-Un Lee[1], San Goh[1], Im Been Lee[1], Jung-Wook Yang[2,*]**

[1]Bioenergy Crop Research Institute, National Institute of Crop Science, Rural Development Administration, Muan 58545, Korea

[2]Crop Cultivation & Environment Research Division, National Institute of Crop Science, Rural Development Administration, Suwon 16613, Korea

[*]Corresponding author: hg369732@korea.kr

[†]These authors contributed equally to this work.

## ABSTRACT

A common post-harvest disease of sweetpotato tuber is root rot caused by *Fusarium solani* in Korea as well as the other countries. Storage root rot disease was monitored earlier on sweetpotato (*Ipomoea batatas*) in storehouses of different locations in Korea. In the present study, an isolate SPL16124 was choosen and collected from Sweetpotato Research Lab., Bioenergy Crop Research Institute, NICS, Muan, Korea, and confirmed the identification as *Fusarium solani* by conidial and molecular phylogenetic analysis (internal transcribed spacer ITS and translation elongation factor EF 1-α gene sequences). The isolate was cultured on potato dextrose agar, and conidiation was induced. The fungus was screened for Fusarium root rot on tuber of 14 different varieties. Among the tested variety, Yenjami, Singeonmi, Daeyumi, and Sinjami showed resistant to root rot disease. Additionally, the pathogen was tested for pathogenicity on stalks of these varieties. No symptom was observed on the stalk, and it was confirmed that the disease is tissue specific.

**Keywords:** *Fusarium solani*, Molecular phylogeny, Resistant variety, Storage root rot, Sweetpotato



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## INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam.] plays a vital role in the economy of many Asian countries, including Korea, China, and Vietnam [1]. It's a high-energy nutritious food with high carbohydrate content, and 98% of its carbohydrate content is easily digestible [2]. Diseases caused by *Fusarium* species are common in all plants and plant products, as well as sweetpotato. *Fusarium solani* has a broad host range, causing root rots, stem cankers, damping-off on 66 host families. The study revealed that severe storage diseases in sweetpotato caused by a number of pathogens and the major pathogenic taxa are

*Rhizopus*, *Ceratocystis*, *Fusarium*, *Plenodomus*, *Diaporthe* and so on. In Korea, Fusarium diseases are one of the major storage diseases among others [3]. *F. solani* caused storage root rot a post-harvest disease in sweetpotato on a large scale and caused a considerable loss to the farmers. The disease is also known as dry rot or end rot [1]. Typical storage root rot lesions along with circular and concentric rings were observed on diseased roots. Visually differentiating between Fusarium surface rot and root rot is challenging [2] as lesions of root rot affected sweetpotato extend through the vascular tissue into the center, while the external appearance of the infected storage root is similar to that of surface rot.

This typical disease reduction is essential to reduce the losses caused by this disease. To minimize the disease, use of resistant variety is necessary. Selecting disease resistance variety could help to choose the best variety. There are many factors involved to choose of a variety, including weather condition and cultural practices [3]. For breeding new variety or cultivar choosing, resistant variety is essential [4], and new different methods have been developed for screening resistance variety selection. Less time consuming and accurate *in vitro* bioassay is an example of resistance variety screening method developed for resistance against Fusarium wilt in banana (*Musa* sp.), and detached leaf assay was applied to detect resistance against *Phytophthora infestans* in tomato (*Solanum lycopersicum*) [5,6]. We attempted to test 14 Korean local sweetpotato varieties against the post-harvest pathogen *F. solani* and tested the pathogenicity on stalks also. So, the objective of the present study was to confirm the pathogen as *F. solani* causes the root rot of sweetpotato in Korea and to screen resistant varieties against this pathogen by a rapid and easy method.

