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Physiological Responses of Soybean Cultivars to *Fusarium solani* f. sp. *glycines* Causing Sudden Death Syndrome

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ABSTRACT

Six soybean cultivars having different SDS susceptibility were planted with sorghum seed inoculum infested with *F. solani* isolate 171 in the greenhouse. First leaf symptoms appeared on unifoliar leaves at 9 days after inoculation and all cultivars showed the typical leaf symptoms at 13 days after inoculation, when trifoliar leaves emerged. Leaf symptoms development in susceptible cultivars was faster than in resistant cultivars. Leaf symptom severities during the period of 25 to 29 days after inoculation showed a significant difference between cultivars which had SDS resistance and susceptibility. In this period, area under the disease progress curve (AUDPC) of Hartz 6686 was the highest and that of PI 520733 was the lowest.

SDS caused serious damage to the growth of soybean in all cultivars. Average reductions of growth rate of root fresh weight and dry weight were greater than those of plant tops. Duyu-kong showed less severe leaf symptoms than that of SDS suscetible cultivars; however, average growth rate of plants top and roots of this cultivar was less but not significantly different than those of SDS susceptible cultivars. In all cultivars, as severity of leaf symptoms increased, plant top weight decreased.

Root rot symptoms were observed in all cultivars before leaf symptoms appeared. Average proportions of tap root reddish-brown discoloration of all cultivars was up to 75 % at 15 days after inoculation; however there was no significant difference between cultivars at each rating date.

Appearances of leaf symptoms on leaves varied in each cultivar. SDS resistant cultivars had a significantly higher level of crinkling than susceptible cultivars and SDS susceptible cultivars had a significantly higher level of necrosis than resistant cultivars. Further study will be needed to identify the relationships between the physiological growth rate and SDS severities in soybeans.

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Sudden death syndrome (SDS) is a soil born disease of soybean(*Glycine max* (L.) Merr.) caused by the *Fusarium solani* (Mart.) Appel & Wollenw. Emend. Synd. & Hans. SDS was first observed in Arkansas, USA in 1971 and has been reported in other states, Mississippi, Kansas, Missouri, Kentucky, Tennessee, Illinois, Indiana and Iowa in United States (Hartman et al., 1995; Jardine & Rupe, 1993; Yang & Rizvi, 1994). Beside the USA, SDS occurs in Argentina, Brazil, and Canada (Anderson & Tenuta, 1988; Ivancovich et al., 1996; Nakajima et al., 1994). In 1983, Hirrel (1983) first reported the occurance of this disease in Arkansas and named it as "Sudden Death Syndrome" in response to perceived rapidity with which above ground leaf symptom developed.

In general, crinkling, mottled appearance and necrosis of interveinal leaves are typical SDS symptoms. Initial leaf symptoms of SDS are circular to irregular shaped, scattered, 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). 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, vascular discoloration of roots and stems (Hirrel, 1983; Roy et al., 1997). In case of severely infected roots, reddish-brown discoloration of the taproot can develop to just above the soil line of stems and lateral roots can not develop (Yang & Rizvi, 1994). External symptoms of SDS are similar to those of Brown stem rot caused by *Phialophora gregata*. However, 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, under the proper environment conditions, affect seed germination (Rupe et al., 1993). Loss estimates due to SDS were collected from 1988, when this disease

were recognized as serious problems, across the southern states in US. Three year average yield loss from 1989 to 1991 due to SDS in north central region in US was ranked 9th out of 20 soybean diseases (Ben Doupnik, 1993).

The SDS pathogen appears to be a unique strain 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 that there are very few if any differences between isolates of *F. solani* f. .sp. *glycines* even when those isolates originate from different states. O'Donnell & Gray (1995) reported, based on molecular studies, that the SDS pathogen was a *F. solani* f. sp. *phaseoli*, but these results were not supported by later pathogenicity data. *F. solani* f.. sp. *glycines* and *F. solani* f. sp. *phaseoli* were used to inoculate to soybean plants in greenhouse and in field for comparing the responses of soybeans (Roy, 1997). *F. solani* f.. sp. *glycines* caused typical SDS leaf symptoms; but *F. phaseoli* did not. However, the SDS pathogen may be related to *F. solani* f. sp. *phaseoli* and the two pathogens do share many cultural characteristics.

Host specifying has been tested with several isolates of *F. solani* on beans. Mung beans and garden beans were susceptible and all plants including soybeans died following inoculation (Gray, 1991). Green bean, limabean and cowpea were not affected by *F. solani* but became infected only after wounded inoculation (Melgar & Roy, 1994). Thus *F. solani* isolates associated with SDS are not host specific and readily infect other hosts.

Rupe et al. (1995, 1996) 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). And, also, 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 toxin has been reported in culture filtrate. This toxin is a 17 kd polypeptide which produces SDS-like foliar symptoms on susceptible cultivars (Jin et al., 1993). Cultivar reactions to the toxin are similar to their reactions to SDS in greenhouse inoculation tests.

Although SDS has not been reported in Korea, the rapid spread of the disease in the USA and later in Argentina and Brazil and the 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 Korean soybean production. The objectives of this study were to compare the reactions of Korean soybean cultivars to *F. solani* f.sp. *glycines* in greenhouse inoculations and to compare selected Korean cultivars with soybeans from the USA with known reactions to the pathogen.

