韓作誌(Korean J. Crop Sci.) 32(2): 173~180

An Evaluation Method for Sesame (*Sesamum indicum* L.) Resistance to *Phytophthora* nicotianae var. *parasitica*

Seong Ho Choi*, Eui Kyoo Cho* and Young Am Chae**

참깨疫病 品種抵抗性 檢定方法 硏究

崔聖浩*·趙義奎*·蔡永岩**

ABSTRACT

Symptom development and disease severity of Phytophthora blight in the sesame plants varied depending upon age of the plants tested, inoculation method, watering method, and inoculum density in both susceptible Suweon 9 and Suweon 26 and moderately resistant B-67 and IS 103 sesame lines to *Phytophthora nicotianae* var. *parasitica* when inoculated. However, successful differentiation of the sesame lines for varietal resistance was possible using 20-day old seedling, inoculation by soil infestation, saturated soil water condition by half immersion of pots in water tank, and 200 sporangia per one ml of inoculum. Spraying or soil inoculation to 70-day old plants also was effective in differentiating the varietal resistance. By the screening method Suwon 26 showed 100% diseased plants and symptom severity index 9.0, while B-67 showed 20% diseased plants and symptom severity index 1.7. The rating scale given was from 0 through 9. For example, the scale 0 signified no symptom development, 5 signified discoloration of basal part of stem, and 9 signified discoloration of stem more than 10 cm high above the soil surface with blighted leaves. Differentiation in symptom severity also was made by percentage of the lesion area. Results evaluated using both parameters were well corresponded in varietal reaction of sesame to Phytophthora blight.

INTRODUCTION

Phytophthora blight of sesame caused by *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterhouse was found to be a major limiting factor in sesame production in Korea[1]. Especially the disease occurred severely during the rainy season July and August, generally mid July after rain, in the sesame field grown continuously. Although improved cultivation practices of high ridge for better drainage and vinyl mulching[2] and regular spraying schedule with the fungicide Ridomil were suggested for control of the disease, the blight appears to be the major disease with the other sesame diseases of Fusarium wilt, Bacterial wilt, Corynespora leaf blight, and Bacterial leaf spot in the sesame fields.

Unfortunately, Korean leading sesame cultivars

* Dept. of Pathology, Agricultural Sciences Institute, R. D. A., Suwon 170, Korea (農業技術硏究所 病理科)

** Dept. of Agronomy, College of Agriculture, Seoul Nat'l Univ., Suwon 170, Korea (서울大學校 農科大學 農學科)

〈1987. 4. 6 接受〉

were susceptible to *P. nicotianae* var. *parasitica* (un-published data). However, some sesame lines such as B-67 and IS 103 presented relatively lower percentage of diseased plants when tested at the seedling stage with some variations in each trial. Gemawat and Prasad[4], and Sehgal and Prasad[9] who tested hundreds of sesame collections for resistance to the disease at both seedling and adult stages also reported inconsistent results depending upon growth stage of sesame and probably inoculation conditions.

Such experimental conditions as plant age[6], inoculum density[5,6,8] and soil water content[6] might lead to variations in reaction of sesame lines to *P. nicotianae* var. *parasitica*. However, no intensive study on the screening method for varietal selection for resistance or tolerance to *P. nicotianae* var. *parasitica* has been reported so far in sesame. Thus this study aimed at developing a reliable screening method prerequisite for evaluation of sesame lines for resistance or tolerance to *P. nicotianae* var. *parasitica*.

