Comparison of Sudden Death Syndrome in Responses to *Fusarium* solani f. sp. glycines between Korea and U.S. Soybean Lines

Joon Hyeong Cho*†, Yong Wook Kim**, and J. C. Rupe ***

ABSTRACT

In order to identify the responses of Korean soybean cultivars to sudden death syndrome (SDS), forty-two Korean cultivars and three check cultivars (Hartwig and PI 520733 are resistant; Hartz 6686 is susceptible) were tested with sorghum seed inoculum infested with Fusarium solani f. sp. glycines isolate 171 in the greenhouse. This isolate has blue pigment cultural shape on potato dextrose agar (PDA) medium. All Korean cultivars inoculated with F. solani isolate 171 showed the typical SDS symptoms and disease severity on soybean leaves in each cultivar varied at 4 weeks after inoculation. Nine cultivars were included in the most SDS susceptible group and six cultivars were included in the most susceptible group based on Duncan's multiple range tests (P≤ 0.05). In results of the LSD analysis for SDS the resistant group, a total of twenty-five Korean cultivars were included in the same SDS resistant group as PI 520733 or Hartwig and fourteen Korean cultivars were included in the same SDS susceptible group as Hartz 6686.

In the second experiment, ten Korean cultivars, ten U.S. cultivars, and one introduced line were compared in the same way as the first experiment. Disease severity ranking of check cultivars, Hartwig, PI 520733, and Hartz 6686, were the same as in the first experiment. Within Korean cultivars, seven cultivars showed the consistent severity proportions of leaf symptoms. Disease rankings of these cultivars in this experiment were the same as those in the first experiment. Three US cultivars: Hartwig, Hartz 5454, and Forrest, three Korean cultivars: Keunolkong, Myeongjunamulkong, and Jinpumkong 2, and one introduced line, PI 520733, were included in the highest SDS resistant group. Shinphaldalkong 2, Milyang 87, and Samnamkong consistently showed the highest SDS susceptibility in both experiments.

Average disease severity in the first and the second experiment were 49.56% and 45.39%, respectively.

Keywords: sudden death syndrome, soybean, Korean cultivars, *Fusarium solani* f. sp. *glycine*, greenhouse test, resistance, leaf symptom.

INTRODUCTIONS

Sudden death syndrome (SDS) is a soil borne disease of soybean (*Glycine max* (L.) Merr.) caused by *Fusarium solani* f. sp. *glycines*. SDS was first observed in Arkansas, USA in 1971 and has been reported in other states in the United States (Hartman et al., 1995; Jardine & Rupe, 1993; Yang & Rizvi, 1994). Besides the USA, SDS occurred in Argentina in 1993, Brazil in 1991, and Canada in 1993 (Anderson & Tenuta, 1988; Ivancovich et al., 1996; Nakajima et al., 1996). Hirrel (1983) first reported the occurrence of this disease in Arkansas and named it as "Sudden Death Syndrome" in response to perceived rapidity with which above ground leaf symptoms developed.

Initial leaf symptoms of SDS are interveinal, pale green to chlorotic spots that produce a mottled appearance at flowering stage. The spots become necrotic and develop into chlorotic streaks, killing the leaflets, which dehisce leaving the petioles attached to the plant (Roy et al., 1989; Roy et al., 1997; Rupe, 1989). Pod abortion occurs in association with defoliation on severely infected plants (Yang & Rizvi, 1994). The typical root symptoms of SDS include root rot, crown necrosis, and vascular discoloration of roots and stems (Hirrel, 1983;, Roy et al., 1989; Rupe, 1989; Yang & Rizvi, 1994). External symptoms of SDS are similar to those of Brown stem rot caused by *Phialophora gregata*, however, the pith of plants infected with F. solani f. sp. glycines remains white (Hirrel, 1983).

Yield loss due to SDS may dramatically increase in high yield potential cultivars. SDS affects yields by reduction in seed size and numbers, and may affect seed germination (Rupe et al., 1993). The three-year average yield loss from 1989 to 1991 due to SDS in the north central region of the United states was ranked 9th out of 20 soybean diseases (Ben Doupnik, 1991). Estimated yield losses due to SDS in the USA, Argentina, and Brazil in 1994 were 89,400, 134,000, and 15,000 metric tons, respectively (Wrather et al., 1997).