# MATERIALS AND METHODS

## Pathogen collection and culture

The fungal pathogen was collected from the fungal herbarium of Sweetpotato Research Lab., Bioenergy Crop Research Institute, National Institute of Crop Science, Rural Development Administration, Muan, Korea. Previously, the pathogen was isolated and reported as the first report from Korea, and we have chosen the best active pathogen explained by Yang et al[4]. The pathogen was maintained in glycerol stock solution before and after use. The isolate SPL16124 was incubated on potato dextrose agar, (PDA; Difco, MD, USA) for seven days at 25°C before doing the agar plug experiment on the tuber. On the other hand, for treating *F. solani* on sweetpotato stalks, the fungus was grown on PDA for 7 days at 25°C before inoculation, and the mycelia were scratched off using a needle and sporulation was induced under 12 hours-12hours light-dark conditions. After two days of culture, spores were collected and counted using a hemocytometer (Marienfeld, Germany).

## Morphology and molecular phylogeny

The isolate SPL160124 was grown on PDA at 25°C for 7 days and induced sporulation to facilitate conidial characteristics study. Randomly selected macro- and micro-conidia (n = 20) were measured and photographed using a light microscope (Olympus, Tokyo, Japan) with an Artcam 300MI digital camera

(Artray, Tokyo, Japan). Total genomic DNA was extracted directly from mycelia obtained from 7–day old colonies have grown previously on PDA at 25 ℃ using the Solg$^{TM}$ Genomic DNA prep.–Kit (Solgent Co. Ltd, Daejeon, Korea) [4]. The identification of the pathogen was confirmed by molecular phylogeny with the available 2 gene sequences of the internal transcribed spacer (ITS) and translation elongation factor (EF 1-α) region of rDNA was amplified using the primer sets ITS5 (5′–GGA AGT AAA AGT CGT AAC AAG G–3′) ITS4 (5′–TCC TCC GCT TAT TGA TAT GC–3′) and EF1 (5′–ATG GGT AAG GAR GAC AAG AC–3′) EF2 (5′–GGA RGT ACC AGT SAT CAT GTT–3′) [7, 8]. Polymerase chain reaction (PCR) amplification was carried out under the following conditions; initial denaturation at 94℃ for 5 minutes, followed by 35 cycles of denaturation at 94℃ for 35 seconds, and annealing at 56℃ for EF 1-α and 55 ° C for ITS for 60 seconds, and a final extension at 72℃ for 2 minutes. PCR products were sequenced by a commercial sequencing service provider (Macrogen, Daejeon, Korea) in both directions. Sequences were subjected to phylogenetic analysis using Clustal_X v.1.83 [9] and BioEdit v. 5.0.9.1 [10]. Phylogenies were assessed using MEGA 7 [11,12]. Maximum parsimony (MP) phylogenetic tree was constructed for the combined datasets of the ITS rDNA and EF 1-α gene sequences. The percentage of sequence identity was obtained by applying the National Center for Biotechnology Information (NCBI) BLAST search tool for the isolate.

## Resistant variety selection

Pathogenicity and disease-resistant variety selection test was performed with the isolate SPL16124 on 14 well-known local varieties- Dahomi, Danjami, Daeyumi, Beniharuka, Singeonmi, Sinjami, Sinhwangmi, Yenjami, Yenhwangmi, Yulmi, Juhangmi. Jinhongmi, Pungwonmi, and Hogammi. A total of three storage roots were used after harvesting for a single variety in each experiment with three replications. Storage roots from each variety were washed in running tap water and surface sterilized following the procedures of Paul et al. [13] with minor modification (dipping in 4% NaOCl for 5 minutes). A 5 mm hyphal colony disk, previously grown on PDA was placed in a wound made by using a 5 mm cork borer. Blank PDA disks were used as a control treatment. Storage roots were then transferred onto a sterilized plastic mesh platform in moistened clean boxes. The inoculated roots in the boxes were incubated for up to 10 days at 25 ℃ in the dark. After incubation, the diameter of necrotic lesions was measured, and the disease severity index was prepared and categorized ranged from 0 to 5 scale. Categories were explained as, 0 = No visible symptoms on tuber; 1 = 1-5% Symptoms; 2 = 6-25%, 3 = 26-50%; 4 = 51-80%; 5 = above 80% rotting. The experiment was conducted three times with three replications.