MATERIALS AND METHODS

This experiments were conducted in the greenhouse of Department of Pathology, Agricultural Sciences Institute (ASI), Suwon, Korea during summer season from late May to late August in 1983. B-67 and IS 103 were used as resistant lines selected in preliminary tests to Phytophthora blight. Suwon 9 and Suwon 26 were used as susceptible lines for comparison in the screening tests. Seeds were obtained from Dr. Lee, Industrial Crop Division, Crop Experimental Station (CES), Suwon, Korea. The resistant lines were originally introduced from the plant introduction of the United States of America, and the susceptible lines were the leading cultivars bred by the staff of the Division, CES. All the seeds were sterilized by dipping seeds in Benlate-T suspension (200 X diluted) for two hours before sowing. The Benlate-T treated seeds were rinsed thoroughly under running tap water and dried in the greenhouse. Thirty seeds of each line for germination test and 10 seeds for 10 and 20 days old seedling tests were sown in plastic pot (8 x 16.7 x 7.2cm, 14 holes, diameter 2mm, in the bottom) containing vermiculite. Growth stage of the tested plant at the time of inoculation were two primary leaves and two first emerging leaves in 10 days old seedlings. Those of the 20 days old seedlings were two first leaves and two second emerging leaves. The average number of seedling set was 7 to 10 plants in a pot when inoculated. For the adult stage, four plants for each line of Suwon 9, Suwon 26, B-67 and IS 103 were transplanted 20-30 days after sowing into plastic pot (diameter 20 x 20.5cm high) containing virgin soil mixed with vermiculite in a volume ratio three to one, respectively.

The isolate no. 313 of *P. nicotianae* var. *parasitica* was used in all experiments. Since the isolate showed variations in virulence during the maintaining on synthetic media, it was re-isolated from diseased tissue after artificial inoculation to susceptible sesame plants to ensure virulence. The isolate was usually maintained in potato sucrose agar medium and transferred at the one month intervals to the fresh media. For inoculum preparation sesame extract liquid medium was used. The medium was prepared by boiling 250 g of fresh tissue of susceptible sesame seedlings in tap water, then filtering through four layers of cheesecloth and adjusted to 1000 ml with tap water. Fifty ml of prepared medium was poured into 250 ml flask and autoclaved at 121°C under 15 psi for 15 minutes. Sterilized medium was inoculated with small mycelial fragment (diameter 0.9 cm) cultured on oat meal agar medium. Inoculated flasks were incubated at 25°C for 20-25 days under continuous fluorescent light. Cultures were blended by homogenizer for one minute. Thus the suspension was consisted of sporangia and mycelia. The inoculum density was adjusted by number of sporangia per one ml of suspension by using microscope. Prepared inoculum was stored at 10°C for 10 minutes to stimulate zoospore release before used. Pots having seeds were inoculated right after sowing and those having

seedlings were inoculated 10-20 days after sowing with a sporangial suspension containing 20-2000 sporangia per one ml. Ten ml of inoculum was pippeted on the soil surface beside each row of plants while five ml of inoculum was used in a pot when seedlings were inoculated by spraying. Soil water content was maintained by spray watering (unsaturation) or maintained by placing pots with plants in a water tank (150 x 50 x 10cm) filled with water one to two cm high (saturation). Plants were placed under a bamboo blinder 30 cm above the plants immediately after inoculation. Adult plants were inoculated 40-50 days after transplanted (70-day old plant) with a sporangial suspension containing 2000 sporangia per ml. Ten ml of inoculum was pippeted on the soil surface beside the bottom of plants, then the soil was watered once in a day with tap water. Plants of another set of each line were inoculated with five ml of inoculum containing 2000 sporangia per ml by sprying method, then the plants were incubated at temperature (27-30°C) and moisture (100% RH) controlled incubator for eight hours immediately after inoculation.

Disease reading were based on disease severity index 0 through 9 or germination ratio: 0=no symptom or 100 % of germination, 1=mild discoloration of roots or 91-99 % of germination, 3=severe discoloration of roots but no stem discoloration or 71-90 % of germination, 5=discoloration of basal part of stem or 51-70 % of germination, 7=discoloration of stem and wilt symptom or 31-50 % of germination, 9=lodging and blight or less than 30 % of germination. Disease severity was observed in roots, stems and leaves of seedlings 4-10 days after inoculation. Roots, stems and leaves were observed for adult plants 20 days after inoculation when inoculated by soil infestation. Disease readings were based on symptom severity index 0 through 9: 0=no symptom, 1=mild discoloration of roots, 3=severe discoloration of roots, but no stem discoloration, 5=discoloration of basal part of stem is less than five cm, 7=discoloration of stem is 5-10 cm or wilt symptom, 9=discoloration of stem is more than 10 cm or blight symptom. Percentage of leaf number and area diseased at 5 and 7 days after inoculation, respectively, were observed when the plants were inoculated by the spraying method. Experiments described were designed with completely randomized with four replicates. The results were analyzed using analysis of variance.

