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Detection of *Alternaria* spp. in Carrot Seeds and Effect of the Fungi on Seed Germination and Seedling Growth of Carrot

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Five seed samples of carrot were tested to detect *Alternaria* spp. by blotter method. *A. alternata* and *A. radicina* were detected from all the seed samples as high as 25.8-70.5% and 37.5-63.5%, respectively. *A. dauci* was detected from four seed samples as low as 0.5-7.5%. The three *Alternaria* spp. were detected from the pericarp and the seed coat and endosperm of the carrot seeds but not from the embryo by component plating test. *A. alternata* and *A. radicina* were much more detected from the pericarp than the seed coat and endosperm. *A. dauci* was detected from the pericarp and the seed coat and endosperm at similar rate. The seed sample which was most severely infected with *A. radicina* showed the lowest rate of germination in the test on top of paper (TP). In the TP test, differences in total infection rate of *A. radicina* and *A. dauci* of the seed samples were very closely correlated with those in incidence of seedling rot on the seed samples. However, there was no correlation between infection rate of *A. alternata* and rate of germination or seedling rot of the seed samples. Soil test for seedling growth revealed that there was no correlation between differences in total infection rate of *A. radicina* and *A. dauci* and those in rate of normal seedlings of the seed samples.

Keywords : *Alternaria alternata, A. dauci, A. radicina,* carrot, detection, germination, seedling growth

Carrot [*Daucus carota* L. subsp. *sativus* (Hoffm.) Arcang.] is one of important vegetables cultivated worldwide. The crop is propagated by seeds. It is recorded that more than ten pathogens are seed-borne in the crop (Richardson, 1990). Among the pathogens, predominant seed-borne fungi are *Alternaria dauci* (Kühn) Groves & Skolko and *A. radicina* Meier, Drechsler & Eddy. *A. dauci* mainly causes leaf blight and leaf spot in carrot, and *A. radicina* black rot (Farr et al., 1989; Farrar et al., 2004; Neergaard, 1977). Maude (1966) reported that the two *Alternaria* spp. caused damping-off of carrot seedlings and when carrots were grown at high densities, *A. dauci* caused severe foliar infection. It was also reported that *A. radicina* caused foliar blight under certain conditions (Farrar et al., 2004).

Seeds infected with pathogens could play a role of transferring the pathogens to a new place, being a primary inoculum source in the field. Detection and infection of *Alternaria* spp. in carrot seeds have been studied by many workers (Maude, 1966, 1996; Netzer and Kenneth, 1969; Scott and Wenham, 1973; Soteros, 1979; Strandberg, 1983). However, detailed infection aspects of the fungi in carrot seeds and their pathological effects on seed germination and seedling growth of carrot have been little studied. In this study, infection of *Alternaria* spp. in carrot seed components was examined, and correlation between seed infection with the fungi and germination or seedling growth of carrot was investigated.

Materials and Methods

Detection of fungi. Five seed samples of carrot obtained from growers in Korea in 1989 were used in 1990 after conservation at room temperature. Four hundred seeds per sample were tested for detection of fungi by the blotter method using alternating cycles of 12 hr near ultraviolet (NUV) light and 12 hr darkness (International Seed Testing Association; ISTA, 1985). Fungi detected on the seeds were observed under a stereo microscope and a compound microscope for identification. Identification of fungi including *Alternaria* spp. was carried out based on the morphological characteristics described by previous workers (Barnett and Hunter, 1998; David, 1999; Ellis, 1971; Ellis and Holliday, 1972).

Component plating test. Fifty seeds per sample were used for detection of *Alternaria* spp. from separate parts of carrot seeds. Each seed sample was soaked in sterile distilled water in 5 cm-Petri dishes for 7 to 8 hr. The soaked seeds were dissected using a scalpel under a stereo microscope and separated into three parts, pericarp, seed coat and endosperm, and embryo. The separate parts of each seed were plated on three layers of blotter papers in a 5 cm-

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plastic Petri dish and incubated in the same way of the blotter method using alternating cycles of 12 hr NUV light and 12 hr darkness.