Surface sterilized stalks were dipped in spore suspensions ($1 \times 10^5$ spores/mL) with 5 replications and incubated at 28 ℃ for three weeks. After 10 days, disease symptoms were monitored and noted. Based on symptoms, a disease severity index was established and categorized ranged from 0 to 5 scale. Categories were explained as 0 = seedlings grew normally without visible symptoms; 1 = ~5% browning in vascular bundle length; 2 = ~25% browning, 3 = ~25% browning in vascular bundle length and yellowing of the bottom leaves; 4 = browning of the entire vascular and discoloration of all the leaves; 5 = seedling collapsed and died [3]. Disease resistant variety were counted by the following disease index scale, 0–0.6 = Resistant;

0.61–1.2 = moderately resistant; 1.21–1.8 = moderate; 1.81–2.4 = moderately susceptible; Over 2.4 = susceptible. The experiment was conducted three times.

# RESULT

## Pathogen selection and molecular phylogeny

The fungal isolate SPL16124 was cultured on PDA and checked it's conidial morphology. In PDA, the fungus produced sparse aerial mycelia, orange pigment in the agar and a whitish to off-white colony color. The shape of the macroconidia was slightly curved and cylindrical to falcate with rounded apical cells, the average length and width of the macroconidia were 23.1 (15.7–31.4) × 5.7 (3.6–6.9) μm (n = 20), respectively, with single to four septations (Fig. 1.). The shape of the microconidia was fusiform to ovoid, and varied in size with no or rare single septation. The microconidial size was 9.3 (6.6–11.3) × 3.7 (3.0–5.1) μm (n = 20). Based on the morphological and conidial characteristics, the fungal isolate was confirmed as *F. solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen [4].To confirm the pathogen as *F. solani*, the sequences of the isolate were collected from GenBank and analyzed molecular data (Table 1). Sequence comparison of the ITS-rDNA and a fragment of translation elongation factor (EF–1α) gene sequences with BLAST revealed sequence similarities of 99–100% in ITS and 100% in EF–1α analyses of all the isolates. Phylogenetic analysis of the combined data set of ITS and EF–1α revealed that the isolates from this study and reference isolates of *F. solani* (*F. solani* CBS 102429 and *F. solani* NRRL 22786$^{T}$) produced a single group with a high bootstrap value (99%) (Fig. 2.). Therefore, the pathogen was identified as *F. solani* based on morphological and molecular data. The GenBank accession numbers of the pathogen are KY796227 (ITS) and KY796233 (EF-1α).

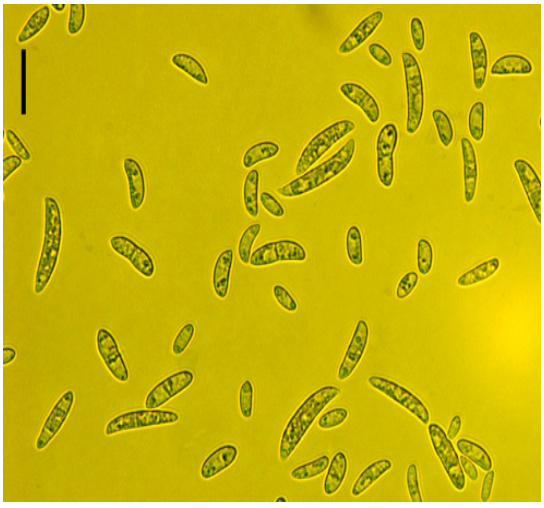

**Fig. 1.** Conidial characteristics of the isolate SPL16124 cultured on potato dextrose agar showing macro- and micro-conidia (Scale bar = 20 μm).

