

博士論文要約 (Summary)

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連合農学研究科

専攻 Course : 農水圏資源環境科学

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タイトル Title	A Study on Genomic Insights into the Dynamics of Amaryllidaceae Family and Their Viruses Collected from All Over Japan
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キーワード Key word (genomic study) (Narcissus viruses) (Allium macrostemon Bunge)

The Japanese *Narcissus* plant (in Japanese call Nihon-zuisen, *Narcissus tazetta* var. *chinensis*) and *Allium macrostemon* Bunge (wild Japanese garlic or no-biru) which belong to the family Amaryllidaceae are well known and widely distributed in Japan. Studies of the genomic of these plants and the viruses that infect them are important for understanding evolutionary analysis, virus-virus interactions, and genetic diversity. Consequently, these investigations will contribute to developing efficient and durable control strategies of the viruses. Furthermore, a better understanding of plant genetic resource could provide the effort of conservation of the plant.

Section 1

Plants of the genus *Narcissus* in the family Amaryllidaceae are known as wild or domesticated plants. Narcissus late season yellows virus (NLSYV) and narcissus degeneration virus (NDV) are major viruses of *Narcissus* plants, causing serious disease outbreaks in Japan. However, the detailed of co-infections of *Narcissus* viruses and the distributed in Japan is still largely unknown. This study focused on the co-infections and evolutionary analyses of *Narcissus* viruses from wild and domesticated *Narcissus* plants in Japan.

Narcissus plant leaves showing mosaic or chlorotic stripe and asymptomatic plants were collected during the winter and spring seasons of 2009–2015 in Japan. Of the 189 plants, 120 (64%) were infected with NLSYV, NDV, narcissus yellow stripe virus (NYSV),

and other *Narcissus* viruses of the genus *Potyvirus* and *Macluravirus* in the family *Potyviridae*. Co- and single infections of *Narcissus* viruses were identified using potyvirus-specific primers. Co-infection with different isolates and quasispecies of the same virus were also found by analyzed nucleotide sequences.

Phylogenetic network was analyzed using NLSYV and NDV coat protein (CP) coding sequences. NLSYV CP coding sequences fell into at least five major clades in the phylogenetic network, whereas the NDV CP coding sequences fell into two major clades. The genomes of NLSYV isolates share nucleotide identities of around 82%, whereas those of NDV isolates are around 94%. Recombination analyzed using polyprotein coding sequences of NLSYV from Japan, Australia, and China. Three recombination sites were identified in the NLSYV genomes: first located in the helper component-proteinase (HC-Pro) coding region, second located in the nuclear inclusion-b (NIb) coding region, and third located in the CP coding region. However, none of the recombination sites were found in NDV genomes.

The spatial diffusion of NLSYV and NDV isolates was analyzed using the polyprotein coding region. The inferred distribution areas of NLSYV and NDV seem different in terms of the range of statistical uncertainty, even though NLSYV and NDV were often found in the same host plant as co-infections. The distributed area of statistical uncertainty for NLSYV is broader, whereas that of NDV is more limited, although both exist in the southwest parts of Japan. This is possibly due to the different periods of introduction into Japan for NLSYV and NDV. NDV might have been more recently introduced into Japan than NLSYV, considering the difference of expanding area and nucleotide diversities of the two viruses collected in Japan. The wild and domesticated *Narcissus* plants in Japan were suggested described co-infections of potyviruses. My findings are essential to understand the co-infections of potyviruses in nature in order to construct the effective strategies to control the viral diseases.

Section 2

Wild Japanese garlic (*Allium macrostemon* Bunge), colloquially known as "no-biru" in Japan, is a ubiquitous wild onion species found throughout the country. Its significance is rooted in ancient Japanese culture, with numerous references in classical literature. In contrast to its culinary use in China and Korea, it remains a neglected crop in Japan. Unraveling the origins of its domestic populations and comprehending their genetic makeup is key to illuminating the plant's historical role in Japan. This study aims to fill this knowledge gap by analyzing 47 *A. macrostemon* samples from diverse Japanese regions, evaluating their genetic diversity through restriction site-associated DNA sequencing (RAD-Seq).

My analysis distinguished unique population structures, dividing the samples into three distinct groups: A, B, and C. Notably, Groups A and B showed clear evidence of bulb propagation, while Group C diverged from this pattern, forming four subgroups C1, C2, C3, and C4. Hybridization between subgroup C1 and either Group A, B, or both, gave rise to subgroups C2, C3, and C4. Thus, Groups A, B, and C1 are posited as the ancestral populations. Additionally, morphological observations indicated distinct differences among these three groups. My findings also suggest that human migration may have influenced the plant's distribution, hinting at active usage in the past that later waned, causing its current underutilized status. In the future, it could improve its desirable traits, such as bulb and leaf quality for culinary purposes, potentially catalyzing a resurgence in its use as a food source in Japan.

The distribution and evolution of *Narcissus* viruses of NLSYV and NDV which appeared major viruses in Japan through evolutionary process during the time. Evolutionary investigation of co-infection revealed that NLSYV is widely distributed whereas the distribution of NDV is limited to the southwest part of Japan. The data of co-infection of *Narcissus* viruses and genetic diversity of *A. macrostemon* suggested that those

species evolved in response to environmental conditions. Both *Narcissus* viruses and *A. macrostemon* are adapted to face the risk of extinction by an evolutionary process. *Narcissus* viruses adapt with host and vector, viral community interactions and abiotic condition in the environment. Meanwhile, *A. macrostemon* adapt from the origin population, migrate to other regions, and being cultivated by human.