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Selection of Resistant Hybrids of *Atractylis* Against *Phytophthora drechsleri*

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Bioassay techniques using young leaves and roots were developed to screen resistance of *Atractylis* spp. against *Phytophthora drechsleri*. Among 638 plants collected from various regions of Korea from 1994 to 1996, 67 were pre-screened in fields naturally infested with *P. drechsleri*, which is the causal pathogen of rhizome rot of *Atractylis*. Among the pre-screened sources, 18 (ca. 26.8%) were highly resistant to the pathogen in leaf inoculation. In the root inoculation test, abundant sporangia were formed in susceptible plant roots, while only a few or no sporangia were produced on the roots which were found resistant in the leaf inoculation test. Among the selected resistant plants, *A. japonica* 96066 and 96104 were used to cross with another species, *A. macrocephala* 96362 that showed high yield with good quality of rhizome but susceptible to the pathogen. The F_1 hybrids designated as HA03 turned out to be resistant to the pathogen, indicating that resistant gene(s) was inherited. Among intra-species hybrids of *A. japonica*, HA07 and HA09 were resistant to the pathogen in leaf inoculation and moderate in root inoculation. However, HA08 was susceptible in both inoculation tests. This result suggests that the parent material might be genetically heterogeneous. Further genetic study should be carried out to verify this phenomenon.

Keywords **:** *Atractylis*, hybrid, leaf inoculation, *Phytophthora drechsleri*, resistant, root inoculation.

Cultivation of resistant plants to control various diseases has been known as the most effective and economical measure for many crops such as rice, potato, etc. Disease resistance is categorized into specific and general resistance (Browning et al., 1977; Thurston, 1971). Specific resistance refers to true resistance and vertical resistance (Vanderplank, 1963), while general resistance refers to horizontal resistance or field resistance (Thurston, 1971; Umaerus et al., 1983). There have been many researches conducted on resistance to *Phytophthora* (Howard et al., 1976; Kannaiyan et al., 1981; Kim and Kim, 1984; McIntyre and Taylor, 1976; Scott et al., 1976; Thomas, 1976). A review of these *Phytophthora* researches showed that many have been carried out in the area of *Phytophthora* resistance. Specifically on *P. drechsleri* resistance, researches have been conducted on several crops, including safflower (Erwin and Ribeiro, 1996).

The rhizome of *Atractylis* spp., which is called "Baegchul" in Korean, has long been used for medicinal purposes in Korea and in other oriental countries. The therapeutic virtues of the rhizome on digestion and diuresis, and as an antiperspirant have been documented in "Dong Ee Bo Gam" (Huh, 1613), Bencao Gangmu (Li, 1578) and so on. The original plant producing "Baegchul" is called "Sabju" in Korea.

Phytophthora diseases in medicinal plants have been less studied compared with other crops in many countries. However, severe rot on the rhizome of Sabju has widely occurred in major cultivation areas in Korea since 1996, and the causal pathogen of the disease was previously identified as *Phytophthora drechsleri* (Kim et al., 1997). Chemical application for the control of Sabju diseases may not be acceptable to consumers due to the usage of the rhizome as medicine. Therefore, the most ideal alternative to control the *Phytophthora* rhizome rot is to exploit host resistance.

In this study, rapid and reliable techniques for bioassay of resistance to *Phytophthora* were developed. Intra-species and inter-species hybridizations were performed to produce *Phytophthora*-resistant cultivars producing high yields with good quality rhizomes.

Materials and Methods

Plant sources. Plant sources used for breeding *Phytophthora*-resistant varieties with high yield and good quality rhizomes were collected from various regions in Korea from 1994 to 1996 including a plant, *Atractylis macrocephala*, which originated from China. The plants surviving in the fields heavily infected with *P. drechsleri* were considered potentially resistant to the pathogen, and a total of 638 plants were collected. The plants were maintained in the field at the Medicinal Plants Experiment Station in Hamyang and screened for resistance through natural infection with the pathogen. Among the surviving plants in the field, 40 lines in 1997 and 30 lines in 1998 were individually tested for

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resistance to *Phytophthora* rhizome rot by artificial inoculation.