MATERIALS AND METHODS

Preparation of sorghum seeds inoculum with *F. solani* isolate 171

Inoculum of *F. solanoi*, SDS pathogen isolate 171 (isolated from the root of SDS symptomatic plant in the field at Marianna, Arkansas, USA : John Rupe, Dept. of Plant Pathology, Univ. of Arkansas) was prepared by growing the isolate on PDA for 5 days at room temperature under flourescent lights (10 hr/day). Three, 1 cm dia plugs were cut from the growing edge of the culture and aseptically transferred to sterilized sorghum seed. In a 500 ml Erlenmyer flask with 150 ml of deionized water, 125 g of sorghum seed was placed. 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 the greenhouse of University of Arkansas in Fayetteville, Arkansas, USA. A total of six cultivars, two resistant cultivars to SDS which are PI 520733 and Hartwig, two susceptible cultivars which are Hartz 6686 and Dillon, and two Korean lines, Eunha-kong and Duyu-kong were used to identify the responses of soybean to *F. solani*. In our preliminary research, Korean cultivars, Eunha-kong and Duyu-kong had the less severe leaf symptom than that of Hartz 6686 and Dillon. Within Korean cultivars, Duyu-kong showed the less severe leaf symptom than that of Eunha-kong, but they didn't show significant differences (Cho, J. H and Rupe, J. C., *unpublished*). Pathogenicity and virulence of *F. solani* isolate 171 were determined in the greenhouse test (Rupe, J. C., *personal communication*).

Plastic flats (30 × 60 × 6 cm) were filled with a steam pasteurized soil mix consisting of 10 parts soil, 4 parts potting mix, 2 parts vermiculite, and 1 part 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 additional 2 cm of soil mix and placed in the greenhouse at 25℃. Highest temperature in the daytime and lowest temperature in the nighttime were 23.5℃ and 27℃, respectively. Every two days after the first leaf symptom appeared in all cultivars, each plant in each row was rated for percent of leaf area with symptoms of SDS using a 0 to 11 scale with 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% (The Horsefall-Barret Scale for assessing disease ; Redman et al., 1969). Tests were conducted in a randomized block design with three replications.

To compare responses of soybean cultivars to *F. solani* isolate 171 uninoculated plants of each cultivar were included as controls with each test of run. For preparing the control, ten seeds of each cultivar were planted in the same way as described above but without inoculum. In addition, controls had three times of run and each run had three replications for each cultivar.

Areas under the disease progress curve (AUDPCs) were compared during the period at which there was significant difference of leaf symptom scales and proportions between resistant cultivars and susceptible cultivars. AUDPC was calculated with average proportions of leaf symptoms in each replication of each run.

Physiological growth rate of infected soybean plants

To determine the physiological growth rate of each cultivar inoculated with *F. solani* isolate 171 to uninoculated control, plant top fresh weight, root fresh weight, plant top dry weight, root dry weight, and plant height were measured when last leaf symptoms were evaluated. For measuring dry weight, plant tops and roots were dried at 55℃ in a dry incubator for 7 days. Relationship between plant top fresh weight and symptom severity proportions were compared with averages of each test run and replication to identify responses of growth rate of each cultivar as disease developed.

Comparisons of leaf responses of soybean plants to *F. solani*

To compare responses of soybean between the resistant cultivar and the susceptible cultivar, the number of plants that showed leaf symptoms of crinkling and necrosis were recorded separately. Plants which had more than 50 % of necrosis area on leaves were included in necrosis plant and plants which had more than 50 % of crinkling were included in crinkling plant. However, asymptomatic plants were not counted.

Rating method for root rot disease development

In addition to rating foliar symptoms, root rot symptoms were rated to determine the relationship between the development of root symptoms and leaf symptoms. For this test, with the sorghum seeds inoculum infested with *F. solani* isolate 171 as described above, each cultivar was tested twice in the greenhouse at the same period as former experiment. Each test was conducted in a randomized block design with three replications.

In order to identify the development of root rot symptom within cultivars caused by *F. solani* isolate 171 as time progressed, the plants were carefully extracted from the soil, the roots were washed in running tap water for 30 minutes, and root rot severity was assessed on each plant using a 0 to 5 scale (Fusarium root rot scale, Hwang et al. 1994) with 0 = 0%, 1 = 0~24%, 2 = 25~49%, 3 = 50~74%, 4 = 75~99%, 5 = 100% of the taproot discolored. The root rot scaled 0 means that whole taproot is healthy: the color of it does not turn reddish brown and is same as that of control. The scale 5 means that discoloration of taproot extends up to the soil line. Root rot of each plant was rated every three days from 6 days after planting with inoculum and potographed every 6 days for comparing the root rot developments of resistant cultivar and susceptible cultivar. Each rating was converted to the mid point range of percentage describe above and percentage of all plants in a replication were averaged before analysis.

Statistical analysis

Average of severity proportions of leaf symptoms and root rot symptoms at each rating time were noted by converting to midpoint percentage in each scale before averaged. The resulting average scales and proportions of leaf symptoms at each rating date, AUDPCs, physiological growth rates, incident rates of two different leaf symptoms on leaves and root rot severties of infected soybean plants 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 AND DISCUSSIONS

All cultivars inoculated with *F. solani* isolate 171 showed the typical leaf symptoms of SDS in the greenhouse test. First leaf symptoms appeared in PI

520733, Hartz 6686, Dillon and Eunha-kong, 9 days after planting with inoculum at unifoliar stage, but not all of the cultivars showed symptoms until thirteen days after inoculation when trifioliar leaves emerged. When trifoliar leaves emerged, all cultivars, but not all plants, showed the pale green chlorotic spots and crinkling on unifoliar leaves and symptom severity of each cultivar increased as time progressed (Table 1). These chlorotic spots extended to trifoliar leaves and some plants showed the leaf symptom on trifoliar leaves rather than unifoliar leaves.

