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Inhibitory Effects of *Ambrosia trifida* L. on the Development of Root Hairs and Protein Patterns of Radicles

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Abstract*—Ambrosia trifida* L. is designated as invasive alien species by the Act on the Conservation and Use of Biodiversity by the Ministry of Environment, Korea. The purpose of present paper was to investigate the inhibitory effects of aqueous extracts of *A.trifida* on the development of root hairs of *Triticum aestivum* L., and *Allium tuberosum* Rottler ex Spreng and the electrophoretic protein patterns of their radicles. The development of root hairs was inhibited by increasing of aqueous extract concentrations. Through SDS-PAGE, the electrophoretic protein bands of extracted proteins from their radicles were appeared in controls, but protein bands of specific molecular weight disappeared or weakened in treatments. In conclusion, inhibitory effects of *A. trifida* made two receptor species changed morphologically, and at the molecular level in early growth stage.

Keywords*—Ambrosia trifida* L., invasive alien species, inhibitory effect, root hair, electrophoretic protein, radicle.

I. Introduction

FARMING system as well as natural ecosystem are influenced by invasive alien species, which displace some of the native species and establish in new habitat [1]-[5]. They are introduced to a new region, and after then have potentials to outcompete native species and become extremely dominant [3]-[6]. Invasive alien plants have inhibitory ability over native plant species through releasing of secondary compound as allelochemicals into the environment [2], [7]-[11]. Their inhibitory effects revealed on seed germination, seedling growth, and root hair development of other plant species [8]-[13].

Great ragweed (*Ambrosia trifida* L., Asteraceae) is an invasive and non-indigenous plant currently, which was introduced via military materials from North America in late 1950s in Korea, where it is a serious allergenic and invasive alien plant species [3], [5], [13]-[14]. It is one of 12 invasive alien plants designated as a harmful IAS (invasive alien species) by the Act on the Conservation and Use of Biodiversity by the Ministry of Environment, Korea [13]. It has invaded and rapidly colonized in open fields including farming fields, grasslands, roadsides, and riverbeds [3], [5]. It often formed dense monotypic stands, and displaced native vegetation [3], [5].

The successful establishment of various aster-family alien plants might be due to inhibitory effects of allelochemicals on native plants [1], [8]-[10], [13], [15]. The aim of this work was to determine inhibitory potentials of *A. trifida* by assessing the effect of aqueous leaf extracts on root hair development and protein producing of radicles in early growth stage of two crops.

II. Materials and Methods

A. Donor Plant Species

Ambrosia trifida L. was used as donor plant for aqueous extracts. The leaves of *A. trifida* were collected in Goyang, Gyeonggi-do, South Korea from September to November in 2009.

B. Receptor Plant Species

Triticum aestivum L. and *Allium tuberosum* Rottler ex Spreng were used as receptor plants. Their seeds were bought from seed store, and sterilized and guaranteed in showing more than 80% germination.

C. Preparation of Aqueous Leaf Extracts

200 g of fresh leaves of *Ambrosia trifida* L., which were within 24 hours after harvesting, were put in a 2 L Erlenmeyer flask with 1 L of distilled water. The flask was incubated for 24 hours at 25 □, and after then the aqueous was filtrated through Whatman No. 40 filter paper. The filtrate was subsequently diluted to 75 %, 50 %, and 25 % with distilled water.

D. Microscopy of Root Hair Development

The seeds of *T. aestivum* L. and *A. tuberosum* were inoculated in Petri dishes with two pieces Whatman No. 40 filter papers. 10 mL of 25 %, 50 %, 75 %, and 100 % aqueous extracts form *A. trifida* exposed to seeds of receptor plants in petri dishes. While 10mL of only distilled water exposed to the controls. They were incubated at 25 □ in the dark, and at the 5th day, after selecting germinated seeds and the development of root hairs of them were observed and taken photos through light microscope (Olympus Corporation, SZX12).

E. Detection of Protein Bands

The patterns of electrophoretic protein bands were examined compared between receptor plant species affected by aqueous

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leaf extracts of *A. trifida*. 25 % and 75 % aqueous extracts, and control were determined to be used. For protein extraction from receptor plants, the radicles of two receptor species were collected for detecting patterns of electrophoretic protein bands at 5th day after germination. They were homogenized in the medicine pestle with liquid nitrogen, transfer into a 1.5 mL eppendorf tube with the extraction solution 500 µL containing 0.3 % SDS, 50 mM Tris-HCl, pH 8.0, and 20 mM DTT, and then treated with boiled water for 10 minutes at 95 ℃. The mixture was cooled for 10 min in a box with ices, and then centrifused for 40 minutes at 4 ℃ at 15,000 rpm. The supernatant was transferred into a 1.5 mL eppendorf tube and mixed with 50 % TCA (1 : 1), remained for 2 hours at -20 ℃, and then the mixture was centrifused for 15 minutes at 4 ℃ at 9,000 rpm. The pellet was mixed with acetone 1 mL, and then centrifused for 1 minute at 4℃ at 9,000 rpm. The pellet was washed with acetone three times, and then with 1.5 M Tris-HCl (pH 8.8) buffer 1 mL once. The mixture was mixed with 1 X SDS-PAGE sample buffer (50 mM Tris-HCl , pH 6.8 : 2 % SDS : 10 % Glycerol : 0.01 % bromophenol blue : 1 % β-mercaptoethanol) 15 µL, remained for 4 minutes at 100 ℃ and then used for SDS-PAGE analysis.

