【別紙２】

論文名：A study on the plant - pathogen interaction between *Brassica oleracea* and *Fusarium oxysporum* f. sp. *conglutinans*（要約）

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（以下要約を記入する）

Plant disease seriously affects the yield and quality of agricultural production. Understanding of pathogen and plant interaction is very important for disease control as well as resistant cultivar breeding. Although such interactions have been demonstrated in many plant-pathogen systems, in *Brassica oleracea* - *Fusarium oxysporum* f. sp. *conglutinans* (Foc) system, to date, few have been known. However, with the increased production of *B. oleracea*, especially in the temperate climates and during warm seasons, Foc infection has become a serious problem. Thus, for further understanding the *Brassica* crops-Foc interaction and controlling Foc in production fields, we conducted (1) identify Foc resistance gene in *B. oleracea* and (2) investigate molecular interactions between Foc and *B. oleracea*.

In chapter 1 and 2, we focused on identification and isolation of Foc-resistant gene in *B. oleracea*. A cross between a susceptible broccoli 'GC' DH line (P04) and a resistant cabbage 'Anju' DH line (P01) was subjected to segregation analysis to characterize the inheritance pattern of fusarium resistance. Foc strain Cong: 1-1 was used as inoculum. The results indicated that resistance was controlled by a single dominant gene. This gene was named *Foc-Bo1* ('Anju' (P01) type) and mapped to linkage group seven (C7) by both the segregation linkage analysis and quantitative trait locus (QTL) analysis. The QTL on C7 was detected with a logarithm of odds (LOD) score of 19.5, which was above the threshold value with genome-wide 1% significance level (2.01). A minor QTL was also detected on C4 with a LOD score of 2.06. Inoculation tests indicated that stable expression of fusarium resistance at high temperatures required *Foc-Bo1* homozygosity. The association between *Foc-Bo1* and the closest simple sequence repeat (SSR) marker (KBrS003O1N10) was analyzed in three F_3 populations. Based on these studies, KBrS003O1N10 represents an effective marker-assisted selection (MAS) tool for breeding fusarium wilt resistance into *B.*

oleracea crops. A cosmid library was constructed and was expected to cover 4-fold of *B. oleracea* genome. Screening using *Foc-Bo1* flanking markers successfully isolated some cosmid clones spanning the *Foc-Bo1* region, indicating the library might cover the whole region of *Foc-Bo1*. One positive clone, pWE#27A7, selected by *Foc-Bo1* closet marker was further used for primer walking sequence method, which directly contributed to disclosing of the gene structure of *Foc-Bo1*.

In chapter 3, we adopted a proteome analysis using xylem sap proteins and focused on the identification of Foc-effectors as well as the plant response against Foc-infection. Commercial cabbage cultivars of YCR-Rinen (Foc-resistant, Nippon Norin Seed Co., Japan) and Delicious (Foc-susceptible, Watanabe Seed Co., Japan) were used for proteome analysis. Xylem sap proteins were precipitated by acetone precipitation and digested by in-solution digestion. After liquid chromatography -tandem mass spectrometry (LC-MS/MS) analysis, proteins were identified by blasting in the database containing plant protein sequences downloaded from Uniprot website and Foc protein sequences predicted from whole genome sequence information of Cong: 1-1. Over 200 hundred proteins were identified in the samples of non-infected samples and Foc-infected Delicious, while 158 proteins were detected in Foc-infected YCR-Rinen. Most of the plant proteins identified in xylem sap were small proteins with a signal peptide denoting secretion. Comparison between the non-infected and Foc-infected samples and taking proteins with fold change >=2 for analysis, the results revealed that Foc infection caused changes in the xylem sap protein composition in *B. oleracea* where repressed proteins accounted for a greater proportion than those of induced in both the susceptible and resistant reactions. Twenty-five Foc proteins were detected in the infected susceptible plant. Eleven of them were estimated having a signal peptide with amino acids less than 300, and ten of them contain cysteine(s). Given the reported fungal effectors are always small secreted cysteine-containing proteins, the ten identified Foc proteins are highly considered as candidate effector proteins.

Overall, our study provides valuable insights into *B. oleracea*-Foc interactions and will contribute to understanding the resistance mechanisms as well as resistant cultivar breeding in *B. oleracea* against Foc.