Germination test. Four hundred seeds per sample were used for their germination test on top of paper (TP) and in soil as indicated by ISTA (1985). For the TP test, 25 seeds were plated on three layers of blotter papers in a 9 cm-plastic Petri dish and incubated in the same way of the blotter method using alternating cycles of 12 hr fluorescent light and 12 hr darkness. For the soil test, 10 seeds were sown in peat soil in a plastic pot (5.0 × 5.0 × 5.5 cm) and incubated under alternating cycles of 12-hr fluorescent light and 12 hr darkness at room temperature. For the germination tests, one hundred seeds per sample were used in four replicates. Final count was made 14 days after sowing. Duncan's multiple range test at 5% was applied for analysis of the data.

Investigation of seedling growth. Diseased seedlings and normal seedlings were rated based on the rot symptoms produced on the seedlings at the final count in the TP test and the soil test. Sporulation of *Alternaria* spp. on the symptoms was examined under a stereo microscope.

Results

Detection of *Alternaria* spp. Three species of *Alternaria* and other fungi were detected from five seed samples of carrot tested (Table 1). *A. alternata* and *A. radicina* were detected from all the seed samples as high as 25.8-70.5% and 37.5-63.5%, respectively. *A. dauci* was detected from four seed samples as low as 0.5-7.5%. Other fungi belonging to genera *Aspergillus, Fusarium, Penicillium*, and *Phoma* were detected from some of the seed samples at low percentage. The morphological features of the three *Alternaria* spp. on carrot seeds observed under a stereo microscope are shown in Fig. 1A-C. Morphology of *A. alternata* was characterized by long conidial chains formed on the seeds. Conidia of the species were obclavate, obpyriform, ovoid or ellipsoidal, with a short beak. Morphology of *A. dauci* was characterized by solitary conidia or 2 conidial chains formed on the seeds. Conidia of the species were straight or curved and obclavate, with a long beak. Morphology of *A. radicina* was characterized by solitary conidia or 2 to 3 conidial chains formed on the seeds. Conidia of the species were straight, ellipsoidal, obclavate or obpyriform, without a beak.

Location of *Alternaria* spp. in the seed. Location of *Alternaria* spp. infection in pericarp, seed coat and endosperm, and embryo of carrot seeds was examined by component plating test (Fig. 1D). The three *Alternaria* spp. were detected from the pericarp and the seed coat and endosperm but not from the embryo (Table 2). *A. alternata* and *A. radicina* were much more detected from the pericarp than the seed coat and endosperm. *A. dauci* was detected from the pericarp and the seed coat and endosperm at similar rate.

Table 1. Detection of *Alternaria* spp. and other fungi in five seed samples of carrot by blotter method

Fungi identified	% detection of fungi from seed samples[a]				
	29852	29853	29854	29855	29856
Alternaria alternata	70.5	34.0	64.8	25.8	53.5
A. dauci	2.8	7.5	0.5	5.3	0
A. radicina	60.8	47.0	37.5	63.5	48.3
Aspergillus sp.	0	0	0	0.3	0.8
Fusarium sp.	0.3	0	0.8	0	0
Penicillium sp.	0	3.3	0	0	10.5
Phoma sp.	0.3	0	0	0	0

[a]Four hundred seeds per sample were tested.

Seed germination and seedling rot. The seed sample 29855 which was most severely infected with *A. radicina* showed the lowest rate of germination in the TP test (Table 3). There was no significant difference in rate of germination among the other seed samples. The seed sample 29855 also showed the highest rate of seedling rot, although there was no significant difference with the seed samples 29852 and 29856. In the TP test, differences in total infection rate of *A. radicina* and *A. dauci* of the seed samples were very closely correlated with those in incidence of seedling rot on the seed samples. However, there was no correlation between infection rate of *A. alternata* and rate of germination or seedling rot of the seed samples.

Seedling growth. The seed sample 29855 showed the lowest rate of normal seedlings in the TP test (Table 4). There was no significant difference in rate of normal seedlings among the other seed samples. Soil test for seedling growth revealed that there was no correlation between differences in total infection rate of *A. radicina* and *A. dauci* and those in rate of normal seedlings of the seed samples.

