論 文 の 内 容 の 要 旨

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論文題名　Studies on Bymovirus Infectivity and Host Resistance
（*Bymovirus* 属ウイルスの感染性と宿主植物の抵抗性に関する研究）

The genus *Bymovirus* in the family *Potyviridae* consists of viruses with a bipartite plus-sense RNA genome. Two bymoviruses *Barley yellow mosaic virus* (BaYMV) and *Wheat yellow mosaic virus* (WYMV) are agriculturally important pathogens causing yellow mosaic diseases of barley and wheat, respectively, with significant yield losses worldwide. BaYMV occurs in East Asia and Europe and WYMV is found in Japan and China. Breeding of resistant cultivars is the only practical and ecological approach to prevent bymovirus diseases. There have been eighteen resistance genes reported for BaYMV and at least three resistance genes for WYMV but the resistance mechanisms are still poorly understood.

In barley, the *rym5* resistance gene encodes a eukaryotic translation initiation factor 4E (eIF4E) on chromosome 3HL and was utilized for breeding resistant cultivars. However, BaYMV pathogenic strains breaking *rym5* resistance have already emerged in fields.

Bymoviruses have restricted host ranges and each species can infect only one type of host; BaYMV infects only barley and WYMV infects only wheat in fields. So far, the host restriction mechanisms of BaYMV and WYMV are unknown. The distinct host ranges of BaYMV and WYMV could also be due to host resistance and susceptibility.

I studied (1) a BaYMV viral determinant for breaking *rym5* resistance, (2) the mechanism for breaking the eIF4E/*rym5*–mediated resistance, and (3) the association of bymoviral VPg with host eIF4E in the host tropism at the cellular level. All the experiments were carried out using infectious *in vitro* transcripts from cDNA clones with isolated protoplasts or intact plants as the hosts.

1 Analysis of a BaYMV Tochigi isolate JT10 and the host resistance

1.1 Genome characterization

A BaYMV isolate JT10 was obtained from *rym5*-carrying cv. Misato Golden leaves in Tochigi. At first the full-length nucleotide sequences of RNA1 and RNA2 were determined. RNA1 was 7,642 nucleotides and RNA2 was 3,585 nucleotides in length, excluding the poly(A) tail at the 3' end. Sequence comparisons with the previously characterized BaYMV-III isolate, another *rym5*-breaking isolate from a different field in Tochigi, showed that isolate JT10 is distinct from isolate BaYMV-III with 1.7% nucleotide differences in RNA1 and 3.0% in RNA2, although both isolates infected *rym5* cultivars and were isolated in different fields in Tochigi Prefecture in different years.

1.2 Infectivity assay at the cellular and whole plant levels using infectious *in vitro* transcripts

Based on the whole genome sequence information, full-length cDNA clones were constructed and designated as pBY-T1 and pBY-T2 for RNA1 and RNA2 of Tochigi isolate JT10, respectively. Inoculating *rym5*-carrying barley plants (cv. Misato Golden) with the *in vitro* transcripts from pBY1-T1 and pBY-T2 resulted in development of yellow mosaic leaf symptoms. Progeny virus genome was examined by PCR/sequencing analysis and it was confirmed that BY-T1 RNA1 + BY-T2 RNA2 were systemically infectious to the *rym5* cultivar.

Next, infectivity of BY-T1 RNA1 + BY-T2 RNA2 to twelve barley varieties was examined at the cellular and whole plant levels. Ryofu and KoA were used as susceptible controls. Express (*rym4*), Mikamo Golden (*rym5*), Misato Golden (*rym5*), Haruna Nijo (*rym6*), Amagi Nijo (*rym6*) and Miho Golden (*rym6*) were systemically infected with BY-T1 RNA1 + BY-T2 RNA2. Ishuku Shirazu (*rym3*) and Haganemugi (*rym3*) supported RNA replication at the cellular level but were not systemically infected, indicating that the *rym3* varieties were resistant at the movement level. Nittakei 68 (*rym1*) showed limited RNA replication at the cellular level and did not develop systemic infection. Mihori Hadaka 3 (*rym2*) did not support RNA replication at the cellular level at all.

1.3 Viral genome mapping analysis and amino acids identification of VPg for breaking *rym5* resistance

The virus factor involved in breaking *rym5* by the Tochigi isolate JT10 was determined. Previous study showed that Kurashiki isolate JK05 (cDNA clones pBY-K1 for RNA1 and pBY-K2 for RNA2) was non-infectious to *rym5* cells. RNA1 replicates autonomously. Therefore, cDNA fragments were replaced in pBY-K1 with those of pBY-T1 to construct chimeric cDNA clones. Protoplasts prepared from *rym5* Misato Golden leaves were transfected with *in vitro* transcripts from chimeric cDNA clones. The result

showed that only chimeric RNA1s containing the JT10 VPg (T-VPg) gene replicated in *rym5* cells. Further, systemic infection in *rym5* plants with BY-K1/T-VPg RNA1 + BY-K2 RNA2 were examined. Progeny viral RNA sequencing results confirmed no additional common spontaneous mutations outside of the T-VPg gene. Thus, T-VPg was identified as a determinant for breaking *rym5* at the whole plant level.