However, increasing rates of symptom severity of each cultivar varied at each date of symptom evaluation. Disease intensities within cultivars at each date were not signigicantly different until 23 days after planting based on both data of scales and proportions of symptom.

Most of all, development of the disease in Hartz 6686 was dramatically increased. Leaf symptom severities during the period of 25 to 29 days after inoculation showed significant difference between cultivars which had SDS resistance and susceptibility. In this period, AUDPC of Hartz 6686 was at highest and that of PI 520733 was at lowest (Table 2).

In result of LSD analysis, AUDPCs of resistant cultivars, PI 520733 and Hartwig, were included in the same group and were distinctly less than those of susceptible cultivars, Hartz 6686 and Dillon. Hartz 6686, Dillon, and Eunha-kong were included in another group of AUDPC. Duyu-kong had less level of AUDPC than that of susceptible cultivars and was significantly different from Hartz 6686. LSD of scales and proportions of leaf symptoms between Hartwig and Dillon were not significant at 31 days after inoculation.

As a result, after planting with sorghum seeds inoculum infested with *F. solani* isolate 171, 25 days

Table 1. Comparisons of disease rating scales and proportions of leaf symptoms of each cultivar as time progressed after inoculation in greenhouse tests.

Cultivars	Days after planting seeds with inoculum†										
	13	15	17	19	21	23	25	27	29	31	33
	Means of disease rating scale of SDS leaf symptoms (0~11)+										
P1520733(R)	0.40^{c}	0.49^{c}	0.96^{c}	1.18^{c}	1.48^{c}	1.84^{c}	2.32^{d}	2.93^{d}	3.50^{d}	3.81^{c}	3.96^{d}
Hartwig(R)	0.29^{c}	0.42^{c}	1.15^{c}	1.85^{bc}	2.36^{bc}	2.71^{bc}	3.30^{cd}	3.76^{cd}	4.25^{cd}	4.97^{b}	5.26^{c}
Hartz 6686(S)	0.72^{b}	0.98^{b}	1.89^{b}	2.96^{a}	4.20^{a}	4.75^{a}	5.26^{a}	5.89^{a}	6.45^{a}	6.64^{a}	6.79^{a}
Dillon(S)	0.38^{c}	0.43^{c}	1.07^{c}	1.85^{bc}	2.44^{bc}	3.31^{b}	4.54^{ab}	5.22^{ab}	5.81^{ab}	5.90^{ab}	6.30^{ab}
Eunha-kong(Kor)	1.59^{a}	1.90^{a}	2.80^{a}	3.50^{a}	4.26^{a}	4.52^{a}	4.99^{a}	5.31^{ab}	5.45^{b}	5.45^{b}	5.87^{bc}
Duyu-kong(Kor)	0.42^{bc}	0.75^{bc}	1.92^{b}	2.70^{ab}	2.91^{b}	3.10^{b}	3.64^{bc}	4.25^{bc}	4.82^{bc}	5.81^{ab}	6.24^{ab}
Avr.§	0.63	0.83	1.63	2.34	2.94	3.37	4.01	4.56	5.05	5.43	5.74
LSD $_{0.05}$¶	0.30	0.38	0.65	0.98	1.23	1.03	1.12	1.12	1.00	1.07	0.91
	Means of severity proportions of SDS leaf symptoms(%)‡										
P1520733(R)	0.77^{c}	0.95^{b}	2.17^{c}	3.05^{d}	4.52^{c}	7.05^{c}	13.13^{c}	19.28^{c}	23.23^{d}	26.88^{c}	29.49^{d}
Hartwig(R)	0.66^{c}	0.89^{b}	3.44^{c}	6.29^{cd}	10.96^{c}	13.05^{c}	18.46^{c}	24.06^{c}	29.19^{d}	37.22^{b}	42.19^{c}
Hartz 6686(S)	2.10^{b}	3.39^{b}	9.03^{b}	17.34^{ab}	32.50^{a}	39.73^{a}	47.68^{a}	56.39^{a}	63.47^{a}	64.70^{a}	66.16^{a}
Dillon(S)	1.43^{bc}	1.60^{b}	3.90^{bc}	9.95^{bcd}	13.81^{c}	21.01^{bc}	36.91^{ab}	44.48^{ab}	52.87^{ab}	53.17^{ab}	58.01^{ab}
Eunha-kong(Kor)	6.04^{a}	9.32^{a}	16.38^{a}	22.21^{a}	31.24^{ab}	34.04^{ab}	39.07^{ab}	44.56^{ab}	46.45^{bc}	46.81^{b}	51.89^{bc}
Duyu-kong(Kor)	0.82^{bc}	1.74^{b}	8.96^{b}	14.11^{ab}	15.71^{bc}	18.6^{bc}	24.77^{bc}	30.86^{bc}	37.37^{cd}	50.18^{ab}	54.49^{b}
Avr.§	1.98	2.98	7.32	12.16	18.12	22.25	30.00	36.61	42.10	46.49	50.37
LSD $_{0.05}$¶	1.12	2.76	5.45	9.19	15.58	16.11	16.77	16.11	14.21	11.70	10.87

†Disease severity presented was evaluated according to a 0~11 rating scale of foliar symptoms, where scale 0=0%, scale1=0~3%, scale 2=4~6%, scale 3=7~12%, scale 4=13~25%, scale 5=26~50%, scale 6=51~75%, scale 7=76~88%, scale 8=89~94%, scale 9=95~97%, scale 10=98~99%, and scale 11=100% of the leaf area with symptoms (The Horsefall-Barret Scale).