**Table 1.** *Fusarium solani* SPL160124 used in the present study and related references from GenBank with their accession numbers to analyze for constructing a phylogenetic tree

| Species | Origin | Host | Isolates/Strain | GenBank Accession No. | |
|---|---|---|---|---|---|
| | | | | ITS | EF |
| *Fusarium armeniacum* | USA | Horse eye | NRRL 43641 | GQ505462 | GQ505430 |
| *F. asiaticum* | USA | *Triticum aestivum* | NRRL 26156$^T$ | NR_121320 | AF212452 |
| *F. bigoniae* | Germany | *Begonia hiemalis* | CBS 452.97$^T$ | NR_111864 | KC514054 |
| *F. bactridioides* | USA | *Pinus leiophylla* | CBS 100057$^T$ | NR_120262 | KC514053 |
| *F. biseptum* | NA[a] | NA | CBS110311$^T$ | NR_137706 | EU926319 |
| *F. cerealis* | Argentina | Barley grains | MAFF 241212 | AB820717 | AB820701 |
| *F. circinatum* | USA | *Pinus radiata* | CBS 405.97$^T$ | NR_120263 | KM231943 |
| *F. commune* | USA | *Lycopersicon esculentum* | NRRL 28387 | NA | HM057338 |
| *F. concentricum* | Costa Rica | *Musa sapientum* | NRRL 25181$^T$ | NR_111886 | AF333935 |
| *F. denticulatum* | USA | *Ipomoea batatas* | CBS 407.97 | NR_138359 | AF160269 |
| *F. fujikuroi* | Japan | *Oryza sativa* | CBS 221.76$^T$ | NR_111889 | KU604530 |
| *F. equiseti* | Germany | Soil | NRRL 26419$^T$ | NR_121457 | GQ505599 |
| *F. graminearum* | USA | *Triticum aestivum* | CBS 130917 | JX162342 | JX118950 |
| *F. guttiformae* | S. America | *Ananas comosus* | CBS 409.97 $^T$ | NA | KC514066 |
| *F. incarnatum* | NA | Human skin | CBS 133024 | KF255449 | KF255493 |
| *F. kyushuense* | Japan | NA | NRRL 6490$^T$ | AB587020 | AB674297 |
| *F. langsethiae* | Japan | NA | CBS 113234$^T$ | NR_121214 | AB674298 |
| *F. mexicanum* | Mexico | *Mangifera indica* | MXMIC-686 | NA | KM823584 |
| *F. miscanthi* | Belgium | *Miscanthus giganteus* | MUCL53238 | NA | HQ683752 |
| *F. nuragi* | Japan | NA | ATCC 200225 | AB586921 | AB674292 |
| *F. nygamai* | NA | NA | NRRL 13448$^T$ | NR_130698 | NA |
| *F. oxysporum* | Poland | NA | CBS 129.24 | DQ453704 | NA |
| *F. oxysporum* | Mexico | *Capsicum annuum* | CPO 3.011 | NA | KR935895 |
| *F. poae* | Japan | *Glycine max* | MAFF 305947 | AB587024 | AB674302 |
| *F. polyphialidicum* | South Africa | Plant debris | CBS 676.94 | X94172 | KR071774 |
| *F. phyllophilum* | Japan | NA | CBS 216.76 | AB587006 | AB674283 |
| *F. proliferatum* | India | NA | CBS 138981 | KT716199 | KT716210 |
| *F. temperatum* | Belgium | *Zea mays* | EFA313A | KC179826 | KC179824 |
| *F. thapsinum* | South Africa | *Sorghum bicolor* | NRRL 22045 | U34560 | AF160270 |
| ***F. solani*** | **Korea** | ***Ipomoea batatas*** | **SPL16124** | **KY796227** | **KY796233** |
| *F. solani* | Australia | Bark | CBS 102429 | KM231808 | KM231936 |
| *F. solani* | Sudan | *Vicia faba* | NRRL 52715 | JF740912 | DQ247657 |
| *F. subglutinans* | USA | *Zea mays* | CBS 215.76 | NA | KC514067 |
| *F. verticillioides* | Germany | *Zea mays* | NRRL 22172 | U34555 | AF160262 |

[a]NA: not available. $^T$: Type strain

Bold letters indicates and accession numbers determined in this study. S. America, South America; SPL, Sweetpotato Laboratory, Bioenergy Crop Research Institute, National Institute of Crop Science, Korea; CBS, The Centraalbureau voor Schimmelcultures-Fungal Biodiversity Centre, the Netherlands; NRRL, Northern Regional Research Service, Agricultural Research Service Culture Collection, USA.