The SDS pathogen appears to be a unique strain

^{*}Post-doc., Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, U.S.A. **Professor, Dongguk University, Department of Plant Resources, Seoul, Korea. ***Professor, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, U.S.A. *Corresponding author: (E-mail) jhcho2001@hanmail.net (Phone)+82-2-2260-3309. Received 1 Nov., 1999.

of the fungus, *F. solani*. Based on pathogenicity tests, Roy et al. (1989) designated the SDS pathogen as *F. solani* f. sp. *glycines*. Various molecular studies have reported that the SDS pathogen is distinct from other *F. solani* strains, but there are very few if any differences between isolates of *F. solani* f. sp. *glycines* even when those isolates originate from different states. Based on molecular studies, the SDS pathogen was *F. solani* f. sp. *phaseoli*, but these results were not supported by later pathogenicity data (O'Donnell, 1995). The SDS pathogen may be related to *F. solani* f. sp. *phaseoli* and the two pathogens share many cultural characteristics.

On potato dextrose agar, *F. solani* f. sp. *glycines* grows slowly producing an appressed colony with a blue center and a white margin. Numerous three to five celled macroconidia are produced, but microconidia are rare. Chlamydospores are produced in hyphae and in macroconidia (Lowlence et al., 1988; Rupe, 1989).

Rupe et al. (1996) reported that causal fungus as blue pigment strain of *F. solani* is considered to be the same as *F. solani* f. sp. *glycines*. Various isolates of *F. solani* produced root rot and leaf symptoms similar to SDS in the greenhouse and field, but the symptoms varied greatly in intensity (Gray, 1996; Rupe, 1989). *F. solani* was recovered from the roots of inoculated plants and infested plants in the field (Killebrew et al., 1988) but not from the stem tissues above the soil line (Gray, 1991). In researches for host specifying with several isolates of *F. solani* on beans, *F. solani* isolates associated with SDS are not host specific and readily infect other hosts (Gray, 1991; Melgar & Roy, 1994).

Various methods have been used to inoculate soybean plants in the greenhouse to determine the virulence of different isolates of the pathogen (Gray, 1996; Killebrew et al., 1988; Lim & Jin, 1991; Melagr & Roy, 1994) to determine the relationship of soybean reactions to *F. solani* between greenhouse tests and field tests and to evaluate soybean cultivar responses to inoculation (Lim & Jin, 1991; Stephens et al., 1993). The effect of *F. solani* isolates and inoculum concentration on disease severity have also been studied (Gray, 1996; Killebrew et al., 1988).

Rupe (1988), Rupe & Gbur (1995) and Rupe et al. (1991) studied the relationship between cultivar susceptibility and disease development. They reported that cultivar susceptibility affected disease development, and SDS severity at R3 growth stage (beginning pod) was significantly correlated with the severity at R6 growth stage (full seed stage). Stephens et al. (1993) reported that SDS leaf symptom severity for field grown plants at R6 growth stages and greenhouse leaf symptom severity at about 3 weeks after inoculation were highly correlated.

The unique foliar symptoms of SDS appear to be

produced by a toxin since the fungus is confined to the root system. A 17 kd polypeptide toxin, which has been reported in culture filtrate, produces SDS-like foliar symptoms on susceptible cultivars (Jin et al., 1996). Cultivar reactions to the toxin are similar to reactions to SDS in greenhouse inoculation tests.

Cultivars vary widely in their response to SDS in both the field and greenhouse. These responses vary from very susceptible to moderately susceptible to resistant. Cultivar reaction to SDS appears to be determined by from one to four genes (Stephens et al., 1993). The use of resistant cultivars is the primary means of controlling SDS.

Although, SDS has not been reported in Korea, sudden death syndrome is being observed in new locations almost every year. From its beginnings in Arkansas in the early 1970's, it now occurs in most of the soybean growing areas in USA, later in Argentina, Brazil, and Canada. High degree of similarities of isolates collected from widely separated locations suggests that the SDS pathogen is being spread. How this spread is occurring is not known, but SDS appears to pose a threat to soybean production in Korea.

The objectives of this study were to establish a proper screening method using a greenhouse assay, to identify the reactions of Korean soybean cultivars to *F. solani* f. sp. *glycines*, and to compare selected Korean cultivars with soybeans from the USA with known reactions to the pathogen.

MATERIALS AND METHODS

In this study, we defined resistance to SDS as a delay in, or lack of, leaf symptom expression after an inoculation period that causes advanced expression of symptoms in susceptible infected genotypes.

Two separate studies were conducted; one identifying the soybean responses to the SDS pathogen with Korean cultivars to search for SDS resistant cultivars which may be available for breeding materials, and the other comparing the cultivar responses to the SDS pathogen between Korean lines and US lines to utilize the introduced elite lines from foreign countries.