RESULTS

Evaluation of resistance at seedling stage: Dis-

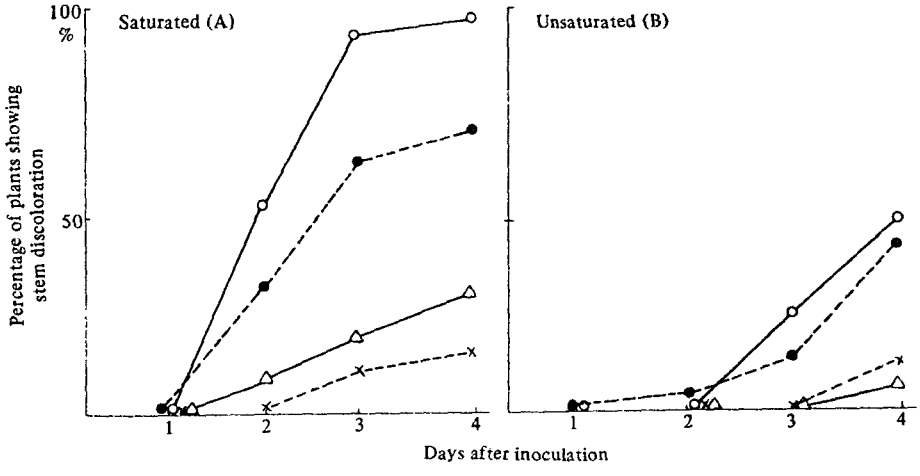

Fig. 1. Symptom development and disease severity in four sesame lines depending upon soil water condition when the soil was infested with sporangial suspension of *P. nicotianae* var. *parasitica* (o——o: Suweon 26, •-----•: Suweon 9, △——△: IS 103, ×-----×: B-67), A: Half immersion of pot in water tank, B: Spray watering – Greenhouse experiment during mid June to mid July in 1983.

coloration of stem was observed two days after inoculation for all the tested lines when inoculated by soil infestation (Figure 1). Symptom development in susceptible lines was faster than that in resistant lines (Figure 1A). All the plants of susceptible line Suweon 26 showed stem discoloration four days after inoculation, whereas only seven plants of the 35 plants showed stem discoloration in resistant line B-67.

Seed germination of all the tested lines was very poor (the lowest 8.7 % in Suwon 9 and the highest 14.4 % in B-67) when the soil was infested right after sowing. Susceptible lines Suwon 9 and Suweon 26 showed its maximum germination ratio four to six days after sowing, but the germinated seedlings were blighted. Resistant lines B-67 and IS 103 also showed its maximum germination ratio four to six days after sowing, but no further symptom was developed in the germinated seedlings until final observation 10 days after sowing. There were no varietal difference in seed germination (Table 1). When 10-day old seedlings were inoculated, the disease incidence in susceptible line Suweon 9 and Suweon 26 was similar to that in 20-day old seedlings. However, symptom development in resistant lines B-67 and IS 103 was faster in 10-day old seedlings than that in 20-day old seedlings. No differences in symptom severity were found among the tested lines when 10-day old seedlings were used (Table 1). However, resistant lines B-67 and IS 103 showed lower symptom severity and slower symptom development compered to susceptible lines Suweon 9 and Suwon 26 when 20-day old seedlings were used (Figure 1A, Table 1).

When the plants were inoculated by the spraying method, severe leaf blight symptoms followed by stem discoloration from the top to the bottom were found in all the tested lines. Successful differentia-

Table 1. Disease severity in four seasme lines when the soil was infested with suspension of *P. nicotianae* var. parasitica at different growth stage[a/].