‡Average of severity proportions at each time were noted by converting to midpoint percentage in each scale before averaged.

§Average scales and proportions of leaf symptomes at each rating date.

¶All means are derived from three seperated 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($P \leq 0.05$) according to the LSD test. a is the highest level of leaf symptom and d is the lowest.

(R) = resistant to SDS

(S) = susceptible to SDS

(Kor) = Korean soybean lines

Table 2. Comparisons of cultivar responses with AUDPCs in rating days which had significantly different disease severities within cultivars.

Cultivar	Disease severity (AUDPC)† from 25 to 29 days after planting with inoculum
PI 520733 (R)	74.91 [d]
Hartwig (R)	95.77 [d]
Hartz 6686 (S)	223.93 [a]
Dillon (S)	178.75 [ab]
Eunha-kong (Kor)	174.63 [ab]
Duyu-kong (Kor)	123.87 [bc]
LSD $_{0.05}$‡	62.19

†Areas under the disease progress curve (AUDPC) were calculated with proportions of leaf symptoms converted from midpoint of a 0~11 rating scale of foliar symptoms, where scale 0=0%, scale1=0~3%, scale 2=4~6%, scale 3=7~12%, scale 4= 13~25%, scale 5=26~50%, scale 6=51~75%, scale 7=76~88%, scale 8=89~94%, scale 9=95~97%, scale 10=98~99%, and scale 11=100% of the leaf area with symptoms.

‡Least significant differences calculated at the P=0.05 level for AUDPCs within cultivars. AUDPCs had significantly different disease severities between cultivars. AUDPCs followed by same letter at each rating date are not significantly different(P≤0.05) according to the LSD test.

[a] is the most severe leaf symptom and [d] is the least.

(R) = resistant to SDS.

(S) = susceptible to SDS.

(Kor) = Korean soybean lines.

to 29 days (3~4 week) were available for rating the leaf symptoms for comparing the susceptibilities, and our results agreed with the results of Stephenes et al. (1993). He reported that light leaf chlorosis could be recognized on greenhouse-inoculated seedlings 10 days after inoculation and that differences of symptom severities between susceptible cultivar Spencer and resistant cultivar Ripley was maximum at about 3 weeks after inoculation. He used the oat-inoculation technique described by Lim & Jin (1991) for producing SDS symptoms on soybean seedling in the greenhouse test.

With plant top fresh weight, root fresh weight, plant top dry weigth, root fresh weight, and plant top height, physiological growth rate of symptomatic plants compared to uninoculated plants of each cultivar were estimated (Table 3). Average reductions of growth rate of fresh weight and dry weight of root were greater than those of plant top in symptomatic plants. Average fresh weight and dry weight of plant top and those of root estimated in all cultivars were 52.73 %, 60.29 %, 33.50 % and 46.16 %, respectively.

In general, growth rates of SDS resistant cultivars, Hartwig and PI 520733, were comparably, but not always significantly, higher than those of SDS susceptible cultivar H6686 and Korean lines, Eunhakong and Duyu-kong. However, in fresh weight and dry weight of plant top and root of SDS susceptible cultivar Dillon were higher than those of other cultivars. In case of plant top height, growth rate of Hartwig was highest. Korean line Duyu-kong showed the greatest reduction rate of growth in all of these five categories estimated.

When soybeans were inoculated with *F. solani* and

Table 3. Proportions of growth of soybean plant inoculated with *F. solani* isolate 171 compared to uninoculated control of each cultivar 33 days after planting in greenhouse.

	Growth rate of inoculated plant compared to control (%)†				
	Plant top			Root	
	Wet weight	Dry weight	Height	Wet weight	Dry weight
PI 520733 (R)	55.80[ab]	64.19[abc]	46.62[b]	39.84[ab]	54.55[ab]
Hartwig (R)	72.36[a]	76.03[ab]	67.61[a]	36.83[ab]	49.09[abc]
H6686 (S)	38.16[b]	42.40[c]	46.67[b]	27.56[ab]	37.51[bc]
Dillon (S)	61.75[ab]	83.99[a]	47.93[b]	40.87[a]	60.85[a]
Eunha-kong (Kor)	49.85[ab]	52.84[bc]	47.01[b]	30.20[ab]	40.26[bc]
Duyu-kong (Kor)	38.47[b]	42.30[c]	40.10[b]	25.70[b]	34.59[a]
Avr.	52.73	60.29	49.32	33.50	46.14
LSD 0.05‡	24.30	30.30	10.43	14.23	17.88

†Growth rate means that the average percentages of the weight of the plant tops and roots and height of plant tops of inoculated plants to control.

‡All means are derived from three 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. a is the highest level of growth rate and d is the lowest.

(R) = resistant to SDS.

(S) = susceptible to SDS.