Greenhouse screening for Korean cultivars

For identifying the susceptibilities of Korean cultivars to SDS, a total of forty-two soybean cultivars, which were grown at the National Crop Experiment Station and National Yeongnam Agricultural Experiment Station, Rural Development Administration (RDA), Korea, were tested in the greenhouse (Table 1). Hartwig and PI 520733, which are SDS resistant cultivars, and Hartz 6686, which is an SDS susceptible cultivar, were used as check cultivars to compare the

Table 1. Responses of Korean soybean cultivars to SDS -pathogen, *Fusarium solani* f. sp. *glycines* isolate 171, in the greenhouse test.

| Soybean Cultivars | Maturing trait † | Source [†] | Disease severity (%) |
|-------------------|---------------------|---------------------|----------------------|
| Shinphaldalkong 2 | M | NCES | 79.09 a |
| Hartz 6686 | - | NOBO | 71.37 ab |
| Milyang 87 | M | NYAES | 70.45 ab |
| Samnamkong | M | NYAES | 70.15 abc |
| Jangsukong | M | NCES | 66.12 abcd |
| Suwon 181 | L | NCES | 64.71 abcde |
| Danyeopkong | м | NCES | 64.30 abcde |
| Suwon 184 | L | NCES | 63.97 abcde |
| Baekwunkong | $\dot{\mathbf{M}}$ | NCES | 63.88 abcde |
| Suwon 185 | L | NCES | 63.02 abcde |
| Mallikong | M | NCES | 60.77 bcdef |
| Milyang 76 | M | NYAES | 60.30 bcdef |
| Suwon 191 | | NCES | 59.03 bcdefg |
| Suwon 190 | L L | | 56.21 bcdefgh |
| Bukwangkong | | NCES | |
| Danwonkong | M | NYAES | |
| Milyang 86 | M | NYAES | |
| Eunhakong | M | NYAES | |
| | M | NYAES | |
| Dankyeongkong | Ē | NYAES | 50.76 defghijk |
| Hannamkong | E | NYAES | 50.62 defghijk |
| Sobaeknamulkong | M | NYAES | 50.49 defghijk |
| Milyang 73 | M | NYAES | 49.02 efghijkl |
| Hwaeumputkong | Ē | NCES | 48.82 efghijkl |
| Taekwangkong | E | NCES | 46.32 fghijklm |
| Keumgangkong | M | NYAES | 45.36 fghijklmn |
| Namhaekong | M | NYAES | 44.83 fghijklmn |
| Pokwangkong | M | NCES | 44.83 fghijklmn |
| Suwon 189 | L | NCES | 43.72 ghijklmn |
| Hwangkeumkong | M | NCES | 43.49 ghijklmn |
| Dajangkong | Ĺ | NYAES | 42.83 hijklmn |
| Suwon 192 | L | NCES | 42.51 hHijklmn |
| Danbaekkong | M | NCES | 42.27 hijklmn |
| Jinpumkong | M | NCES | 41.97 hijklmn |
| Saealkong | M | NYAES | 41.37 hijklmn |
| Jinpumkong 2 | M | NCES | 40.77 hijklmn |
| Muhankong | M | NCES | 39.46 ijklmn |
| Suwon 188 | L | NCES | 37.89 jklmn |
| PI 520733 | - T2 | NISTATIO | 37.02 klmno |
| Milyang 81 | E | NYAES | 36.59 klmno |
| Duyukong | E | NYAES | 35.44 klmno |
| Myeongjunamulkong | | NCES | 35.40 klmno |
| Keunolkong | E | NYAES | 34.15 lmno |
| Hartwig | ~ | NINTATIO | 31.46 mno |
| Milyang 83 | E | NYAES | 29.48 no |
| Kwangankong | M | NCES | 21.00 o |
| Hartz 6686 (C) | | | 0.00 p |
| Avr [¥] | | | 49.56 |
| LSD 0.05* | | | 16.31 |
| LOD 0.00 | | | 10.01 |

[†]Maturing traits of all Korean cultivars were evaluated by National Crop Experiment Station and National Yeongnam Agricultural Experiment Station, Rural Development Administration. E = early maturing, M = middle maturing, L = late maturing

"Uninoculated control

Average proportions of leaf symptoms in all cultivars. All means are derived from two separated test, which has three replications, with each test considered a replication in the ANOVA. Means followed by same letter are not significantly different (P<0.05) according to the LSD test. is the highest level of leaf symptom and be the lowest.

symptom developments between cultivars. PI 520733 is an introduced line from Korea (Hartman et al. 1995). Korean cultivars used in this test had early, middle, and late maturing traits. The number of each maturing cultivars of Korean lines used in this test were 8 cultivars of early, 25 cultivars of middle, and 10 cultivars of late maturity group, respectively.

Comparisons of responses of Korean and U.S. cultivars to the SDS pathogen

To compare the soybean responses to SDS between Korean lines and US lines, 3 cultivars showing the highest disease severities, 4 cultivars showing the lowest disease severities, and 3 moderate cultivars were selected based on the results of the above experiment. Also, ten US cultivars from maturity groups (MG) V and VI and one introduced line (PI 520733) which is MG III (*informed from* SoyBase), including check cultivars used in the above experiment, were selected based on the results of a former study (Dr. J. C. Rupe, *unpublished data, personal communications*) (Table 2.). For this study, Korean and U.S. cultivars were tested under the same conditions in the greenhouse.

