

Diversity of VC and incidence of hypovirulence-associated ds-RNAs in the chestnut blight fungus *Cryphonectria parasitica* in Korea

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Chestnut blight, caused by *Cryphonectria parasitica*, is the most destructive disease of American and European chestnut trees. A total of 672 *C. parasitica* was isolated from blight lesion on chestnut twigs, which were collected from major chestnut plantations all over Korea in 1999. Isolation rates were over 30% in Kyunggi-, Kyongnam-, and Chonnam-do. The highest isolation rate was 37.4% and recorded in Kyongnam-do. On the other hand, Chonbuk-do had the lowest isolation rate as 13.5%. In grouping of *C. parasitica* by colony shape and color, yellow colony with irregular margin were the most dominant colony type with a frequency of 65.2%. When the 672 isolates were inoculated on the chestnut twigs, 380 isolates (56.5%) caused lesions larger than the standard virulent isolate EP155-2, while 158 isolates (23.4%) caused smaller lesions than the standard hypovirulent isolate UEP-1. In Bavendamm test that determines phenol oxidase activity, 87.1% of all the isolates resulted the same or darker discoloration than EP155-2, and only 12.2% resulted the same or lighter discoloration than UEP-1. In the vegetative compatibility (VC) tests, total 670 isolates were divided into 121 VC groups (VCGs). Kyongnam-, Chonnam-, and Chungnam-do, the three principal chestnut plantation area, had 49, 33, and 27 VCGs, respectively. Among the VCGs, the biggest VCG, KR-VC104, was composed of 164 isolates and the second biggest VCG had 62 isolates. But, 64 of 121 VCGs consisted of sole member. More than 65.8% of KR-VC104, was isolated from the three provinces, Kyongnam-, Kangwon-, and Chungbuk-do. In KR-VC104, 62.8%, 59.1%, and 85.9% of the isolates looked like virulent in colony type, pathogenicity test, and Bavendamm test. In ds-RNA detection tests using cellulose chromatography, 77 of total 650 isolates were ds-RNA positive and detected ds-RNA segments were approximately 12kb, 3kb, 2.7kb, 2kb, and 1.8kb in size. Among the 77 isolates, 46 isolates had 12kb and 25 isolates had 12kb and 2.7kb. Other 6 isolates had small ds-RNA segments. Kyongnam-, Chonnam-, and Chungnam-do had 43, 16, and 5 ds-RNA positive isolates, respectively. Among the 121 VCGs, only 29 VCGs had ds-RNA positive isolates.

Over 32% of KR-VC104 isolates were the ds-RNA positive isolates. Ds-RNA was detected 31.5%, 11.3%, 52.3% from hypovirulent-like isolates in colony type, pathogenicity test, and Bavendamm test. On the other hand, in virulent-like isolate, ds-RNA detection rate was 1.7%, 11.6%, 5.4%. In ITS-RFLP tests of ds-RNA positive isolates, 4 of total 75 isolates revealed different band pattern from other isolates. Furthermore, 4 isolates had small ds-RNA segments. In RT-PCR of 13 ds-RNA positive isolates that collected from different localities, RT-PCR products for two different regions of viral genome (5 non-coding region and ORF B) were obtained using primer sequences of ds-RNA CHV-1 which was isolated from EP713, the hypovirulent type-species. In the comparison of RT-PCR product sequences with those of other countries, some Korean isolates were closely related to foreign isolates in phylogenetic analysis of 5 non-coding region

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