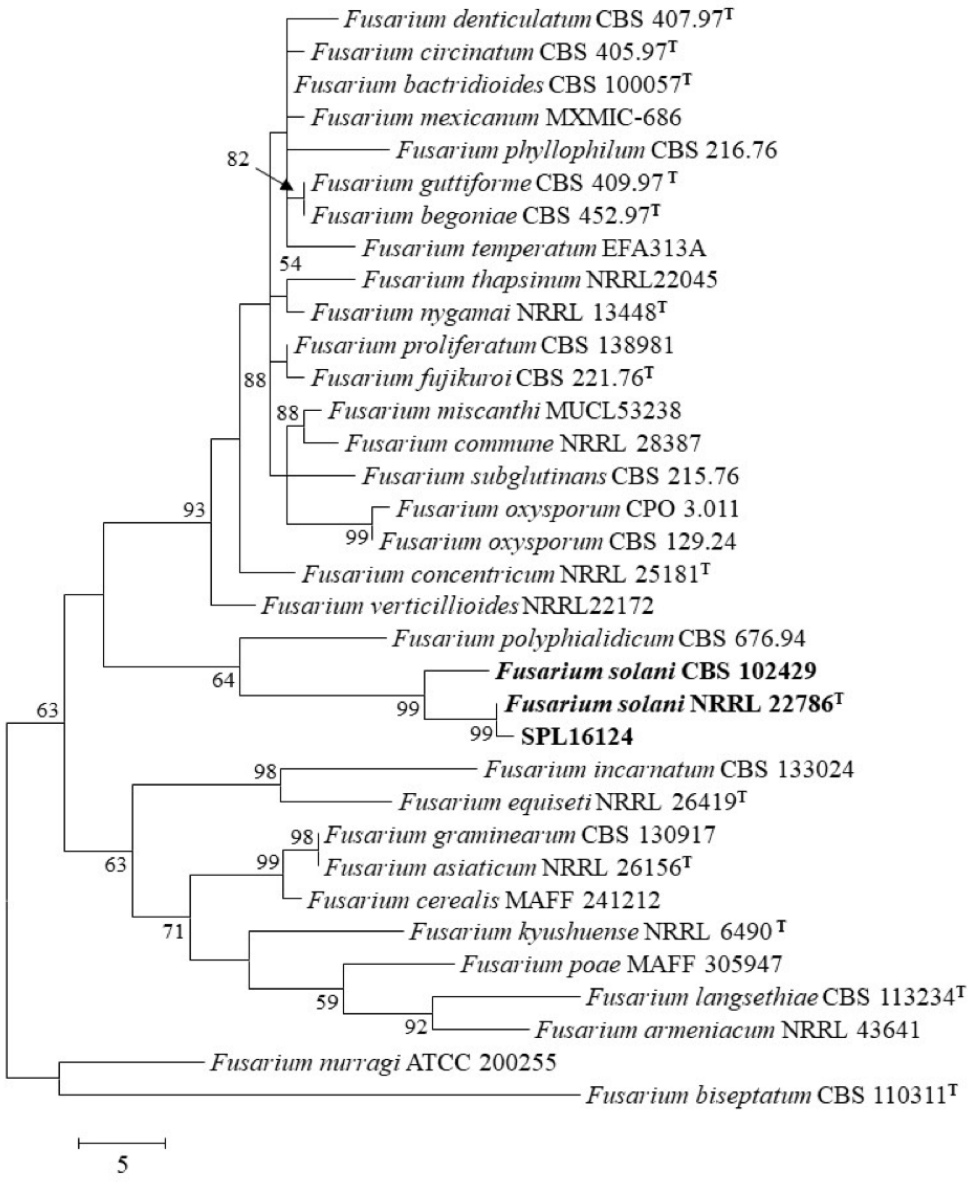


**Fig. 2.** A parsimonious tree of the combined datasets of the ITS and EF-1α region of *Fusarium* and it's related species from GenBank. Consistency, retention, and composite indexes are 0.546392, 0.738872, and 0.451170, respectively, for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Bootstrap values > 50% is indicated above branches. The analysis involved 34 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 273 positions in the final dataset. 'T' indicates type strain, and present study isolate is in bold.