Preparation of sorghum seed inoculum with the SDS pathogen

Inoculum of F. solanoi, SDS pathogen isolate 171 (isolated from the root of an SDS symptomatic plant in the field at Marianna, Arkansas, USA: Dr. J. C. Rupe, Dept. of Plant Pathology, Univ. of Arkansas) was prepared by growing the isolate on potato dextrose agar (PDA) medium for 7 days at room temperature under flourescent lights (10 hr/day). Three 1 cm diameter plugs were cut from the growing edge of the culture and aseptically transferred to sterilized sorghum seed. 125 g of sorghum seed was placed in a 500 ml Erlenmyer flask with 150 ml of deionized water. The seeds were autoclaved for 50 minutes on two consecutive days. Once cooled, the plugs of the isolate were added and the inoculum was incubated for 15 days with daily shaking to insure complete colonization of the seed.

Inoculation and leaf disease rating method for soybean SDS

These experiments were conducted in greenhouse at the University of Arkansas in Fayetteville, Arkansas, USA. Pathogenicity and virulence of *F. solani* isolate 171 were determined in the greenhouse test (J. C. Rupe, *personal communication*).

Plastic flats $(30 \times 60 \times 6 \text{ cm})$ were filled with a steam pasteurized soil mix consisting of 10 parts soil, 4 parts potting mix, 2 parts vermiculite, and 1 part

Growing institution; NCES = National Crop Experiment Station and NYAES = National Yeongnam Agricultural Experiment Station, Rural Development Administration, Korea Disease severity presented was evaluated according to a 0 to 11 scale of foliar symptoms, where scale 0 = 0%, 1 = 0~3%, 2 = 4~6%, 3 = 7~12%, 4 = 13~25%, 5 = 26~50%, 6 = 51~75%, 7 = 76~88%, 8 = 89~94%, 9 = 95~97%, 10 = 98~99%, and 11 = 100% of the leaf area with symptoms (The Horsefall-Barret Scale for assessing disease). Average of severity proportions were noted by converting to midpoint percentage in each scale before averaged.

Table 2. Comparisons of Korean and U.S. cultivars soybean responses to Fusarium solani f. sp. gly-cines.

| C -1 - 14 | | Carran | Maturity group | | D:(0/) † | SDS [§] |
|-----------------------------|-------|---------------------|----------------|--------------|---------------------|------------------|
| Soybean cultivars | | Source – | USA | Kor | Disease severity(%) | |
| Shinphaldalkong | Korea | | _ | - M | 76.94 a | |
| Dillon | USA | Clemson University | 6 | - | 74.82 a | S |
| Milyang 87 | Korea | | _ | M | 72.81 a | |
| Hartz 6686 | USA | Hartz seed co. | 6 | _ | 70.06 a | S |
| Samnamkong | Korea | | _ | M | 69.89 a | |
| Asgrow 5547 | USA | Asgrow seed co. | 5 | _ | 66.17 ab | S |
| NK S 5960 | USA | Northrup King co. | 5 | - | 64.98 ab | S |
| Milyang 83 | Korea | | _ | E | 50.84 bc | |
| Delta Pine DP 3640 | USA | Delta&Pine land co. | 6 | - | 49.15 cd | |
| Bukwangkong | Korea | | | ${f M}$ | 44.33 cde | |
| Taekwangkong | Korea | | - | \mathbf{M} | 39.89 cdef | |
| Asgrow 6297 | USA | Asgrow seed co. | 6 | - | 37.51 cdef | |
| Kwangankong | Korea | | | M | 37.03 cdef | |
| Hartz 5350 | USA | Hartz seed co. | 5 | _ | 36.94 cdef | R |
| Jinpumkong 2 | Korea | | - | \mathbf{M} | 35.51 cdefg | |
| PI 520733 | Korea | | 3 | _ | 33.65 defg | R |
| Myeongjunamulkong | Korea | | _ | \mathbf{M} | 30.06 efg | |
| Keunolkong | Korea | | _ | \mathbf{E} | 29.62 efg | |
| Forrest | USA | _ | 5 | - | 29.40 efg | R |
| Hartz 5454 | USA | Hartz seed co. | 5 | _ | 26.85 fg | R |
| Hartwig | USA | | 5 | - | 19.67 g | R |
| Hartz 6686 (C) [¶] | | | | | 0.00 h | |
| Avr¥ | | | | | 45.39 | |
| LSD 0.05* | | | | | 17.02 | |