SDS-PAGE analysis was carried out using 12 % acrylamide resolving gels, 5 % acrylamide stacking gels according to the method of Laemmli [16]. Molecular weight marker was PRO-STAINTM(I) Prestained Protein Marker (iNtRON BIOTECHNOLOGY, Cat. No. 24051). The patterns of electrophoretic protein bands were visualized by staining the gels for 30 minutes with Coomassie Brilliant Blue G240 (Bio-Rad Co., No. 161-0406) and destained for 2 hours by shaking with the mixture of acetic acid : methanol : distilled water (3 : 10 : 2).

III. Result

The root hair development of *T. aestivum* and *A. tuberosum* exposed with different concentrations of aqueous leaf extracts of *A. trifida* was much more inhibited in comparison with control treatments (see Fig. 1 and 2). In leaf extracts, the length of root hairs of receptor plant seedlings were reduced, but in control treatment, root hairs of them were developed well and regularly arranged. The number of root hairs of receptor plants decreased proportional to the concentration of aqueous leaf extracts used.

The root hair development of *T. aestivum* was remarkably inhibited in 25 %, 50 %, 75 % and 100 % of *A. trifida* aqueous leaf extracts. The length and density of root hairs of both *T. aestivum* were dramatically reduced in 50 %, 75 % and 100 % treatments of *A. trifida* aqueous leaf extracts. Especially, root hairs of *T. aestivum* in the 75 % and 100 % treatment didn't develop at all, it revealed that the root hair development of *T. aestivum* was perfectly inhibited.

The development of root hairs of *A. tuberosum* was inhibited in 25 %, 50 %, 75 % and 100 % of *A. trifida* aqueous leaf extracts as well. But, the length and density of root hairs of *A. tuberosum* were slightly reduced in 25 % treatment of *A. trifida* aqueous leaf extracts.

The growth of root hairs of receptor plants was inhibited and number of these decreased proportional to concentration was reduced, and the elongation of radicles of those was inhibited only in higher concentration of aqueous leaf extracts. Aqueous leaf extracts of *A. trifida* have more inhibitory ability on the root hair development of *T. aestivum* than that of *A. tuberosum*.

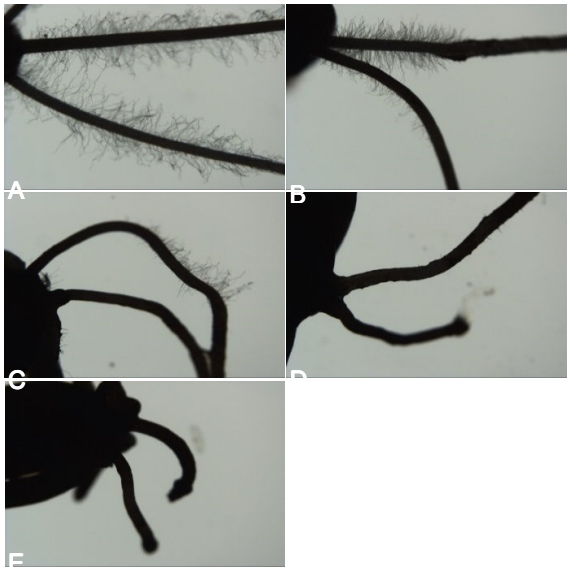

Fig. 1 The root hair development of *Triticum aestivum* by aqueous leaf extracts from *Ambrosia trifida* (A : control , B : 25 % treatment, C : 50 % treatment, D : 75 % treatment, E : 100 % treatment, x25)

Fig. 2 The root hair development of *Allium tuberosum* by aqueous leaf extracts from *Ambrosia trifida* (A : control , B : 25 % treatment, C : 50 % treatment, D : 75 % treatment, E : 100 % treatment, x25)

The gel patterns of electrophoretic protein bands obtained by SDS-PAGE from radicles of *T. aestivum* and *A. tuberosum*

exposed with *A. trifida* aqueous leaf extracts are shown in Fig. 3 and 4. Total protein banding patterns of them treated with aqueous leaf extracts differed from the controls.

For *T. aestivum* exposed with 25 % of *A. trifida* aqueous leaf extracts, electrophoretic protein band 18.5 kDa were disappeared, and 9 kDa and below 9 kDa protein bands were thinned. In 75 % treatments, 18.5 kDa and 9 kDa protein bands were disappeared and below 9 kDa protein band were thinned.