Sesame entry	Symptom severity[b/]		
	20 day	10 day	0 day
Suweon 9	6.2	6.0	9.0
Suweon 26	9.0	7.7	9.0
IS 103	2.9	6.5	9.0
B-67	1.7	3.6	9.0
Mean LSD at 5%	2.67	NS	NS

a/ Ten ml of inoculum containing 200 sporangia per ml was pippeted on the soil surface. Pots with sesame plants were placed in a water tank filled with water one to two cm high right after inoculation for four days in 10 and 20 days old seedlings and for 11 days in seed germination test (Abbrev. 0day).

b/ Symptom severity was based on seed germination rate and symptoms of root, stem and leaves from 0 through 9 as follows: 0 = No symptom or 100% of seed germination, 1 = Mild discoloration of root or 91-99% of seed germination, 3= Severe discoloration of roots but no stem discoloration or 71-90% of seed germination, 5= Discoloration of basal part of stem or 51-70% of seed germination, 7=Discoloration of stem with wilt symptom or 31-50% of seed germination, 9= lodging and blight or less than 30% of seed germination.

* This experiment was conducted in Suwon during mid June to Mid July in 1983.

Table 2. Varietal resistance of four sesame lines inoculated with suspension of *P. nicotianae* var. *parasitica* by different inoculation method to 20-day old seedlings.

Sesame entry	Symptom severity[a/]	
	Soil inoculation[b/]	Spray inoculation[c/]
Suweon 9	6.2	9.0
Suweon 26	9.0	9.0
IS 103	2.9	9.0
B-67	1.7	9.0
Mean LSD at 5%	2.67	NS

a/ Disease reading was made four days after inoculation and symptom severity ranged from 0 through 9 (see materials and methods or Table 1).

b/ Each pot was inoculated with 10ml of inoculum containing 200 sporangia per ml.

c/ Each pot was inoculated with five ml of inoculum containing 200 sporangia per ml.

* This experiment was conducted in Suwon during mid June to mid July in 1983.

tion in symptom severity between resistant lines B-67 and IS 103 and susceptible lines Suweon 9 and Suweon 26 was possible when inoculated by the soil infestation (Table 2). However, both resistant lines B-67 and IS 103 failed to show resistance when inoculated by the spraying method (Table 2).

Twenty one plants among the 40 plants in Suweon 26 showed stem discoloration at two days after inoculation when the soil was saturated to the maximum water holding capacity by half immersion of pot in a water tank, whereas only one plant among the 30 plants showed stem discoloration when the soil was watered lightly by spraying (Figure 1A,B). Symptom development of both resistant lines B-67 and IS 103 also was faster in the saturated condition of soil water than that in the unsaturated condition (Figure 1A,B). However, susceptible and resistant lines were distinguished with both watering method, even though symptom severity was higher in the saturated condition of soil water compared to that in the unsaturated condition with the average symptom severity index of 5.0 and 2.8 respectively (Table 3).

Table 3. Effect of watering method on disease incidence in four sesame lines when the soil was infested with suspension of *P. nicotianae* var. *parasitica* at 20 day old seedling stage.

Sesame entry	Symptom severity[a/]	
	Pot half immersion[b/]	Spraying
Suweon 9	6.2	4.2
Suweon 26	9.0	4.9
IS 103	2.9	0.6
B-67	1.7	1.4
Mean LSD at 5%	2.67	2.69

a/ Disease reading was made four days after inoculation and symptom severity ranged from 0 through 9 (see materials and methods or Table 1).

b/ Pots with sesame plant were placed in a water tank filled with water one to two cm high after inoculation.

* This experiment was conducted in Sueon during mid June to mid July in 1983.

Symptom severity index of the susceptible and resistant lines inoculated with different inoculum densities is shown in Table 4. Symptom development was faster and symptom severity also was severer in the high inoculum density compared to those in the lower inoculum densities with the average symptom severity index of 7.6 vs. 5.3 vs. 2.3 respectively (Table 4). Statistically significant difference in symptom severity in all the tested inoculum densities was found among the tested lines (Table 4). However, many plants of the susceptible lines Suwon 9 and Suwon 26 survived when inoculated with low density of inoculum containing 20 sporangia per ml (percentage of plants survived= 51.3 vs. 50 respectively), whereas resistant lines B-67 and IS 103 showed severe disease incidence when inoculated with high density of inoculum containing 2000 sporangia per ml (percentage of

Table 4. Effect of inoculum density on disease incidence in four sesame lines when the soil was infested with suspension of *P. nicotianae* var. *parasitica* at 20 day old seedling stage.