[†]Maturing traits of all Korean cultivars were evaluated by National Crop Experiment Station and National Yeongnam Agricultural Experiment Station, Rural Development Administration (E=early, M=middle, and L=late maturing).

sand (V:V). Six equally spaced furrows were dug across the width of the flat to a depth of 5 cm, and 20 ml of inoculum was evenly spread along the bottom of the furrow. The inoculum, was covered with 2 cm of soil mix and ten seeds of each cultivar were placed in the furrow. The seeds were covered with an additional 2 cm of soil mix and placed in the greenhouse. Average temperature conditions in the greenhouse were 28°C for the first experiment and 24°C for the second experiment. The lowest temperature at night and the highest temperature during the day were 23°C and 30°C , respectively. Plants were watered from the top twice daily.

For this study, each cultivar was tested twice in

the greenhouse in a randomized block design with three replications. To compare responses of soybean cultivars to *F. solani* isolate 171, uninoculated plants of Hartz 6686 (SDS susceptible cultivar) was included as an untreated control in each replication. For preparing the untreated control, ten seeds of each cultivar were planted in each plot in the same way as described above but without inoculum.

Rating of foliar symptom severities were conducted once for each test of cultivars, 3 to 4 weeks after planting with inoculum, when leaf symptoms were clearly distinguishable between inoculated cultivars of SDS resistant and SDS susceptible plants. Each plant in each row was rated for percent of leaf area

[†]Disease severity presented was evaluated according to a 0 to 11 scale of foliar symptoms, where scale 0 = 0%, $1 = 0 \sim 3\%$, $2 = 4 \sim 6\%$, $3 = 7 \sim 12\%$, $4 = 13 \sim 25\%$, $5 = 26 \sim 50\%$, $6 = 51 \sim 75\%$, $7 = 76 \sim 88\%$, $8 = 89 \sim 94\%$, $9 = 95 \sim 97\%$, $10 = 98 \sim 99\%$, and $11 = \sim 00\%$ of the leaf area with symptoms (The Horsefall-Barret Scale for assessing disease). Average of severity proportions was noted by converting to midpoint percentage in each scale before averaged.

Data of soybean responses to SDS; former result from Dr. J. C. Rupe, University of Arkansas, Department of Plant Pathology. (S=SDS susceptible, R=SDS resistant)

[¶]Uninoculated control.

^{*0} Average proportions of leaf symptoms in all cultivars.

^{*}All means are derived from two separated test, which has three replications, with each test considered a replication in the ANOVA. Means followed by same letter at each rating date are not significantly different ($P \le 0.05$) according to the LSD test. a is the highest level of leaf symptom and b is the lowest.

with symptoms of SDS using a 0 to 11 scale with 0 = 0%, 1 = $0 \sim 3\%$, 2 = $4 \sim 6\%$, 3 = $7 \sim 12\%$, 4 = $13 \sim 25\%$, 5 = $26 \sim 50\%$, 6 = $51 \sim 75\%$, 7 = $76 \sim 88\%$, 8 = $89 \sim 94\%$, 9 = $95 \sim 97\%$, 10 = $98 \sim 99\%$, and 11 = 100% (The Horsefall-Barret Scale for assessing disease).

In order to identify the development of SDS symptom on soybean plants caused by 171 as time progressed, unifoliar leaf symptoms, trifoliar leaf symptoms, petiole drops of leaf, and taproot discoloration due to root rot were photographed.

Statistical analysis

Average of severity proportions of leaf symptoms at the rating date was noted by converting to midpoint percentage in each scale before averaged. The resulting average scales and proportions of leaf symptoms were then analyzed by ANOVA and the means compared using the Least Significant Difference test (LSD). The analysis was conducted using Proc GLM of SAS (SAS Institute, Cray, NC).

RESULTS

Cultural morphology of SDS pathogen

Cultural colony shape of *F. solani* isolate 171, SDS pathogen, is shown as Fig. 1. The colony of *F. solani* isolate 171 grew to 2.8 cm diameter by 7 days, and to 7 cm diameter by 14 days after transfer to potato dextrose agar (PDA) medium in 9 cm plastic petri-dish at the room temperature. At less then 7 days old colonies had a distinct bluish color in the central part of colony in contrast to grayish white aerial mycelium of edge of the colony. As colonies became older, grayish-white aerial mycelium turned to grayish blue, but it had still more dark blue color in the central part. These morphological characteris-

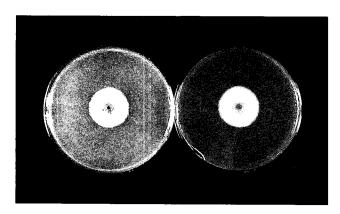


Fig. 1. Surface (left) and reverse (right) of 7-day -old cultural colony shape of *Fusarium solani* f. sp. *glycines* isolate 171, sudden death syndrome (SDS)-pathogen, on potato dextrose agar (PDA) medium.

tics were similar to the results of Lowlence et al. (1988) and Melgar et al. (1994).

