| | |
|---|---|
| **氏　　名** | ANNISA' AULIA |
| **授与した学位** | 博　士 |
| **専攻分野の名称** | 農　学 |
| **学位授与番号** | 博甲第　　６２０８　　号 |
| **学位授与の日付** | ２０２０年　３月２５日 |
| **学位授与の要件** | 環境生命科学研究科　　農生命科学専攻<br>（学位規則第４条第１項該当） |
| **学位論文の題目** | Interactions between two naturally co-infecting mycoviruses in the chestnut blight fungus *Cryphonectria parasitica*<br>（クリ胴枯病菌に自然共感染する二種のウイルスの相互作用） |
| **論文審査委員** | 教授　　鈴木　信弘　　教授　　ガリス　イバン　　准教授　近藤　秀樹 |

## 学位論文内容の要旨


Mixed infections in single isolates of filamentous phytopathogenic fungi by multiple viruses are frequently detected. Such co-infections allow heterologous viruses to interact with each other in either a synergistic or antagonistic way. A comprehensive understanding of such virus/virus interactions would enable us to design environment-friendly management strategies against fungal plant pathogens exploiting fungal viruses as biological control (virocontrol) agents. *Cryphonectria parasitica* is an important fungal pathogen of the blight disease in chestnut. A US field isolate of *C. parasitica*, strain C18, has previously been shown to be infected with a 11-segmented double-stranded RNA virus, mycoreovirus 2 (MyRV2, family *Reoviridae*). Here, I report the molecular and biological characterization of a newly detected positive-sense (+) single-stranded RNA (ssRNA) virus Cryphonectria hypovirus 4 (CHV4-C18, family *Hypoviridae*) in C18, and describe an interesting commensal interplay between MyRV2 and CHV4-C18 in *C. parasitica*. In this novel interplay, CHV4-C18 facilitates stable infection by and efficient vertical transmission of MyRV2.

Molecular analyses showed CHV4-C18 to have a single open reading frame, sharing 99.4% sequence identity at both nucleotide and amino acid levels to a previously characterized exemplar strain, CHV4-SR2, of the species *Cryphonectria hypovirus 4*. Single infection by MyRV2 and double infection by CHV4-C18 + MyRV2 similarly resulted in reduced hyphal growth and greater orange pigmentation. A difference between the single and double infections was the infection stability and transmission efficiency of MyRV2. MyRV2 was maintained more stably inside host during subculturing, and transmitted more efficiently via asexual spores when accompanied by CHV4-C18. It was assumed that host antiviral RNA silencing was responsible for this phenomenon, as CHV4-C18 suppressed the transcriptional induction of one of the RNA silencing key genes, dicer-like 2 (*dcl2*), which was otherwise highly upregulated in fungal strains singly infected by MyRV2. To test this hypothesis, RNA silencing-deficient fungal strains with the C18 genetic background (C18 Δ*dcl2*) were prepared by targeted disruption of *dcl2*. Comparative analyses with C18 wild-type WT and C18 Δ*dcl2* strains showed that the deletion of *dcl2* resulted in enhanced stability of MyRV2 during subculturing and increased its vertical transmission via sporulation. These results suggest that CHV4-C18 facilitates the stable infection of MyRV2 in the C18 genetic background by suppressing the host antiviral RNA silencing pathway. Furthermore, I could identify the papain-like protease, p24, of CHV4-C18 as an RNA silencing suppressor using a newly developed green fluorescent protein-based reporter system.

These combined results unveiled a previously unsuspected interesting virus/virus interaction, where the stable infection and efficient vertical transmission of a dsRNA reovirus, MyRV2, were facilitated by a co-infecting (+)ssRNA hypovirus, CHV4-C18, which appears to impair (by using its p24 protein) host antiviral RNA silencing through suppressing the induction of *dcl2*. Indeed, this study will provide a platform for exploring the molecular mechanism by which RNA silencing is suppressed in filamentous fungi by virus-encoded silencing suppressors.

## 論文審査結果の要旨

自然界では，１つの宿主個体が複数のウイルスに共感染していることが観察される。菌類での混合感染は，特に高頻度に観察される。共感染個体では，多様なウイルス間相互作用が考えられ，それはウイルスを利用した植物病原菌の生物防除（ヴァイロコントロール）の成否を決定する重要な因子となる。本研究では，世界３大樹病の病原・クリ胴枯病菌で繰り広げられる全く異なる２種のウイルス，すなわちマイコレオウイルス２(MyRV2)とハイポウイルス４（CHV4），の相互作用の特徴付けとその相互作用を制御している機構の解明に取り組んだ。まず，CHV4が無病徴感染すること，さらに，共感染するMyRV2の安定維持，効率的胞子伝搬に寄与することを明らかにした。一方，MyRV2は宿主菌の生育低下，クリへの病原性の低下をもたらすが，CHV4への影響がないことが示された。すなわち，CHV4は安定的に，C18菌株で維持されるが，MyRV2の方はCHV4無しでは，宿主から除去されてしまう。この現象を規定している宿主側因子（抗ウイルスRNAサイレンシング），ウイルス側の因子（RNAサイレンシング抑制タンパク質，p24）を同定した。本成果は，新規ウイルス間相互作用とそれを規定する因子を明らかにしたもので，独創性が高い。

Aulia女史は，博士号に価する上記のように十分な研究成果を挙げ，またその過程で十分な研鑽を積んだことを学位論文審査員として認める。