For *A. tuberosum* exposed with 25 % and 75 % *A. trifida* aqueous leaf extracts, protein banding patterns were the same as the control except 18.5 kDa protein band disappeared of both treatments, and about 19 kDa protein band thinned of 75 % treatment.

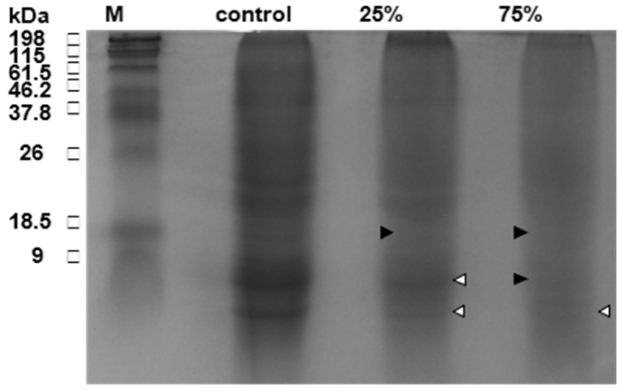

Fig. 3 Total electrophoretic protein bands in *Triticum aestivum* radices according to different concentration of aqueous leaf extract from *Ambrosia trifida* by SDS-PAGE (M : Molecular weight marker, control : distilled water treatment, 25 % : 25 % treatment , 75 % : 75 % treatment, ▸ : disappeared ◃ : weakened)

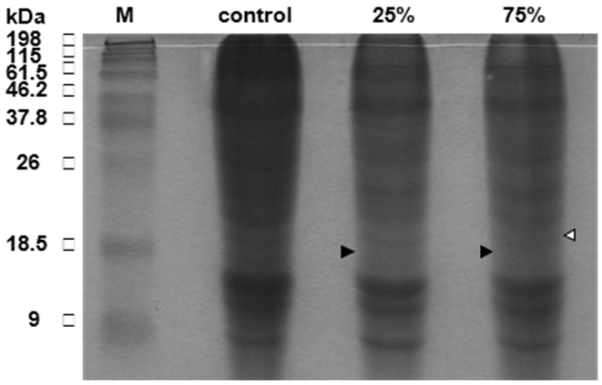

Fig. 4 Total electrophoretic protein bands in *Allium tuberosum* radices according to different concentration of aqueous leaf extract from *Ambrosia trifida* by SDS-PAGE (M : Molecular weight marker, control : distilled water treatment, 25 % : 25 % treatment , 75 % : 75 % treatment, ▸ : disappeared ◃ : weakened)

IV. Discussion

This study clearly shows that there are inhibitory effects of *A. trifida* aqueous leaf extracts on the development of root hairs and protein production of radicles in early growth stage of *T. aestivum* and *A. tuberosum*. Great ragweed (*A. trifida,* Asteraceae) is invasive and non-indigenous plant species currently invading in South Korea, and designated as an invasive alien species by the Act on the Conservation and Use of Biodiversity.

Higher concentrations of aqueous leaf extracts had a higher inhibition on root hair development, because some allelochemicals present in leaves of *A. trifida* might have caused. These results were confirmatory to previous studies conducted with the reduction in root length, and elongation of radicles by aqueous leaf extract has been reported [8]-[13].

Identifying extracted proteins from radicles of *T. aestivum* and *A. tuberosum* exposed to *A. trifida* aqueous leaf extracts through one-dimensional SDS-PAGE revealed that aqueous leaf extracts of *A. trifida* have inhibitory effect on the protein synthesis of radicles of two receptor plants. It was shown that aqueous leaf extracts from *A. trifida* have phytotoxic chemicals to inhibit root hair development and early stage growth by reducing protein synthesis of radicles. Phytotoxic chemicals of aqueous leachates have inhibitory effects on the development and growth of root hairs and root as well as the growth of seedlings [8], [17]-[19]. Some allelochemicals interrupted the protein synthesis and the mitotic activity of young cells, and caused reduction in seedling growth [9], [20]-[21]. Allelochemicals released by invasive alien plant species can help them outcompete native plants by inhibiting the development and growth of root hairs of native plant seedlings, because of inhibiting nutrient uptake of root hairs, and prohibiting establishment of them in soil [9], [19], [22].

A. trifida aqueous leaf extracts have more inhibitory effect on the root hair development of *T. aestivum* than that of *A. tuberosum*. Protein amounts and number of protein bands of radicles of *T. aestivum* decreased more than those of radicles of *A. tuberosum* by *A. trifida* aqueous leaf extracts. The aqueous leaf extracts of *A. trifida* have more inhibitory effects on root hair development as well as protein synthesis of radicles of *T. aestivum* than that of *A. tuberosum*. The secondary metabolites present in invasive alien plants causing this inhibitory ability could be used as potential natural resources to outcompete native plants.

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