Symptom progression on soybean plant

The first leaf symptoms were observed on soybean leaves at about 10 days after planting with inoculum in the greenhouse test. Several days later, these symptoms appeared more distinct. All Korean cultivars and check cultivars inoculated with *F. solani* isolate 171 showed the typical leaf symptoms and root rot symptoms 12 days after planting with sorghum seed inoculum (Fig. 2).

In general, in the early stage of infection, the pale green colored and irregular shaped chlorotic spots appeared on unifoliar leaves (Fig. 2, A) or mosaic like mottling and crinkling appeared on trifoliar leaves of soybean plants (Fig. 2, B). As disease developed, this chlorotic spots developed into interveinal chlorotic streaks, became necrotic on unifoliar and trifoliar leaves, and only the leaf veins remained green (Fig. 2, C and D). In the case of severely affected plants by SDS or in older plants, leaflets abscise from the petioles, which dehisce leaving the petioles attached to the stem (Fig. 2, E).

Greenhouse-inoculated seedlings, however, did not always go through the mottling or crinkling stage before becoming chlorotic (28). We also observed that mottling or crinkling appearance were rare on SDS susceptible cultivars but common on resistant cultivars. Leaves of susceptible cultivars such as Shinphaldalkong 2 and Hartz 6686 developed interveinal chlorosis that progressed rapidly to interveinal necrosis. Resistant cultivars such as Hartwig showed the mosaic like mottling and crinkling or some chlorosis in early affected stage, but this chlorosis developed mere slowly than for the SDS susceptible cultivars (data unpublished).

Root systems were also affected by *F. solani*. The color of the taproot of infected plants turned reddish brown and this discoloration extended up to the soil line and even lower stem while uninoculated plants did not show the discoloration of the taproot. Also, lateral roots of inoculated plants did not develop as well as lateral roots of uninoculated plant (Fig. 2, F).

Responses of Korean cultivars to *F. solani* in the greenhouse

To identify the responses of Korean cultivars to SDS pathogen, forty-two soybean cultivars were planted with sorghum seed inoculum infested with F. solani isolate 171 in the greenhouse and compared with check cultivars.

Disease severities results for each Korean cultivar are shown as Table 1. Proportions of disease severities on soybean leaves in each cultivar varied at 4 weeks

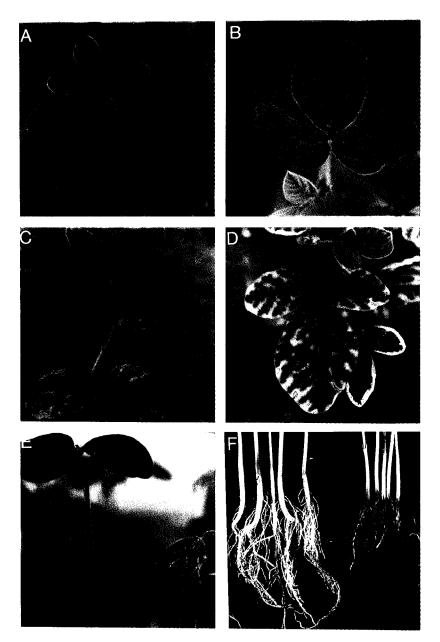


Fig. 2. Progression in severity of leaf symptoms and the typical root rot symptom of sudden death syndrome (SDS) on soybean plant after inoculation with *Fusarium solani* f. sp. *glycines* in the greenhouse test. A, Chlorosis on unifoliar leaves (12-day old); B, mottling and crinkling on trifoliar leaves (12-day old); C-D, interveinal necrosis on unifoliar or trifoliar leaves (19-day old); E, leaf abscission (arrow) at juncture of petiole and leaflet (15-day old); F, root rot and lower stem necrosis (28-day old).

after planting with inoculum and significant differences within cultivars were observed at $P \le 0.05$, according to Duncan's multiple range tests. the resistant check cultivar, Hartwig, showed the lowest susceptible traits to SDS pathogen (31.36%) and the susceptible check cultivar, Hartz 6686, showed the highest (71.37%). PI 520733 showed a low level of disease severity on leaves (37.02%) and there was no significant difference between disease severities of these two SDS resistant

check cultivars, Hartwig and PI 520733. Disease severity of Hartz 6686 was significantly higher than that of Hartwig and PI 520733. This cultivar was significantly different from the SDS resistant cultivars at $p \le 0.05$.