(Kor) = Korean soybean lines.

grown under the controlled environment in the greenhouse, plant height of soybeans was hardly affected by SDS disease. According to the results suggested by Melgar & Roy (1994), Roy et al. (1989), and Rupe (1989), plant height was not affected by SDS disease in the field; however, plant height reductions occurred on artificially inoculated plants in the greenhouse.

In these results present here, SDS caused serious damage to soybean plants in all cultivars. The results of SDS leaf symptoms do not always lead to the similar results of growth rate in each cultivar. In some cases, even lower levels of leaf symptom appeared in one cultivar, this cultivar may be damaged seriously by this disease in growth. The Korean line Duyu-kong had a lower level of leaf symptoms than that of SDS susceptible cultivars Hartz 6686, Dillon and Eunha-kong (Table 1, and 2). However, in this greenhouse test, physiological growth rate of Duyu-kong had similarly or comparably less levels than those of SDS susceptible cultivars in all of the five categories (Table 2). Comparison of SDS symptomatic plants with the uninoculated control of each cultivar is shown as Fig. 1.

SDS development affected the plant top weight in all cultivars. Relationship between plant fresh weight and severity proportions of SDS leaf symptoms in each cultivar is shown as Fig. 2. As severity proportions of leaf symptoms increased, plant top weight decreased.

To identify the root disease development, root rot of each cultivar was rated every 3 days from 6 days after planting with inoculum. First leaf symptoms were observed at about 9 days after inoculation in this greenhouse test, and all of the cultivars showed the SDS leaf symptoms at 13 days after inoculation. However, discoloration of taproot was observed in all cultivars before leaf symptoms appeared. Average proportion of taproot discoloration of all cultivars was up to 75 % at 15 days after inoculation (Fig. 3.). There was no significant difference between cultivars at each rating date. Colors of taproot of infected plants were reddish brown to dark brown in contrast to white of uninoculated roots. Color of the tip of the taproot turned brown by infection of *F. solani*, 6 days after inoculation. Gradually, this symptoms extended up to the soil line and the whole taproot had symptoms but not above the soil line (Fig. 4). Also, lateral roots of infected plants were not developed as well as the lateral roots of control.

Incidence rate of two typical leaf symptoms, crinkling, and interveinal necrosis, were compared in each cultivar. All of six cultivars tested in the greenhouse showed both symptoms; however, the incident rate of these symptoms varied between cultivars (Table 4, Fig. 5). In each symptom, SDS resistant cultivars had a significantly higher level of

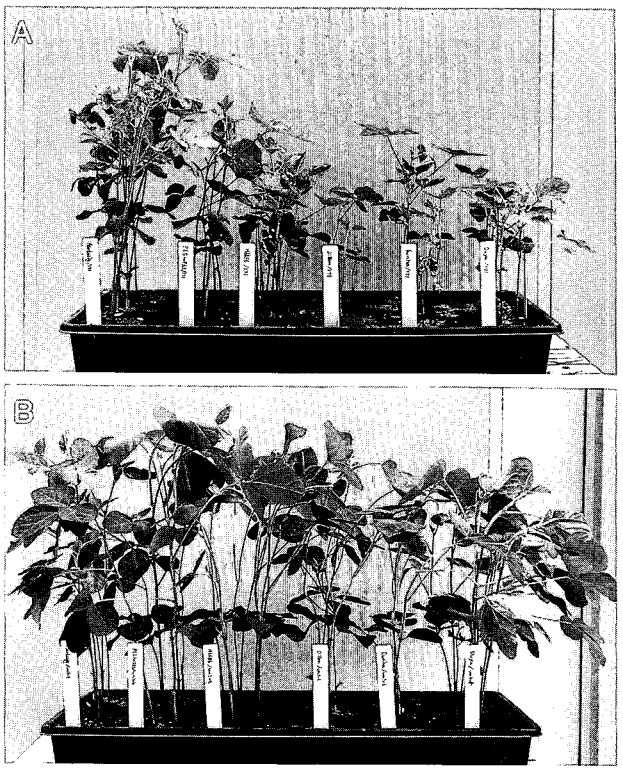

Fig. 1. Comparisons of plants inoculated with *F. solani* isolate 171 to plants uninoculated in each cultivar. Plants were grown for 29 days with sorghum seed inoculum in the greenhouse at 25℃. Hartwig, PI 520733, Hatrtz 6686, Dillon, Eunha-kong, and Duyu-kong from the left, respectively.
A : Plants inoculated with *F. solani* isolate 171. B : Uninoculated controls.

crinkling than SDS susceptible cultivars and SDS susceptible cultivars had a significantly higher level of interveinal necrosis than SDS resistant cultivars.

SDS resistant cultivar Hartwig had the highest rate of crinkling (64.1 %) but had lowest level of interveinal necrosis (35.9 %) while SDS susceptible cultivar Hartz 6686 and Dillon had lowest rate of crinkling (13.1 % and 11.8 %, respectively) but highest rate of interveinal necrosis (86.9 % and 78.6 %, respectively). In average incident rate of each symptom in all cultivar, necrosis symptom was higher than crinkling.