Discussion

Incidence of *A. alternata* infection in the seed samples tested was as high as 25.8-70.5%. However, there was no correlation between rate of the fungal infection and that of germination or seedling rot of the seed samples. The results suggest that the fungus is not virulent on seeds and seedlings of carrot. It has been reported that *A. alternata* has

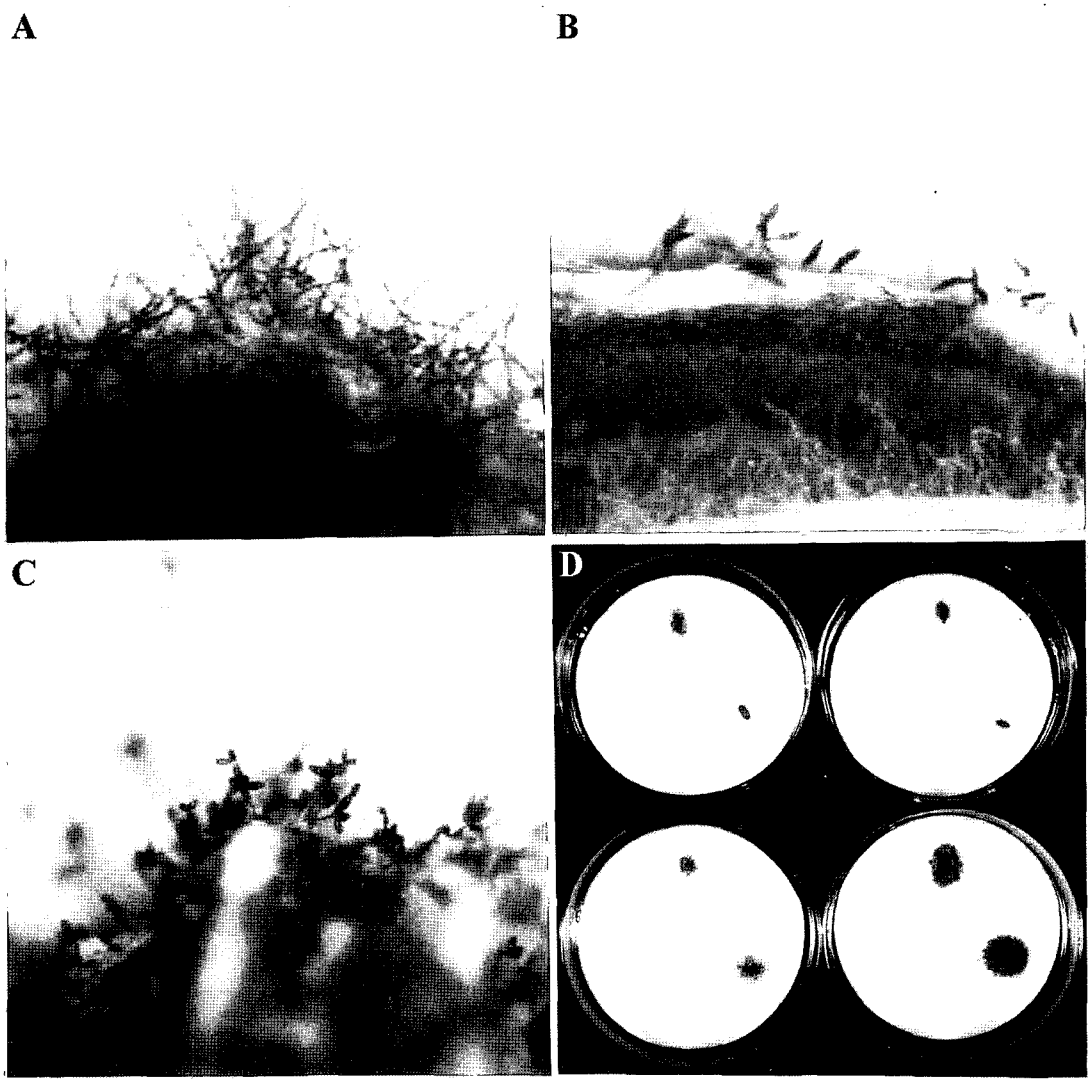

Fig. 1. Morphological features of *Alternaria alternata* (A), *A. dauci* (B), and *A. radicina* (C) on carrot seeds observed under a stereo microscope and detection of the *Alternaria* spp. from the pericarp, the seed coat and endosperm, and the embryo of carrot seeds by component plating test (D).