Nine Korean cultivars, Shinphaldalkong 2, Milyang 87, Samnamkong, Jangsukong, Suwon 181, Danyeopkong, Suwon 184, Baekwunkong, and Mallikong, were included in the most SDS susceptible group and six cultivars,

which are Kwangankong, Milyang 83, Keunolkong, Myeongjunamulkong, Duyukong, and Milyang 81, showed the most SDS resistant based on the results of Duncan's multiple range test. The ranges of disease proportions of the most susceptible group and the most resistant group were from 63.02 % to 79.09 % and from 21.00 % to 37.02 %, respectively.

Kwangankong and Milyang 83 showed less disease severity than Hartwig and PI 520733 and Shinphaldalkong 2 showed more disease severity than Hartz 6686. But, these differences were not significantly different in each comparison.

In results of LSD analysis for SDS resistant group, a total of twenty five Korean cultivars were included in the same SDS resistant group as PI 520733 or Hartwig and fourteen Korean cultivars were included in the same SDS susceptible group as Hartz 6686. Only one cultivar, Danwonkong, was not significantly different from either SDS resistant and susceptible groups. While all cultivars inoculated with the SDS pathogen showed leaf symptoms, the Hartz 6686 uninoculated control did not showed the SDS symptoms. The average proportion of leaf symptom severity in all cultivars was 49.56%.

Comparisons of Korean and U.S. cultivars soybean responses to SDS

To compare the soybean responses to SDS between Korean lines and US lines, ten Korean cultivars were selected based on the results of above experiment and tested with ten US cultivars and one introduced line (PI 520733) in the greenhouse. Four cultivars, Kwangankong, Milyang 83, Keunolkong, and Myeongjunamulkong, were selected from the highest resistant group; three, Shinphaldalkong 2, Milyang 87, and Samnamkong, from the highest susceptible group; and three, Bukwangkong, Taekwangkong, and Jinpumkong 2, from the middle range of SDS disease severity proportions (40~60%). Three cultivars selected from the middle range, disease severity of Bukwangkong (55.40%) was not significantly different from that of Hartz 6686 (71.37%), and disease severity of Taekwangkong (46.32%) and Jinpumkong 2 (40.77%) were not significantly different from those of SDS resistant cultivars, which are Hartwig (31.46%) and PI 520733 (37.02%).

The U.S. soybean lines tested, four cultivars and one introduced line we selected were SDS resistant, Hartwig, Hartz 5454, Hartz 5350, Forrest, and PI 520733 and four cultivars were SDS susceptible, Dillon, Hartz 6686, Asgrow 5547, and NK S-5960 (from Dr. J. C. Rupe, *unpublished data, personal communication*). Disease severities for Deltapine DP 3460 and Asgrow 6297 were not tested.

Disease severity ranking of check cultivars, Hartwig, PI 520733, and Hartz 6686, were the same as in first experiment. Within Korean cultivars, seven cultivars

showed the consistent severity proportions of leaf symptoms. Keunolkong, Myeongjunamulkong and Jinpumkong 2 were included in the same group as SDS resistant check cultivars and Shinphaldalkong 2, Milyang87, and Samnamkong were in another group of Hartz 6686. Disease rankings of these cultivars in this experiment were same as those in the first experiment.

Three cultivars, Kwangankong, Milyang 83, and Bukwangkong, showed the inconsistent results. In first experiment, Kwangankong and Milyang 83 were ranked first and second in the highest SDS resistant group. In second experiment, disease severities of these two cultivars were higher than those of SDS resistant check cultivars. The disease severity of Milyang 83 dramatically increased and that was higher than Bukwangkong in second experiment. Otherwise, Bukwnagkong showed the higher level of SDS susceptibility and included in the same group of Hartz 6686 in first experiment, but, in second experiment, disease severity of this cultivar was significantly higher than that of Hartz 6686.

Soybean responses of US lines were similar to our former result. The five resistant cultivar were included in the same group of Hartwig and PI 520733 and the four SDS susceptible cultivars were included in the same group as Hartz 6686. Three US cultivars, Hartwig, Hartz 5454, and Forrest, three Korean cultivars, Keunolkong, Myeongjunamulkong, and Jinpumkong 2, and one introduced line, PI 520733, were included in the highest SDS resistant group. Shinphaldalkong 2 consistently showed the highest SDS susceptibility in both experiments.

Deltapine DP 3460 and Asgrow 6297 were included in the second experiment. Disease severities of these two cultivars were not significantly different from that of PI 520733, but significantly higher than that of Hartwig. Average disease severity due to SDS in this experiment was 45.39 %. Uninoculated control Hartz 6686 did not show SDS symptoms.