Isolates and inoculum preparation. *Phytophthora drechsleri* 9601, which has been previously reported (Kim et al., 1997), was used in this study. The pathogen was grown on V-8 juice agar at 25°C for 3 days in the dark. The margin of colony was cut off to mycelial discs (5 mm in diameter) with a cork borer and then used for the leaf inoculation test.

For root inoculation, three mycelial discs (5 mm in diameter) from the colony margins were transferred to a petri dish containing 20 ml of sterile distilled water to induce massive sporangia production under fluorescent light illumination for 8-12 h.

Development of bioassay techniques for screening of *Phytophthora* resistant plants. Plant resistance was evaluated by artificial inoculation of the pathogen to leaves and roots. Newly developed young soft *Atractylis* leaves (15 days old) were detached from plants and placed in a moistened petri dish. Then, each leaf was inoculated with an agar disc obtained from 3-day-old mycelia of *P. drechsleri*. The petri dish was incubated at 15, 20, or 25°C for 2-3 days.

Plant resistance to the pathogen was evaluated by rot lesions, which developed on the leaves. When rot lesions appeared on the leaf, the plant was considered as susceptible, and vice versa.

Young white roots were sampled from each plant and washed with running tap water. The roots were dipped in zoospore suspension of *P. drechsleri* (ca. 100 spores/ml) and incubated at 25°C for 2-3 days. Plant resistance was evaluated by the sporangial formation on root surface examined under a microscope at magnification × 100. If sporangia of the fungus were formed on the roots, the plants were considered as susceptible, and vice versa.

Resistant hybrids of Sabju to *P. drechsleri*. Inter-species hybrids of Sabju, which were resistant to *Phytophthora* and producing high yields with good quality rhizome, were made by artificial crossing between the maternal parent *Atractylis japonica* 96104, a dioecious plant that does not produce pollen, and the paternal parent *A. macrocephala* 96035. The pollens of *A. macrocephala* were donated onto the stigma of *A. japonica* including 96066, a dioecious plant, which was resistant to the pathogen. Hybrid plants designated as HA01, 02, 03, 04, 05, and 06 were developed. However, only lines HA01 and HA03 survived for 2 years, while other lines degenerated before the test.

Pollens of susceptible plants of hermaphroditic *A. japonica* including 96070, which produced good quality rhizomes but susceptible to the pathogen, were crossed with 96066 to determine the inheritance of resistance. The F_1 plants were designated as HA07, HA08, and HA09, respectively. The F_1 plants were clarified by observing leaf and flower morphology; selfing plants were removed. Resistance of the hybrid F_1 plants to the fungus was estimated by artificial inoculation on leaves and roots as described above.

Results

Plant sources. As shown in Table 1, among 638 plants collected from various regions in Korea from 1994 to 1996, 67 were pre-screened in a naturally infested field with *P. drechsleri*, which is the causal pathogen of the disease.

Table 1. Susceptibility of *Atractylis* spp. (Sabju) collected from various regions in Korea to *Phytophthora drechsleri* in leaf disc inoculation method

Year tested	No. of plants		
	Total	Susceptible	Resistant
1997	40	30 (75.0%)	10 (25.0%)
1998	27	19 (66.7%)	8 (33.3%)

Among the selected plants, 18 (ca. 26.8%) showed high resistant reaction to the pathogen in the leaf inoculation. Most of the resistant plants in the field were dioecious, having only female flowers.

Bioassay results. The newly developed young soft leaves (2 weeks old) were more susceptible to *P. drechsleri* than old and hardened leaves. Rot lesions developed around the fungal agar disc in 2 days on susceptible plant leaves. However, on resistant plant leaves, the lesions did not develop. The lesions enlarged more rapidly at 20°C than at 25°C, but did not develop at 15°C. The lesions which developed at 20°C and 25°C were measured as 16.6 × 9.7 mm and 7.7 × 6 mm in average, respectively.