Soybean root symptoms to SDS were similar and did not have significant difference within cultivars (Fig. 3); however, soybean leaf symptoms were varied. In these results, Crinkling may be a self-defense reaction of soybean. Even crinkling were occurred on soybean leaves, color of leaves did not turn yellow and petiole drop did not occurred, although petiole drop occurred followed by necrosis in

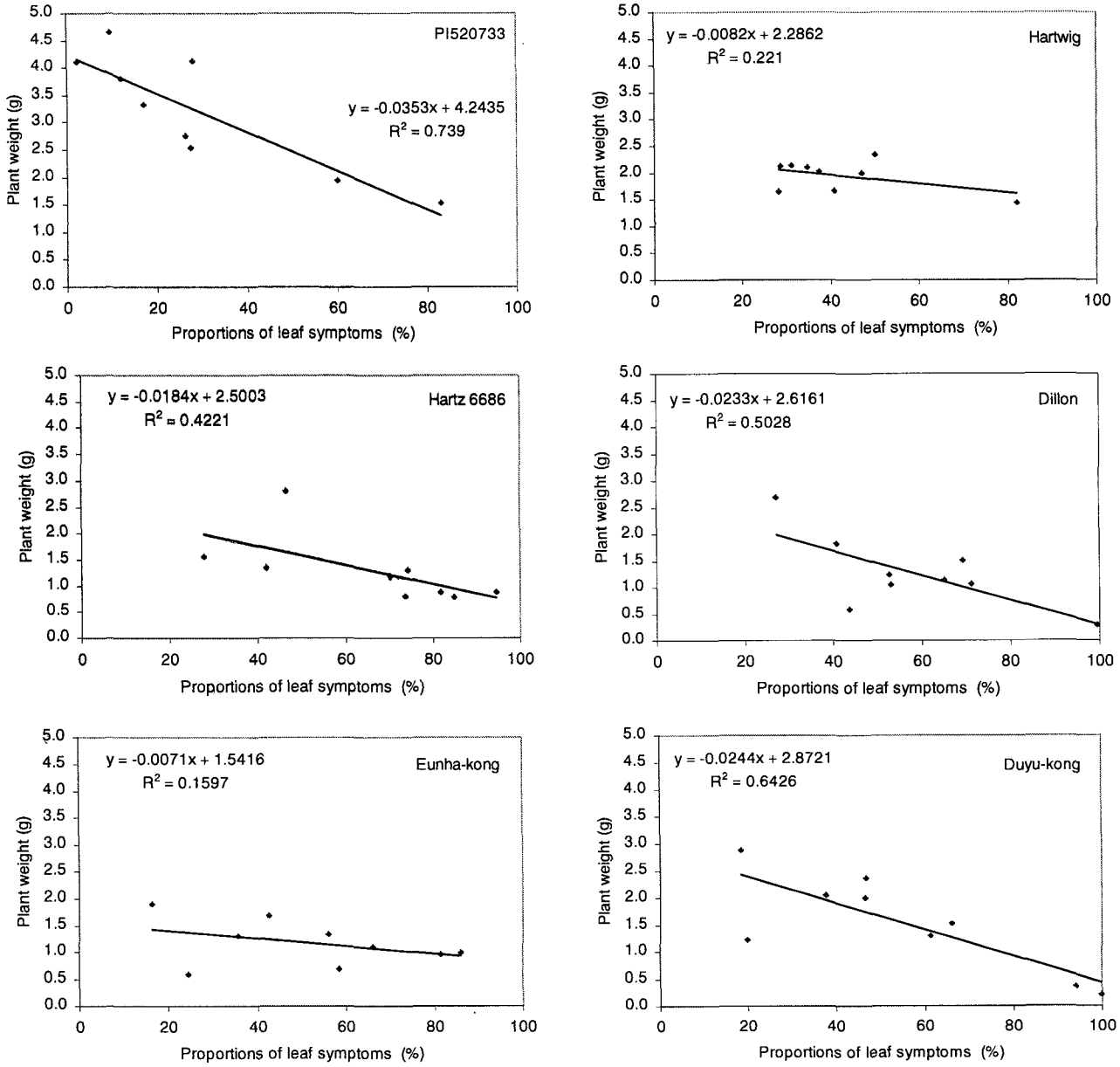

Fig. 2. Comparisons of relationship between plant top fresh weight and proportions of SDS leaf symptoms of each cultivars. These were graphed with average of proportions of leaf symptoms and average of fresh weight of plant top in each replication of each run.

severely infected plants. Sometimes, crinkling occurred on unifoliar leaves at early stage of infection (7 to 10 days after inoculation) and in less severe infected plants in susceptible cultivars. In these cases, leaf symptoms were recovered and disappeared as soybean plants grown up. It seems that symptomatic leaves with crinkling did not lose their physiological functions, such as photosynthesis, and, also, necrosis development was delayed.

From the results presented in this study, we have shown that there are significant differences in leaf symptoms due to SDS in each cultivar, but not in root symptoms. Results of leaf symptom severity did not correspond well with the growth responses in each cultivar. A cultivar which showed less severe leaf symptom could be damaged seriously in growth by SDS. It appears that these differences are due to the traits of each cultivar. Thus, we need a more detailed standard to determine SDS susceptibility. Some possible parameters to examine would be the