## Resistant variety selection

A total of 14 varieties were tested for resistant variety selection on sweetpotato tuber. Among them, Daeyumi, Singeonmi, Sinjami, and Yenjami found resistant based on disease severity index (Fig. 3.). Sinjami did not

show any disease incidence in each treatment and replication at all. So, this variety is termed as complete resistant variety. Pungwonmi, Jinhongmi, Dahomi, and Danjami were moderately resistant. These four well known and quality varieties showed nearly resistance activity. Moderate and moderately susceptible varieties were Yulmi & Hogammi and Jugwabgmi & Yenhwangmi, respectively. Two susceptible varieties were detected and confirmed as Sinhwangmi and Beniharuka (Fig. 4.). Beniharuka is known to have a susceptibility to many fungal diseases.

On the other hand, all the varieties evaluated for the wilting of stalks showed no disease incidence (Fig. 5.). A complete resistance was observed in the stalk. *Fusarium solani* might not affect stalk, and Fusarium storage rot disease caused by *F. solani* is specific to the tuber.

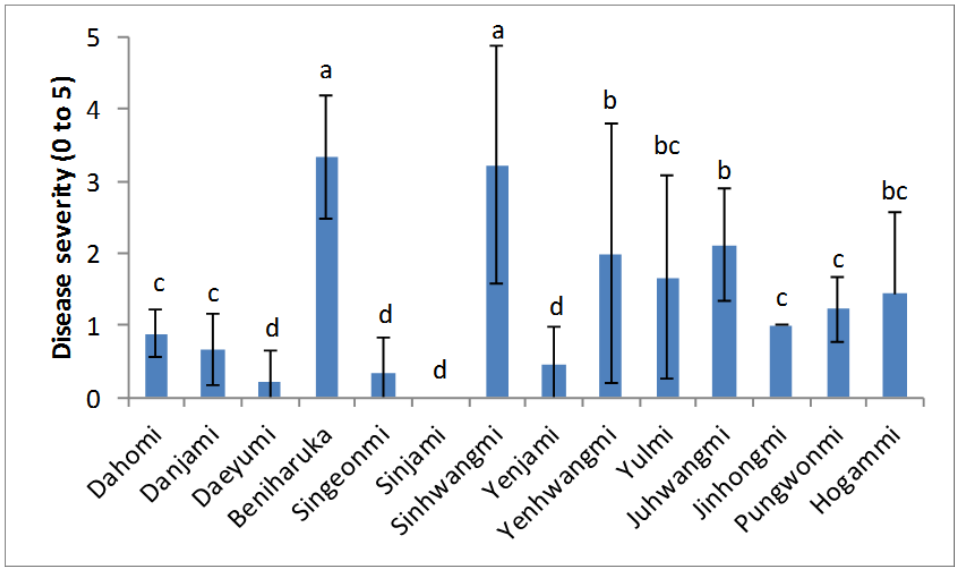


**Fig. 3.** Disease severity index and resistance variety selection from 14 different sweetpotato experimented by *Fusarium solani* isolate SPL160124 on the tuber. Letters indicate the level of resistance or susceptibility- 'a' susceptible.

