

Establishment of Seedling Test for Resistance to Phytophthora capsici Leonian in Cucurbita.

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In Japan, the commercial varieties of pumpkin classified into Cucurbita maxima are widely cultivated. C. maxima seems to be susceptible to Phytophthora capsici compared with C. moschata on field observation. It is necessary to transfer Phytophthora resistance gene of C. moschata to C. maxima. The purpose of this study was to establish the method of seedling test to screen Cucurbita cultivars for resistance to Phytophthora capsici.

Materials and Cultivation. Five varieties were used for this study; C. maxima: Utsugiwase-akaguri and Kurokawaguri, and C. moschata: Heiankogiku, Hyuga 14 and Bizenchirimen. The seeds after forced sprouting were sown in plastic pot (9 cm diameter) filled with perlite. Experiments were designed for two replications of eight plants per plot.

Preparation of Pathogen and Inoculation. Thirty ml of liquid medium consisted of vegetable juice in 100 ml Erlenmeyer flask was sterilized. Mycelia of Phytophthora were inoculated in this medium. Mycelial mat, which was produced by 2-week incubation at 28°C, was rinsed on a Buchner funnel with sterile distilled water and put on the filter paper moistened by sterile distilled water in the petri dish. The petri dish was covered with a Japanese paper lid and placed in a 28°C incubator illuminated with fluorescent lamp. Numerous zoospores were formed on the surface of mycelial mat by 20-30 hour incubation. The zoospores were collected with a small brush after pouring of sterile distilled water into the petri dish (1). About 50 ml of the zoospore suspension was poured into each pot.

Evaluation. At 10-14 days after inoculation, results of the observation were recorded.

Effect of Inoculum Concentration (Table 1). The six kinds of inoculum were prepared as 0,  $4 \times 10^1$ ,  $2 \times 10^2$ ,  $1 \times 10^3$ ,  $5 \times 10^3$  and  $1 \times 10^4$  zoospores/ml. Two weeks after sowing, plants were inoculated. With the inoculation of suspensions above  $1 \times 10^3$  zoospores/ml, all of the plants in C. moschata were diseased but the percentage of died plants were low. At low inoculum concentrations ( $4 \times 10^1$  and  $2 \times 10^2$  zoospores/ml), the percentage of diseased plants and the percentage of died plants were high C. maxima and low in C. moschata.

Effect of Seedling Stage (Table 2). Plants were grown to the 8-day, 15-day and 22-day seedling stage before inoculation. Inoculum concentrations were prepared for  $2.5 \times 10^2$  zoospores/ml in C. maxima and for  $1 \times 10^3$  zoospores/ml in C. moschata. In C. maxima, all of the plants inoculated at all of the seedling stages were diseased and died. In C. moschata, the percentage of diseased plants and the percentage of died plants were low compared with those of C. maxima, and the percentage of the 15-day seedling was higher than those of the 8-day and 22-day seedling stages.

Effect of Temperature Condition (Table 3). Plants had been kept in 30°C-23°C (day temperature-night temperature), 25°C-18°C and 20°C-13°C during 10 days from 2 days before inoculation. Plants were inoculated with  $1 \times 10^3$  zoosporangia/ml at 2 weeks. In C. maxima, the percentage of diseased plants and the percentage of died plants were 100% or nearly 100% at high and middle temperature conditions. In C. moschata, the percentage of diseased plants was very low and the percentage of died plants was 0%, at all of the temperature conditions.

Conclusion. It seems likely that the appropriated zoosporangia concentration may be in the range from  $2 \times 10^2$  to  $1 \times 10^3$  zoosporangia/ml, because of stability of disease appearance. It was estimated that the uniform seedlings about 2 weeks old may be available to inoculate Phytophthora pathogen. Desirable temperature condition for inoculation and nursery of inoculated seedlings seems to be in the high or middle range; 30°C-25°C at day and 23°C-18°C at night.

#### Literature cited

1. Katsura, K., Y. Miyata and T. Mitani. 1968. A new method for the numerous formation of zoosporangia in Phytophthora spp. Sci. Rep. Kyoto Pref. Univ., Agric. 20:32-36.

Table 1. Effect of inoculum concentration of Phytophthora capsici on percentage of diseased plants and percentage of died plants.

Species	Variety	Inoculum Concentration (zoosporangia/ml)					
		0	$4 \times 10$	$2 \times 10^3$	$1 \times 10^3$	$5 \times 10^3$	$1 \times 10^4$
<u>C. maxima</u>	Utsugiwase-akaguri	0/0 <sup>2</sup>	100/88	100/100	100/100	100/100	100/100
	Kurokawaguri	0/0	88/88	100/100	100/100	100/100	100/100
<u>C. moschata</u>	Heiankogiku	0/0	0/0	63/13	100/25	100/38	100/38
	Hyuga 14	0/0	50/25	50/25	100/63	100/50	100/38

<sup>2</sup>Left; Percentage of diseased plants.

Right; Percentage of died plants.

Table 2. Effect of seedling stage when inoculated on percentage of diseased plants and percentage of died plants.

Species	Variety	Seedling Stage When Inoculated (Days after sawing)		
		8	15	22
<u>C. maxima</u>	Utsugiwase-akaguri	100/100 <sup>Z</sup>	100/100	100/100
	Kurokawaguri	100/100	100/100	100/100
<u>C. moschata</u>	Heiankogiku	31/0	81/69	69/44
	Bizenchirimen	38/6	63/56	44/31

<sup>Z</sup>Left; percentage of diseased plants.  
Right; Percentage of died plants.

Table 3. Effect of temperature condition on percentage of diseased plants and percentage of died plants.

Species	Varieties	Temperature Condition (°C, Day-Night)		
		30-23	25-18	20-13
<u>C. maxima</u>	Utsugiwase-akaguri	100/100 <sup>Z</sup>	100/94	88/75
	Kurokawasguri	100/100	100/100	81/75
<u>C. moschata</u>	Heiankogiku	19/0	19/0	6/0
	Bizenchirimen	13/0	6/0	31/0

<sup>Z</sup>Left; Percentage of diseased plants.  
Right; Percentage of died plants.