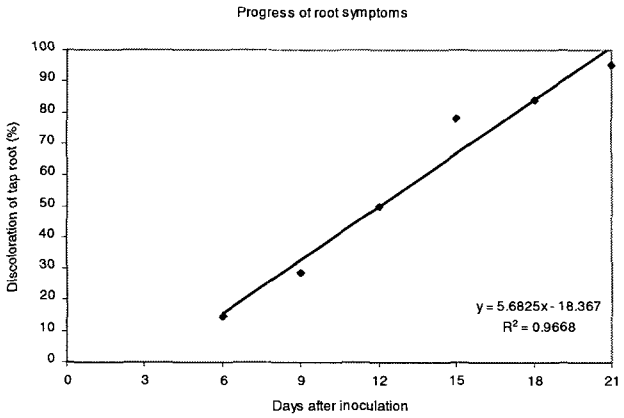

Fig. 3. Observation of root symptom development as time progressed after inoculation.
Disease severity of root presented was evaluated according to a 0~5 rating scale (Fusarium root rot scale, Hwang et al. 1994) of root symptoms, where scale 0=0%, scale1=0~24%, scale 2=24~49%, scale 3=50~74%, scale 4=75~99%, scale 5=100% of the taproot area with symptoms. Averages of severity proportions at each time were noted by converting to midpoint percentage in each scale before averaged. Root disease was rated every three days from 6 days after inoculation.
This was graphed with the average proportion of root discoloration in all cultivars at different rating date, since, there was no significant differences between cultivars in root disease severities at $P \leq 0.05$.

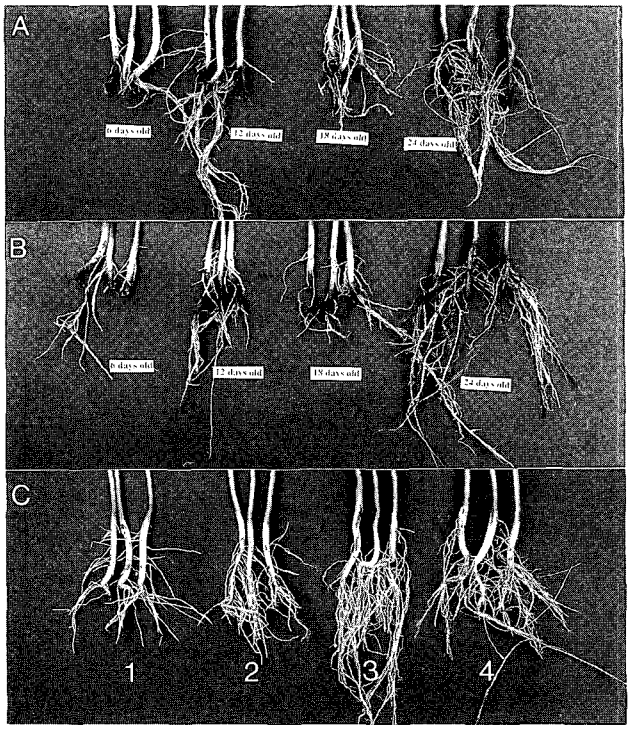

Fig. 4. Comparisons of developments of root symptom of Hartwig (SDS resistant) and H6686 (SDS susceptible) caused by *F. solani* isolate 171 to uninoculated control. These plants were grown in the greenhouse at 25℃. Samples were taken every 6 days after inoculation. Controls were prepared with H6686 (SDS susceptible cultivar) without inoculum.
From the left, 1 = 6 days, 2 = 12 days, 3= 18 days and 4 = 24 days after inoculation, respectively.
A : Hartwig (SDS resistant) inoculated with *F. solani* isolate171.
B : Hartz 6686 (SDS susceptible) inoculated with *F. solani* isolate171.
C : Hartz 6686 (SDS susceptible) uninoculated control.

estimations of physiological growth rate such as plant top weight, root weight, and plant height. Plant weight, especially, would have a negative relationship with leaf symptom severities (Fig. 2).

Another possibility would be suggested by the utilization of phytotoxin which is produced by *F. solani*, SDS pathogen. Jin et al.(1996) already identified the characteristics of this phytotoxin which is 17 kd polypeptide and reported that this polypeptide phytotoxin caused the same leaf symptoms as typical SDS symptoms in the greenhouse and the field. We have examined soybean responses to a phytotoxin filterate produced by *F. solani* and observed the typical SDS leaf symptoms. However effects of medium and buffer, which are for growing *F. solani*, have also been observed. Sometimes, leaf symptoms effected by medium would be confused with SDS leaf symptoms.

In addition, with the inoculation method described here, soybean responses in Korean lines have been studied and compared with US cultivars. There has been no report for SDS in South Korea, however, because, SDS is being spread widely in the world, establishing the screening methods for SDS susceptibility of soybeans would be important.

REFERENCES

Anderson, T. R. and A. U. Tenuta. 1988. First report of *Fusarium solani* f. sp. *glycines* causing sudden death syndrome of soybean in Canada. Plant Dis. 82: 448.

Ben Doupnik, Jr. 1993. Soybean Production and disease loss estimates for North Central United States from 1989 to 1991. Plant Dis. 77: 1770-1771.

Gray, L. E. 1991. Alternate hosts of soybean SDS strains of *Fusarium solani*. (Abstr.). Phytopathology 81: 1135.

Table 4. Comparisons of development proportions of two typical leaf symptoms, crinkling and interveinal necrosis, in each cultivar.

	Incidence rate of each symptoms on leaves†	
	Crinkling (%)	Interveinal necrosis (%)
PI 520733 (R)	37.9[b]	53.9[cd]
Hartwig (R)	64.1[a]	35.9[cd]
Hartz 6686 (S)	13.1[c]	86.9[a]
Dillon (S)	11.8[c]	78.6[ab]
Eunha-kong(Kor)	25.9[bc]	64.9[bc]
Duyu-kong(Kor)	32.8[b]	63.5[bc]
Avr.‡	31.0	63.9
LSD 0.05§	16.3	20.8

†The number of plants that showed more than 50% each leaf symptoms of crinkling or necrosis were recorded separately. For example, the plant which had more than 50% of necrosis was included in necrosis group. Asymptomatic plants were not counted.