Sesame entry	Symptom severity[a/] at inoculum density[b] of		
	2.0×10^1	2.0×10^2	2.0×10^3
Suweon 9	3.6	7.3	9.0
Suweon 26	4.1	9.0	9.0
IS 103	0.9	2.5	6.5
B-67	0.4	2.3	5.7
Mean LSD at 5%	1.59	1.94	0.76

a/ Disease reading was made four days after inoculation and symptom severity ranged from 0 through 9 (see meterials and methods or Table 1).

b/ Number of sporangia per one ml of inoculum.

* This experiment was conducted in Suwon during early July to early August in 1983.

plants diseased=75.3 vs. 80.9 respectively). Only inoculum density of 200 sporangia per ml ensured both complete loss of the susceptible line Suwon 26 and reliable survival of the resistant lines B-67 and IS 103 (Table 4).

Varietal resistance at adult stage: When adult plants were inoculated using soil infestation, all the plants showed discoloration of roots followed by stem discoloration, wilt, and blight symptom in the susceptible lines. Clear difference in symptom severity was found among individual plants especially in both resistant lines B-67 and IS 103. Among the diseased plants no significant difference in symptom severity was found. However, differentiation in symptom severity among the tested lines was possible with statistical significance (Table 5).

Difference in symptom severity among individuals was clearer when adult plants were inoculated by spraying method than that inoculated by soil infestation. Symptoms occurred mainly on leaves. Sympoms were severer in lower position of leaves compared to those on upper position of leaves, but no differences between middle and bottom position of leaves. All the plants of susceptible lines Suwon 9 and Suweon 26 showed severe blight symptoms on leaves, whereas only seven plants among the 16 plants and three plants among the 11 plants showed blight symptoms in the resistant lines B-67 and IS 103, respectively. But there was no difference in symptom severity among the diseased plants. However, differentiation of resistant lines B-67 and IS 103 from susceptible lines Suweon 9 and Suweon 26 in symptom severity was possible with statistical significance (Table 5). Both parameters of percentage of lesion area and percentage of leaf number diseased were useful to assess the results (Table 5).

DISCUSSION

No sesame lines immune to Phytophthora blight was found in the previous tests (un-published data). However, there were differences in symptom severity of sesame lines inoculated with *P. nicotianae* var. *parasitica*. Selection of tolerant sesame lines, if immune lines are not available, may well be a great contribution for efforts to reduce yield losses due to the disease. For the purpose of selection of tolerant sesame lines, our standardization of the screening methods for resistance of sesame lines will be the most important key since reaction of sesame lines were reported to be variable depending upon inoculation technique[4,9].

Table 5. Disease incidence in four sesame lines inoculated with suspension of *P. nicotianae* var. *parasitica* by different method at 70-day old adult stage.

Sesame entry	Disease severity when inoculated by different methods		
	Soil inoculation	Spray inoculation	
	Symptom severity[a/]	Percentage of lesion area	Percentage of leaf number diseased
Suweon 9	7.9	20.3	58.0
Suweon 26	8.6	25.2	70.4
IS 103	4.2	6.7	16.2
B-67	5.5	6.9	18.9
Mean LSD at 5%	2.33	15.08	23.37

a/ Symptom severity ranged from 0 through 9 as follows: 0=No symptom, 1=Mild discoloration of roots, 3= Severe discoloration of roots, but no stem discoloration, 5=Discoloration of basal part of stem is less than 5 cm, 7=Discoloration of stem is 5-10 cm or wilt symptom, 9=Discoloration of stem is more than 10 cm or blight symptom.

* This experiment was conducted in Suwon during late May to late August in 1983..

Most Korean farmers have been aware of that continuous cultivation of sesame in a field could bring hazardous yield losses probably due to sesame diseases. In Korea, Fusarium wilt, Corynespora leaf blight, Bacterial wilt and Phytophthora blight found in 1981 in the major sesame production areas[1] are the major disease constraints. In fact, rotation, improvement of cultivation methods including vinyl-mulching, chemicals application and use of resistant sesame lines might help farmers for safe production as parts of the integral control measures.

