Science and Technology Group
Annual Report FY2017

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1 Introduction

Sexual reproduction transmits genetic information to the next generation and increases genetic diversity among offspring. Successful sexual reproduction in flowering plants depends on accurate germ cell differentiation. Molecular mechanisms of germ cell development during pre-meiotic stages remain unknown in plants.

We have identified over 700 types of long intergenic non-coding RNAs (lincRNAs), expressed during specific rice reproductive stages in which germ cell differentiation occurs. Furthermore, lincRNAs that contain consensus sequences complementary to microRNA 2118 (miR2118) are cleaved in the miR2118 site. Cleaved lincRNAs are processed via DICER-LIKE4 (DCL4) protein, resulting in production of 21-nucleotide (nt) small RNAs (Figure 1, Komiya *et al.*, 2014).

The study of non-coding RNAs (ncRNAs) is currently an active research topic in biology. More than 90% of the genomes of higher organisms are comprised of intergenic regions. Endogenous ncRNAs play diverse and important roles at various developmental stages in many organisms. However, most ncRNA functions and those of molecules that interact with them remain unknown in plants.

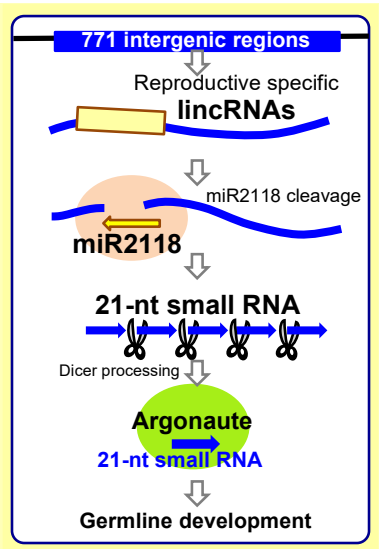

Fig1. Biogenesis of small RNAs derived from lincRNAs.

To reveal reproductive roles of ncRNAs in plants, I am engaged in two main projects using rice:

1. Determining the roles of over 700 lincRNAs, miR2118 and 21-nt small RNAs in early reproduction

2. Chromatin regulation between germ cells and somatic cells

By integrating **1** and **2**, my goal is to construct an RNA/chromatin network model in germ cells to better understand plant reproductive systems. In FY2017, I focused on project 1 in relation to reproduction-specific ncRNAs.

2 Activities and Findings

2.1 Diversification of reproductive specific lincRNAs

Biogenesis of small RNAs triggered by miR2118 is conserved in monocots, dicots, and gymnosperms. However, precursors of small RNAs differ between monocots and dicots. Unlike monocots, in dicots most precursors are derived not from lincRNAs, but from leucine-rich repeat (NB-LRR) family genes, coding-genes that serve as pathogen-defense genes. These results reveal diversification and adaptation of small RNA pathways, which include miR2118 triggers and DCL processing in land plants (Zhai *et al.*, 2011; Komiya *et al.*, 2017).

In FY2017, to reveal the homology of reproductive lincRNAs in the family Poaceae, we searched ortholog lincRNAs and compared their sequences using genomes of 7 species in the Poaceae. We identified 52 lincRNAs in *Brachypodium*, 64 in maize, 132 in barley, 82 in wheat, and 191 in sorghum, all of which have high homology to rice lincRNAs. However, only 6 lincRNAs are conserved in all 7 species; Komiya *et al.*, unpublished data).

Next, a phylogenetic tree was constructed to detect subgroups with high homology among rice lincRNAs. This analysis showed that there is no global relationship among rice lincRNAs (Komiya *et al.*, unpublished data). That is, most rice reproductive lincRNAs are unique sequences. Furthermore, lincRNA sequences within the Poaceae display low homology, even though 21-nt small RNA pathways including miR2118-trigger and DCL processing, are conserved.

2.2 Roles of microRNA2118 (miR2118) in rice reproduction

In the rice genome, there are 18 members of the miR2118 family. These trigger biogenesis of 21-nt small RNAs, and they are expressed specifically during early meiosis (Figure 2A). To reveal the function of miR2118 family members, we produced mutants with 14 of the 18 miR2118s deleted by gene targeting. In these mutants, miR2118 expression is decreased and most lincRNAs are increased. Furthermore, these miR2118 mutants show low fertility (Figure 2B). Their anthers were tiny (Figure 2C) and anther wall development was especially decayed or hypertrophied (Komiya *et al* unpublished data). These results suggest that miR2118s are required for early reproduction, especially for anther wall development in rice.

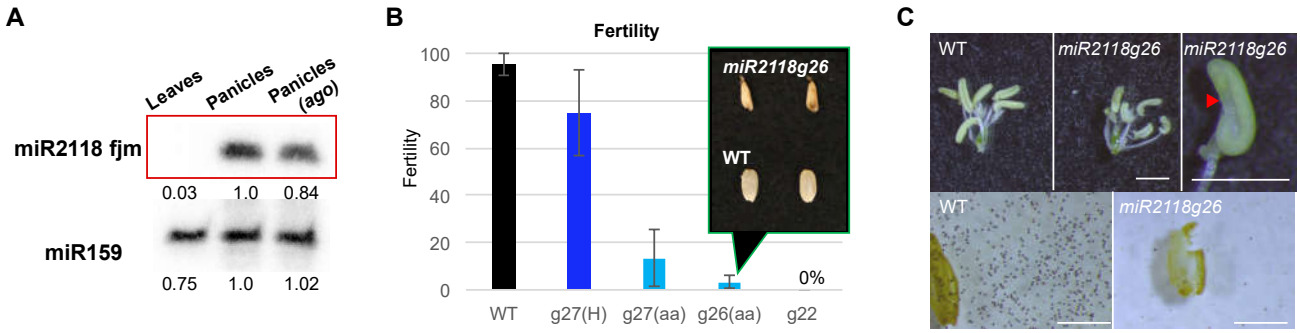

Figure2. miR2118 family members are required for early reproduction in rice.

A. miR2118fjm are expressed at premeiotic stages.
B. Fertility of miR2118g27 (H), miR2118g27 (aa), miR2118g26 (aa), miR2118g22 (aa) and WT.
C. Anthers and pollen of *miR2118g26* mutants and WT. I_2KI staining of mature pollen in anther of *miR2118g26* and WT. bars, 1mm.

3 Collaborations

3.1 Dr. Tu N. Le, OIST. Homology analysis of lincRNAs.
3.2 Dr. Masaki Endo and Ms. Masahiro Mikami, National Institute of Agrobiological Sciences (NIAS). CRISPER CAS9 vectors were provided by Dr. Endo.
3.3 Dr. Alejandro Villar Briones, OIST. Proteomics support using mass spectrometry.

4 External Funding

5.1 JST PRESTO, PI: **Komiya, R.** 2017, September ~ 2021, March.
5.2 JSPS Innovative area (RNA Taxonomy), PI: **Komiya, R.** 2017, April ~ 2019, March.