Among 67 plants bioassayed by leaf inoculation in 1997 and 1998, 18 plants (ca. 26.8%) were considered as resistant to the pathogen, while the rests (49 plants, ca. 73.2%) were susceptible (Table 1).

In young root inoculation, abundant sporangia were formed on the roots of the susceptible plants including 96006. On the other hand, only a few sporangia were observed on the roots of the resistant plants 96053, 96064,

Table 2. Comparison of resistant reaction of *Atractylis* spp. (Sabju) to *Phytophthora drechsleri* in leaf inoculation and root inoculation

Plant accession	Incidence of *Phytophthora* rot in field[a]	Reaction in leaf inoculation[b]	Sporangial formation in root inoculation[c]
96006	+++	S	+++
96011	–	R	+
96053	–	R	+
96064	–	R	+
96066	–	R	–
96104	–	R	+
96101	–	R	+
96299	–	R	+
98001	–	R	+
98002	–	R	+

[a] +++ severely infected, – not infected

[b] S susceptible, R resistant.

[c] +++ sporangia abundantly formed, + a few sporangia formed, – no sporangium formed.

Table 3. Resistant reaction of hybrid plants between *Atractylis japonica* and *A. macrocephala* to *Phytophthora drechsleri* by leaf inoculation and root inoculation methods

Hybrid	Parent	Reactions[a]	
		leaf inoculation	root inoculation
HA01 F_1	–	R	M
	P_1 *A. japonica* 96104[b]	R	M
	P_2 *A. macrocephala* 96035[c]	S	S
HA03 F_1	–	R	R
	P_1 *A. japonica* 96066[b]	R	R
	P_2 *A. macrocephala* 96035	S	S
HA07 F_1	–	R	M
	P_1 *A. japonica* 96066	R	R
	P_2 *A. japonica* 96070[c]	S	S
HA08 F_1	–	S	S
	P_1 *A. japonica* 96066	R	R
	P_2 *A. japonica* 96070	S	S
HA09 F_1	–	R	M
	P_1 *A. japonica* 96066	R	R
	P_2 *A. japonica* 96070	S	S

[a] R: resistant, M: moderately resistant, S: susceptible.
[b] a dioecious female plant without producing pollen.
[c] a hermaphroditic plant with male and female organs in one flower.

96104, and 98001, while no sporangia were formed on the roots of 96066 and 98002 (Table 2).

The results of leaf and root inoculation method were compared with those screened in severely infested field with *P. drechsleri*. The leaf inoculation result was consistent with the reaction to field screening. Furthermore, root inoculation was more sensitive than leaf inoculation.

Resistant hybrids of *Atractylis* to *P. drechsleri*. By the artificial crossing between *A. japonica* 96104, which was found resistant in leaf inoculation but moderately resistant in root inoculation, and *A. macrocephala* 96035, which was susceptible in both inoculations, the HA01 was selected as a resistant plant to the pathogen *P. drechsleri* in leaf inoculation, and moderately resistant in root inoculation. The hybrid HA03 was also selected as another resistant plant by both inoculation methods (Table 3). Among intra-species hybrids of *A. japonica*, HA07 and HA09 were resistant to the pathogen in leaf inoculation, and moderately resistant in root inoculation. However, HA08 was susceptible in both inoculation tests.

Discussion

In many crops, screening for resistance to diseases is done on seedlings, especially for plants, which grow slowly and require long-term cultivation period (Thomas and Hill, 1977). Since many medicinal plants including Sabju need to be cultivated for several years, screening on adult plants is practically impossible. Moreover, resistance screening fields for epidemic diseases such as *Phytophthora* rhizome rot of Sabju, is especially difficult because of rapid dispersal of the pathogen and contamination of fields.

