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SUMMARY OF DOCTORAL THESIS

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Title: **Pathogenic variation, toxicity, and molecular characterization of *Fusarium* species isolated from wilted Welsh onion in Japan**

(日本 国内の萎ちょうネギから分離されたフザリウム属菌の病原性バリエーション、毒素生産、および分子生物学性質)

Welsh onion, also known as Japanese bunching onion (*Allium fistulosum* L.), is one of the commonly cultivated vegetables in East Asia, including Japan, China, and Korea. Fusarium wilt, also known as Fusarium basal rot, of Welsh onion was firstly reported in Japan in 1977 and the causal pathogen was identified as *Fusarium oxysporum* f. sp. *cepae*. Fusarium wilt of Welsh onion has not been considered as an important disease in the country. However during the summer of 2006 and 2007 wilted Welsh onion plants were frequently found in large number of fields in the major Welsh onion growing areas of Japan. Thus, we needed a method to identify the pathogens that cause Fusarium wilt-like symptoms in Welsh onion.

Fifty-one isolates of *Fusarium* species were obtained from wilted Welsh onion and identified as *F. oxysporum* (30 isolates), *F. proliferatum* (7 isolates), and *F. solani* (14 isolates). The pathogenenicity of 51 isolates was tested on five Welsh onion lines using seedling assay in a green house. Although *Fusarium* isolates analyzed in this study showed varied degrees of disease severity to cultivars tested, *F. oxysporum* isolates were more pathogenic on Welsh onion cultivars than the isolates of other two *Fusarium* species, suggesting that *F. oxysporum* is the major pathogen causing the severe wilt in Welsh onion. Eight *F. oxysporum* isolates (08, 15, 17, 22, 30, 37, 41 and 45) had a higher virulence potential to the tested cultivars than the other isolates.

The pathogenecity assays showed different pattern in disease severity for Welsh onion cultivars, suggesting the presence of various populations of *F. oxysporum* with different virulence. Genetic relationships among the *F. oxysporum* isolates were analyzed using partial DNA sequencing of the ribosomal DNA (rDNA) intergenic spacer (IGS) region. The partial sequence of IGS revealed four groups (clades A-D) with considerable correlation between phylogeny and pathogenicity. These results suggest that *F, oxysporum* isolates from wilted Welsh onion are

polyphylogenetic. The low-virulence isolates were clearly separated into two groups (subgroup C2 and clade D) except for isolate 07, which was grouped in clade A. All moderate-virulence isolates were grouped into two separate clades (A and B) with high-virulence isolates, suggesting that some of high- and moderate-virulence isolates may share the similar genetic background. These results suggest that low-virulence isolates can phylogenetically be differentiated from high- and moderate-virulence ones. Mating type analysis revealed that all isolates were *MAT1-1* idiomorph suggesting that there is no sexual recombination within the population.

The association of *F. proliferatum* with Welsh onion plants and seeds may be a serious health risk as a toxigenic species and as well as potentially serious pathogen of *Allium* plants including bulb onion and garlic worldwide. Fumonisin B_1 (FB_1) levels in 20 *F. proliferatum* isolates obtained from Welsh onion plants and seeds of seven commercial cultivars were determined by high-performance liquid chromatography (HPLC). 65% of the *F. proliferatum* isolates from Welsh onion analyzed in this study produced FB_1 in variable levels, suggesting that both FB_1-producing and nonproducing isolates of *F. proliferatum* are associated with Welsh onion.

The identity of the isolates was confirmed by PCR amplification with species-specific primers and sequence analysis of mitochondrial small subunit (mtSSU) of rDNA. All isolates analyzed showed more than 96% sequence identity with that of *F. proliferatum*. Results of PCR analysis using primer pairs, VERT-1/VERT2 and PRO1/PRO2, were inconsistent with morphological identification and sequence analysis of isolates, demonstrating that the species-specific primers can lead to the misidentification of *F. verticillioides* and *F. proliferatum*. Therefore, care should be taken when identifying these two species by using species specific PCR amplification.

PCR assay with *FUM1* gene-specific primers was performed to discriminate fumonisin-producing strains from nonfumonisin-producing. Thirteen out of 20 isolates of *F. proliferatum* produced FB_1. PCR assay amplified a single DNA fragment of the *FUM1* gene only from the 13 isolates. The present study revealed that *F. proliferatum* producing FB_1 is associated with Welsh onion plants and that commercial cultivar seeds of Welsh onion may be contaminated with the fungi. The PCR method used in the present study could be a useful tool for rapid identification of FB_1-producing *F. proliferatum*.