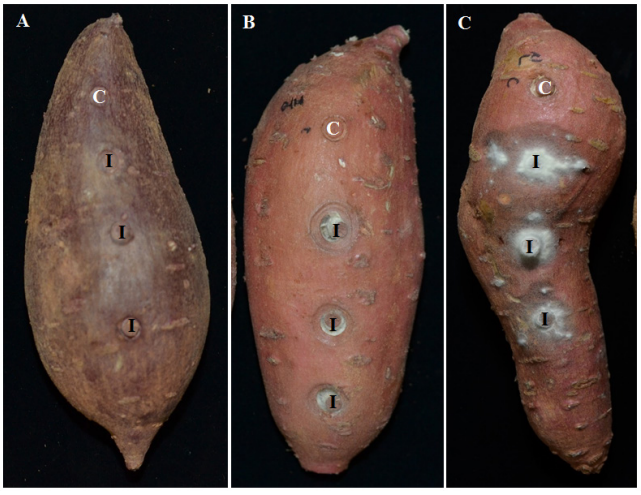


**Fig. 4.** Photographs of the resistant, moderate and susceptible varieties of Sinjami (A), Pungwonmi (B), and Jinhwangmi (C), respectively. Here C indicates control, and I indicate inoculated.


**Fig. 5.** Fusarium storage root rot-resistant variety test on stalks of Dahomi and Jinhongmi inoculated with spore suspensions of $1 \times 10^5$ spores/mL.

## DISCUSSION

Fusarium storage root rot is a common postharvest disease in sweetpotato, sunken lesions are observed in the surface of the tubers, which extended into the periderm of the root [3]. In severe cases, hollow cavities formed under the lesion surfaces and white mycelium observed inside the cavities. Fusarium surface rot and storage root rot are closely related and show similar symptoms. Fusarium surface rot is restricted to the cortex of the storage roots, whereas root rot is penetrating the disease into the inner part [14]. This is the only differentiating characteristic of these two diseases. The storage root rot disease explained clearly by Yang et al. [3] and isolated from different locations in Korea. The authors identified through morphological & molecular methods tested pathogenicity and confirmed the pathogen as *Fusarium solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen.

In the present study, we collected an isolate SPL160124 from Sweetpotato Research Lab., Bioenergy Crop Research Institute, NICS, Muan, Korea, and confirmed its identity by molecular phylogenetic analysis. The internal transcribed spacer (ITS) and translation elongation factor (EF 1-α) region used to identify the pathogen. Romberg and Davis [15] identified *F. solani* based on these gene sequences and confirmed that these two gene sequences are enough to identify *F. solani* species complex. The use of the molecular technique for the identification of this pathogen species isolated from different solanaceous crops (Eg. tomato, potato, sweetpotato, pepper, and eggplant) could help clarify the taxonomic positions of this species [15,16].

*F. solani* causes diseases in many plants such as potato tuber dry rot, a variety of wilt and root rots in eggplant, pepper, and tomato. So, it was necessary to find out root rot and wilt-free resistant variety selection. The method we applied to screen resistant variety was easy, fast and inexpensive. Fourteen local and well known sweetpotato varieties were used in the present study. The highest degree of resistance found was Sinjami followed by Daeyumi, Singeongmi, and Yenjami. As these varieties are resistant to *F. solani*, the storage period could increase the tuber. A similar experiment was conducted by Leach & Webb [17], and they mentioned to apply proper cultivation techniques in the field to reduce the injury and damage.

In contrast, the present study suggested that some prevalent varieties are susceptible to *F. solani* pathogen-

Beniharuka and Yulmi are two of them. These two varieties are well known for their taste and color. It is suggested that an excellent agronomic practice strategy is needed to reduce storage root rot disease. Almost 90% of the cultivars were susceptible to *Fusarium* diseases tested by Corsini and Pavek [18]. In the present study, we also observed the percentage of disease incidence among varieties was very high. It was found that the disease occurs only on tubers. We tested the disease occurrence on stalks. The result revealed that the *F. solani* pathogen could not infect stalks of sweetpotato. The disease showed tissue specificity on sweetpotato. In conclusion, we could improve the level of storage root rot resistance or inhibit the disease incidence by practicing proper agronomic strategy and by confirming a clean storage environment.

## ACKNOWLEDGMENTS

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