Table 2. Detection of *Alternaria* spp. in seed parts of carrot by component plating method

Alternaria spp.	Seed part	% detection of *Alternaria* spp. from separated seed parts of seed samples[a]				
		29852	29853	29854	29855	29856
A. alternata	Pericarp	72.0	12.0	72.0	14.0	48.0
	Seed coat and endosperm	40.0	2.0	56.0	0	6.0
	Embryo	0	0	0	0	0
A. dauchi	Pericarp	6.0	2.0	0	6.0	0
	Seed coat and endosperm	6.0	0	2.0	4.0	0
	Embryo	0	0	0	0	0
A. radicina	Pericarp	42.0	44.0	32.0	48.0	36.0
	Seed coat and endosperm	24.0	8.0	6.0	2.0	0
	Embryo	0	0	0	0	0

[a]Fifty seeds per sample were tested.

Table 3. Seed germination and seedling rot of five seed samples of carrot studied by TP test[a]

Seed sample	% germination	% seedling rot
29852	77.3 a[b]	17.0 ab[b]
29853	78.8 a	13.8 bc
29854	73.5 a	9.0 c
29855	62.0 b	23.3 a
29856	78.3 a	16.0 abc

[a]One hundred seeds per sample were tested in four replicates at room temperature. Final count was made 14 days after sowing.
[b]In a column, means followed by a common letter are not significantly different by Duncan's multiple range test at 5%.

Table 4. Seedling growth of five seed samples of carrot studied by two methods[a]

Seed sample	% normal seedlings in TP test	% normal seedlings in soil test
29852	60.3 a[b]	55.8 b[b]
29853	65.0 a	63.5 a
29854	64.5 a	57.8 ab
29855	38.7 b	63.8 a
29856	62.3 a	57.3 ab

[a]One hundred seeds per sample were tested in four replicates at room temperature. Final reading was made 14 days after sowing.
[b]In a column, means followed by a common letter are not significantly different by Duncan's multiple range test at 5%.

many hosts and mostly causes leaf blight or spot on a variety of plants (Farr et al., 1989; Rotem, 1994). However, the fungus is also known as a weak and opportunistic pathogen or a saprophyte in many plants. There has been no report that the fungus causes a disease in carrot.

A. dauci and *A. radicina* cause leaf blight and black rot of carrot by seed transmission, respectively (Scott and Wenham, 1973). It was reported that *A. dauci* was found in the inner pericarp layer of carrot seeds (Netzer and Kenneth, 1969) or confined to the outer surface and tissues of dried pericarp and did not penetrate the seed coat and endosperm (Strandberg, 1983). Soteros (1979) reported that hyphae of *A. radicina* were carried in the inner pericarp layers of the seed and in the testa. In the present study, *A. dauci* and *A. radicina* were detected from the seed coat and endosperm as well as the pericarp of carrot seeds. However, it was not revealed where the *Alternaria* spp. were located in the seed coat and endosperm because the seed coat and the endosperm were fused. It needs further study with anatomical examination to clarify location of the *Alternaria* spp. in the seed coat and endosperm.

Incidence of *A. radicina* infection in the seed samples was 37.5-63.5%, whereas that of *A. dauci* was 0.5-7.5%, suggesting that the former species mainly affects germination and seedling growth of the seed samples. The tendency of difference in the infection rate between the two *Alternaria* spp. was similar to that reported by Soteros (1979). Carrot seeds infected with *A. radicina* result in germination failure or seedling blight (Grogan and Snyder, 1952). In the present study, the seed sample which was most severely infected with *A. radicina* showed the lowest rate of germination, the highest rate of seedling rot, and the lowest rate of normal seedlings in the TP test. However, seed infection of the fungus did not affect normal seedling growth of carrot in the soil test. Pryor et al. (1998) reported that *A. radicina* survived in the soil as solitary conidia or as conidia associated with organic debris. It is considered that the fungus could propagate in the soil along with growth of the infected seedlings of carrot and cause black rot at the late stage of carrot growth. Further study is needed to reveal a virulence mechanism in the soil by seed transmission of the fungus.

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