SEMINAR-III

PCR-SSCP ANNALYSIS OF ASPARAGUS DECLINE PLANTS IN JAPAN

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Summary

Asparagus, *Asparagus officinalis* L. is a perennial plant with high value vegetable crop in Japan. The asparagus decline in Japan is mainly caused by the pathogen, *Fusarium* spp. But the pathogenecity and population diversity of it is unclear. In this study, SSCP (Single-Strand Conformational Polymorphism) analysis of *Fusarium* population in asparagus decline was carried out in order to estimate the existence of fusarial species from the North to South part of Japan and to determine the pathogenecity of *Fusarium* species those were isolated predominantly from the diseased plants as well as to clarify the mechanisms for asparagus decline.

Under the intensive sampling regime, 712 fusarial isolates were obtained from the fields of North to South part of Japan, which regularly showed pronounced symptoms of asparagus decline. The sampling locations were Hokkaido, Fukushima, Nagano, Kagawa and Saga. The SSCP analysis of the ITS2 gene region proved to be a rapid and objective method for identifying fusarial isolates associated with asparagus decline. Over 712 fusarial isolates were obtained from the field, the most were easily differentiated by using PCR-SSCP analysis into three principal species, *F. oxysporum* f. sp. *asparagi* (Foa), *F. proliferatum* (Fp) and *F. solani* (Fs). Among them, *F. oxysporum* f. sp. *asparagi* (Foa) was the most frequently isolated from the domestic five regions (68.4%) and then *F. proliferatum* (28.4%), whilst *F. solani* (Fs) was found very few only in the Hokkaido (5%) and Nagano regions (3.5%). In this study, individual field showed considerable intraregional variation. As determined by the SSCP profiles of the Hokkaido regions, the most dominant isolates was Foa (82.5%) and then Fp (12.5%) and Fs (5%) were detected, which were isolated partially from the single root sample. SSCP profiles of Nagano samples showed large distribution density variation between the fields and kinds of isolates. Though three kinds of fusarial isolates were isolated from the Nagano region but the most frequently obtained isolates were Foa (63.1%) and Fp (33.4%). In Fukushima, Kagawa and Saga sample, Foa and Fp were obtained. Though Foa was dominant but remarkable fusarial isolates population density variations were observed among these three regions. The fusarial isolates were highly pathogenic. The average pathogenicity scores of Hokkaido, Fukushima, Nagano, Kagawa and Saga were 5, 5, 4.49, 4.86

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and 4.92, respectively. In this study the distribution tendency of *F. proliferatum* increased gradually from the north part to south part of Japan except Saga. The ratio of Foa and Fp might be changed in the field of each region by the age of plantation and cultivar difference which were reflected in the result. On the other hand, there are possibility of environmental (soil temperature, soil moisture etc.) and soil chemical factors which might regulate the distribution density of fusarial isolates between the field of each region. However, the use of SSCP allowed to large numbers of samples to be rapidly screened for sequence variation without need for sequencing. Form the above result it was revealed the *F. oxysporum* f. sp. *asparagi* (Foa), *F. proliferatum* (Fp) and *F. solani* (Fs) were the most important cause of asparagus decline in Japan.