DISCUSSIONS

Cultivar resistance is probably the most important biotic factor affecting SDS beyond presence or absence of the pathogen. Cultivars range in response to SDS from displaying no symptoms in the field to complete defoliation. While the use of resistant cultivars is the primary means of controlling SDS, the dependency on field screening can make screening cultivars for resistance very difficult. This is due to the failure of the disease to occur because of weather conditions or, to the uneven distribution of the disease in the field. Some of the environmental variability can be reduced by proper irrigation, but adequate cultivar evaluation in non-irrigated fields usually requires tests at multiple site and multiple years.

Fortunately, cultivar reactions are fairly consistent across locations where severe levels of SDS occur. Stephens et al. (1993) tested twelve cultivars at three locations and found that the relative disease ratings were similar across the locations. There were some differences in ranking, but these differences were relatively small so that cultivars that were resistant at one location were resistant at all locations.

Although greenhouse screening of cultivars has also been frustrating, for many of the same reasons as screening in the field, the variability of cultivar reactions in the field suggested that the use of environmentally controlled screening tests might be more effective in identifying resistant or susceptible cultivars. Sometimes, high temperatures (>30C) and inconsistent watering can result in little SDS development. In addition, greenhouse screening has suffered from high frequencies of escapes. These escapes make separation of cultivar reactions and identification of segregation in breeding lines very difficult.

However, with sorghum seed inoculum infested with *Fusarium solani* f. sp. *glycines*, Hartman et al. (1997) developed an inoculation technique to reduce this variability and make routine greenhouse screening possible. The most important factor in this technique is that the seed not be direct contact with inoculum because excessive root rot prevents sufficient plant growth to allow development of typical SDS-like symptom. In greenhouse test, disease ratings are usually higher than in the field even with those culivars that have few if any SDS symptoms in the field, and, also, greenhouse evaluation of these cultivars with isolates obtained from each location gave similar rankings as those from the field (Stephens et al., 1993).

Our results presented here suggested that greenhouse screening is a valid alternative to field screening to evaluate the cultivar resistance. At least these results could serve as an adjunct to field screening or means of prescreening and selecting cultivars and lines for field screening or possibly selecting breeding materials. However, there were some discrepancies between cultivar reactions in the greenhouse and in those the field. Results of disease severity obtained from controlled environment are more indicative of genetic differences among cultivars, because unlike field conditions such as temperature, soil moisture, and presence of soybean cyst nematode (SCN), which interact with cultivar resistance, can be more carefully controlled.

The genetics of SDS resistance has been studied with three different sources of resistance. Using a greenhouse assay, Stephens et al. (1993) found a single dominate nuclear gene, designated *Rfs*, for resistance in crosses with the SDS-resistant cultivar, Ripley. Other studies have found resistance to SDS to be multigenic nature of resistance found in these studies.

Using field results and molecular analysis, four quantitative trait loci (QTL) have been identified in the cultivar 'Forrest' (Hnetkovsky et al. 1996). One of these QTL's is also associate with QTL for SCN resistance (Chang et al, 1993). Study for SDS resistant introduced line, PI 520733, have been conducted in the greenhouse and in molecular analysis. This introduced line originates from Korea. Normally the seedlings are more vigorous than other genotypes (Hartman et al., 1995), and resistance appears to be controlled by three genes (Clay Sneller; Department of Agronomy, University of Arkansas, personal communication). The wide range in cultivar responses to SDS agrees with the multigenic nature of resistance found in these studies.

Maturity group of soybean should be considered to identify the reactions of soybean to SDS pathogen. Maturing traits in each cultivar may be interacted with SDS development (Rupe et al., 1991). It is difficult to compare responses to SDS between Korean cultivars and US cultivars, because evaluation standards for maturing trait are different. The maturity group (MG) information of PI 520733 varied. According to the information from the result of Hartman et al. (1995), PI 520733 is included in the MG V, but the information from the SoyBase (database system in USDA and Iowa state university), is MG III. Most of all Korean cultivars might be included in one MG in United States, because latitude of Korea is similar to the cultivated regions for later MG III to earlier MG IV in US.

Most research on soybean resistance to SDS pathogen has been based on lack of leaf symptoms. We observed that there were significant differences in leaf symptoms due to SDS between resistant and susceptible cultivars, but not in root symptoms (data unpublished) in former research. Similar result was obtained by Gray (1996). F. solani caused root rot of soybean, and differences in foliar symptoms, but not root rot severity, were observed between the resistant cultivar Ripley and susceptible cultivar Spencer.

It might be possible to screen for SDS resistant by the utilizing the phytotoxin which is produced by F. solani (Jin et al., 1996). It appears that resistance in soybeans to the foliar disease phase of SDS has relationship with this toxin. The mechanism of this resistance is unknown, but could be related to reduced toxin accumulation, reduce translocation, detoxification or a combination of these factors.

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