Alignment of amino acid sequences of K-VPg (VPg of Kurashiki isolate JK05) and T-VPg showed that there are six amino acid differences out of 187 amino acids; Tyr in K-VPg and Phe in T-VPg at the position 73; Asp in K-VPg and Glu in T-VPg at the position 85; Thr in K-VPg and Ser in T-VPg at the position 118; Lys in K-VPg and Thr in T-VPg at the position 120; Tyr in K-VPg and His in T-VPg at the position 142; Ala in K-VPg and Val in T-VPg at the position 175.

To precisely identify the key amino acid(s) of VPg for breaking *rym5*, six T-VPg mutants were constructed, in which a single amino acid was substituted with that in K-VPg such as T-VPg_F73Y, T-VPg_E85D, T-VPg_S118T, T-VPg_T120K, T-VPg_H142Y and T-VPg_V175A. Infectivity of each of these VPg mutants was examined in *rym5* cells. The results showed that VPg mutations of F73Y, E85D and V175A negatively affected virus replication (less than 40% was lost) of T-VPg, mutation of T120K sharply reduced virus replication (more than 50% was lost) and mutations of S118T and H142Y were lethal to T-VPg in *rym5* cells, indicating that serine at the position 118 and histidine at the position 142 of VPg were mostly important for virus replication in *rym5* cells. However, converse pBY-K1-derivative mutant of VPg_T118S/Y142H was not able to replicate in *rym5* cells, suggesting that more amino acid mutation(s) may be required for replication of Kurashiki isolate JK05 in *rym5* cells. Later, the pBY-K1-derivative mutant of VPg_T118S/K120T/Y142H containing three amino acid mutations could replicate in *rym5* cells but failed to cause systemic infection to *rym5* plants, indicating that threonine at the position 120 is required for replication and triple VPg mutations at the positions118, 120 and 142 are insufficient for systemic infection to *rym5* plants.

1.4 Expression of susceptible barley eIF4E in *rym5* protoplasts

Previously the *rym5* resistance gene has been reported to code for eIF4E. To testify whether *rym5* resistance is conferred by eIF4E, an eIF4E gene of a susceptible variety KoA (KoA_eIF4E) was introduced into pBY-K2 cDNA clone. Co-expression of the susceptible KoA_eIF4E enabled BY-K1 replication in *rym5* cells, indicating that the resistance by *rym5* is indeed conferred by host eIF4E. However, Kurashiki isolate JK05 could not break *rym5* at the whole plant level even if KoA_eIF4E was co-expressed. Therefore, there must be other resistance factor(s) affecting virus movement in *rym5* plants.

This result shows that host eIF4E functions in bymovirus replication.

1.5 Viral genome mapping analysis to break *rym2* resistance

rym2 cells supported replication of the Kurashiki isolate JK05 but not of the Tochigi isolate JT10. To identify the virus factor responsible for breaking *rym2* at the cellular level, mapping analysis between these two isolates was also done in *rym2* cells. The C-terminal region of P3 of Kurashiki isolate JK05 was identified as a determinant for replication in *rym2* cells, also suggesting a role of P3 in BaYMV replication.

2 Association of VPg and eIF4E in the host tropism of BaYMV and WYMV at the cellular level

The infectious full-length cDNA clones of WYMV RNA1 and RNA2 were constructed and designated as pWY1 and pWY2, respectively. BaYMV and WYMV replication in barley and wheat mesophyll protoplasts was first examined. The results showed that BaYMV replicated in both barley and wheat cells, but WYMV replicated only in wheat cells not in barley cells. Next, the role of eIF4E in determining the host tropism of BaYMV and WYMV at the cellular level was examined. Two derivative mutant pBY-K2.P1/barley_eIF4E and pWY2.P1/wheat_eIF4E modified from pBY-K2 and pWY2 were constructed, in which viral P2 genes were replaced with barley and wheat eIF4E genes, so that barley and wheat eIF4E genes could be expressed in wheat and barley protoplasts, respectively. Co-expression of wheat eIF4E enabled WYMV replication in barley protoplasts, and co-expression of barley eIF4E increased BaYMV replication in wheat protoplasts, suggesting that eIF4E is an important factor that determines the host tropism of BaYMV and WYMV at the cellular level. The role of VPg was also examined. Replacing the BaYMV VPg gene with that of WYMV abolished BaYMV replication in barley protoplasts, whereas the additional expression of wheat eIF4E from BaYMV genome restored the replication of the BaYMV mutant in barley protoplasts. These results indicate that both VPg and the host eIF4E are involved in the host tropism of BaYMV and WYMV at the replication level.

In this study, I experimentally proved for the first time that (1) BaYMV VPg is the bymovirus factor for breaking eIF4E–mediated resistance in barley, (2) the host eIF4E is functional in bymoviral RNA replication, and (3) VPg/eIF4E association plays an important role in the host tropism at the cellular level. Furthermore, bymoviral P3 was shown to function in RNA replication and involved in *rym2* resistance. This study broadens our understanding in breaking host resistance against bymovirus infection and sets a useful model for understanding other resistance mechanisms.