‡Average incidence rate of each symptom.

§All means are derived from three separated test, which has three replications, with each test considered a replication in the ANOVA. Means followed by same letter in each category are not significantly different ($P \leq 0.05$) according to the LSD test. [a] is the highest level of each symptom and [d] is the least.

(R) = resistant to SDS.

(S) = susceptible to SDS.

(Kor) = Korean soybean lines.

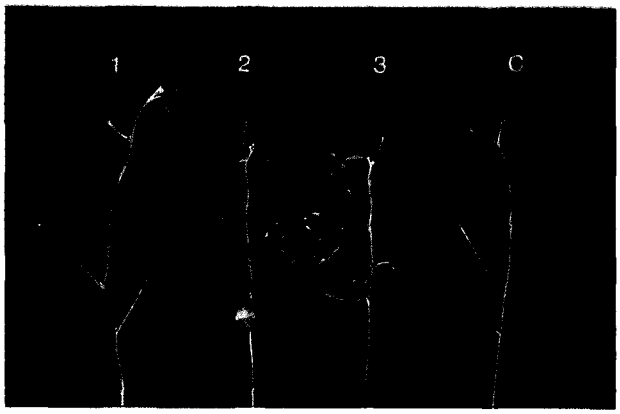

Fig. 5. Comparisons of soybean leaf responses to *F. solani* on different cultivars.

These plants were grown for 33 days after planting with inoculum in the greenhouse at 25℃. 1=Hartwig, SDS Resistant cultivar, showed the crinkling on leaves. 2=Eunha-kong, Korean cultivar, showed the both symptoms of crinkling and necrosis on leaves. 3=Hartz 6686, SDS susceptible cultivar, showed the interveinal necrosis on leaves. C=Hartz 6686, uninoculated control, did not show mottling and necrosis on leaves.

Hartman, G. L., G. R. Noel, and L. E. Gray. 1995. Occurrence of soybean sudden death syndrome in East-Central Illinois and associated yield losses. Plant Dis. 79: 314-318.

Hirrel, M. C. 1983. Sudden death syndrome of soybean - A disease of unknown etiology. (Abstr.). Phytopathology 73: 501.

Ivancovich, A., G. Botta, and J. Annone. 1996. Sudden death syndrome in the Northern region of the province of Buenos Aires, Argentina. (Abstr.) Phytopathology 86: 12.

Jardine, D. J. and J. C. Rupe. 1993. First report of sudden death syndrome of soybeans caused by *F. solani* in Kansas. Plant Dis. 077: 1264.

Jin, H., G. L. Hartman, C. D. Nickell, and J. M. Widholm. 1996. Characterization and purification of phytotoxin produced by *Fusarium solani*, the causal agent of soybean sudden death syndrome. Phytopathology 86: 227-282.

Lim, S. M., and H. Jin. 1991. Pathogenic variability in *Fusarium solani isolated* from soybeans with sudden death syndrome symptoms. (Abstr.) Phytopathology 81: 1236.

Melgar, J., and K. W. Roy. 1994. Soybean sudden death syndrome : Cultivar reactions to inoculation in a controlled environment and host range and virulence of causal agent. Plant Dis. 78: 265-268.

Nakajima, T., T. Mitsueda, and M. J. D. Charchar, 1996. First occurrence of sudden death syndrome of soybean in Brazil. JARQ 30: 31-34.

O'Donnell, K. and L. E. Gray. 1995. Phylogenetic relationships of the soybean sudden death syndrome pathogen *Fusarium solani* f. sp. *phaseoli* inferred from rDNA sequence data and PCR primers for its identification. MPMI 8: 709-716.

Roy, K. W. 1997. *Fusarium solani* on Soybean roots : Nomenclature of the causal agent of Sudden Death Syndrome and identity and relevance of *F. solani* form B. Plant Dis. 81: 259-266.

Roy, K. W., D. E. Hershman, J. C. Rupe, and T. S. Abney. 1997. Sudden death syndrome of soybean. Plant Dis. 81: 1100-1111.

Roy, K. W., G. W. Lawrence, H. H. Hodges, K. S. Mclean, and J. F. Killebrew. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incident and relation of *Heterodera glycines* to disease severity. Phytopathology 79: 191-197.

Rupe, J. C. and Gbur, E. E., Jr. 1995. Effect of plant age, maturity group, and environment on disease progress of sudden death syndrome of soybean. Plant Dis. 79: 139-143.

Rupe, J. C., E. E. Gbur, and D. M. Marx. 1991. Cultivar responses to sudden death syndrome of soybean. Plant Dis. 75: 47-50.

Rupe, J. C., W. E. Sabbe, R. T. Robbins, and E. E. Gbur, Jr. 1993. Soil and plant factors associated with sudden death syndrome of soybean. J. Prod. Agric. 6: 218-221.

Stephens, P. A., C. D. Nickell, C. K. Moots, and S. M. Lim. 1993. Relationship between field and greenhouse reactions of soybean to *Fusarium solani*. Plant Dis. 77: 163-166.

Yang, X. B. and S. S. A. Rizvi. 1994. First report of sudden death syndrome of soybean in Iowa. (Abstr.). Plant Dis. 78: 830.