Our results confirm that such factors as inoculation method, growth stage of the test plants, soil water condition, and inoculum dosage affect varietal reaction to the disease. Determination of resistance in seedling stage would permit rapid identification of resistant germplasm. Consistent results in varietal resistance between seedling and adult stages have been reported in alfalfa[3,7], soybean[10], and safflower[6] to Phytophthora disease, but no one in sesame[4,9]. However, consistent and successful differentiation of varietal resistance in both seedling and adult stage tests using the greenhouse technique described here could be used as a rapid and reliable screening method for varietal resistance of sesame to Phytophthora blight.

摘　　要

本 시험은 참깨 疫病(病原菌 : *Phytophthora nicotianae* var. *parasitica*)에 대한 品種抵抗性 檢定方法 確立을 위해 실시하였다. 供試作物의 苗齡, 接種方法, 土壤水分狀態 및 接種源의 濃度는 供試한 中度抵抗性系統 B-67, IS 103과 羅病性系統 水原 9號, 水原 26號 모두의 病進展速度와 發病甚度에 영향을 주는 것으로 나타났다. 그러나, 播種後 20日된 幼苗에 1 *mℓ*當 200個 胞子囊의 接種源을 폿트(8 × 16.7 × 7.2 cm)當 10 *mℓ*씩 土壤表面에 接種한 後 底面灌水로 土壤水分을 飽和狀態로 維持하였을 때 系統間 抵抗性의 差異를 잘 볼 수 있었다. 苗齡 70日의 成苗를 使用하였을 때는 土壤接種을 하였을 때나 噴霧接種을 하였을 때 모두 系統間 抵抗性 差異를 잘 볼 수 있었다.

前述한 幼苗檢定方法에 依해 羅病性系統인 水原 26號는 罹病株率로는 100%, 發病甚度로는 最大値인 9.0을 보였다. 反面에 中度抵抗性系統인 B-67은 罹病株率로는 20%, 發病甚度로는 1.7을 보였다. 發病甚度는 0에서 9까지 指數化하여 나타내었다. 例를 들면, 發病甚度 0은 病徵이 없는 狀態, 5는 地面部 줄기가 變色된 경우, 9는 잎마름症狀과 함께 줄기變色이 地面部로부터 10cm 以上인 경우이다. 品種間 抵抗性의 差異는 病斑面積率로도 調査를 하였으며, 이 結果는 發病甚度로 調査한 檢定結果와 잘 一致하였다.

LITERATURE CITED

1. Cho, E. K., N. Y. Heo, S. H. Choi and S. C. Lee. 1981. Studies on sesame diseases in Korea. I. Incidences of Phytophthora blight. Korean J. of Plant Prot. 21: 211-215.
2. Choi, S. H., E. K. Cho and W. T. Cho. 1984. Epidemiology of sesame Phytophthora blight in different cultivation types. Res. Rept. ORD 26-2 (S.P.M.U): 64-68.
3. Frosheiser, F. I. and D. K. Barns. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Science 13: 735-738.
4. Gemawat, P. D. and N. Prasad. 1965. Tests with different varieties of sesamum for resistance to Phytophthora blight of sesamum. Indian Phytopath. 18(2). 128-132.
5. Gooding, G. V. and G. B. Lucas. 1959. Effect of inoculum level on the severity of tobacco black shank. Phytopathology 49: 274-276.
6. Heritage, A. D. and E. K. S. Harrigan. 1984. Environmental factors influencing safflower screening for resistance to *Phytophthora cryptogea*. Plant Disease 68: 767-769.
7. Hohrein, B. A., G. A. Bean and J. H. Graham. 1984. Greenhouse technique to evaluate alfalfa resistance to *Phytophthora megasperma* f. sp. *medicaginis*. Plant Disease 67: 1332-1333.
8. Kannwischer, M. E. and D. J. Mitchell. 1981. Relationship of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infection and mortality of tobacco. Phytopathology 71:

69-72.

9. Sehgal, S. P. and N. Prasad. 1972. Note on testing of different varieties, strains and species of sesamum for resistance to Phytophthora blight. Indian Jour. Agri. Sci. 42(2): 122.

10. Walker, A. K. and A. F. Schmitthenner. 1984. Comparison of field and greenhouse evaluation for tolerance to Phytophthora rot in soybeans. Crop Science 24: 487-489.