However, newly developed bioassay techniques for screening of Sabju resistance to *P. drechsleri* are very simple, rapid, and repeatable. Since rot lesions on leaves developed only around the agar discs in susceptible but not resistant plants, the leaf inoculation method readily distinguished the resistant plant *in vitro*. Root inoculation method with zoospore suspension was also highly valuable. Only a few or no sporangia of the pathogen were formed on the roots of resistant plants in 2-3 days at room temperature, but abundant sporangia were produced on the roots of susceptible ones. The techniques used in this study were considered reliable because resistance evaluated by both techniques was consistent.

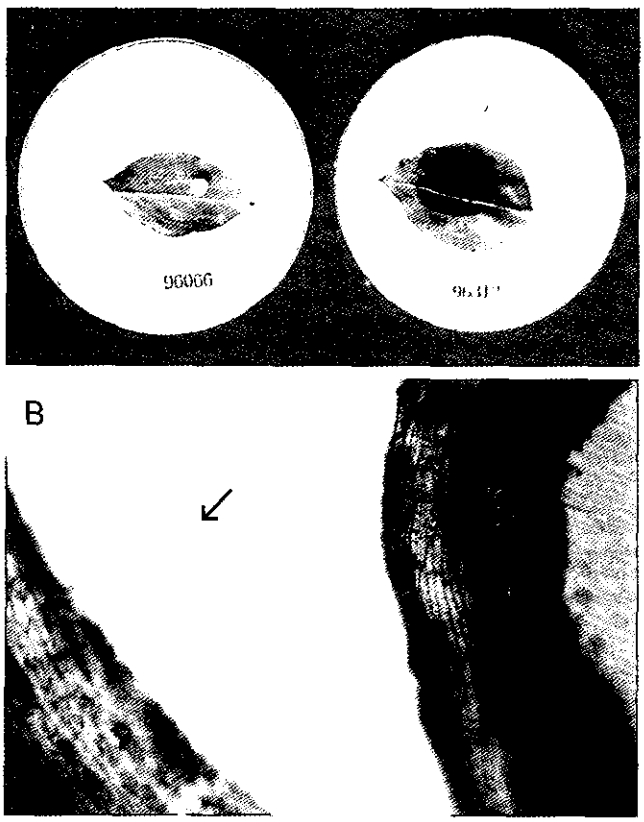

Fig. 1. Bioassay techniques for screening resistance of *Atractylis* spp. (Sabju) to *Phytophthora drechsleri*. (**A**) Leaf inoculation method, (**B**) Root inoculation method in zoospore suspension. The black color on the leaf shows lesion infected by inoculating mycelial agar disc, which was considered susceptible; the leaf on the left without color change was considered resistant. In (B) Zoosporangial production (arrow) on the root infected with *P. drechsleri* is also evaluated susceptible.

Keeling (1976) reported that stem-wound inoculation was an efficient method to screen resistance of soybean to *P. megasperma* var. *sojae*. For potato late blight, Stewart (1990) suggested that the detached leaf inoculation method on soft young leaf was efficient. The method used in this study relied on introducing mycelial agar disc to detached soft leaf without wounding. In this method, the excised root, if not washed carefully enough, could be contaminated by protists, and repeatable results may not be achieved due to predators of zoospores.

A few Sabju plants collected from the wilderness showed high resistance to the pathogen, indicating that resistant gene(s) to *Phytophthora* exists in the plant. When the resistant plants of *Atractylis japonica* 96104 and 96066 were crossed with A. *macrocephala* 96035, resistant traits were inherited to F_1 hybrids. Unexpectedly, in case of intra-species hybridization, HA08 was susceptible to *P. drechsleri* as opposed to the case of HA07 and HA09, which were derived from the same crossing combination (Table 3). This result may be due to the genetic heterogeneity of parent materials. Moreover, the prominent phenotype characteristics of the latter, such as high yields and good quality rhizomes, were also inherited by the hybrids. However, the hybrids were dioecious female plants that do not produce pollens. Therefore, gene analysis by selfing the F_1 plant was impossible. Subsequently, a mass multiplication technique should be developed for practical use.

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