

Science and Technology Group Annual Report FY2021

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1 Introduction

The genome of higher organisms is composed of more than 90% of non-coding genomic regions. A recently large number of non-coding RNAs transcribed from these intergenic regions have been identified in many organisms. However, most biological functions remain unknown in plants and animals.

I identified more than **1300 types of reproductive long non-coding RNAs and numerous small RNAs derived from these long non-coding RNAs in rice** (Komiya *et al.*, 2014; Fig.1A). However, the functions of these non-coding RNAs remain unknown. We have successfully generated mutants for these non-coding RNAs by genome editing in the recent few years. These mutants have shown reproductive abnormalities (Komiya *et al.*, 2020; unpublished data), suggesting the significant functions of these non-coding RNAs during reproduction.

The major aim of this research is to elucidate the comprehensive reproductive system through the non-coding RNAs research.

2 Activities and Findings

2-1 Non-coding RNA mutants

To elucidate the biological functions of these reproductive long non-coding RNAs and small RNAs, we generated mutants via genome editing. These mutant lines that affect the multiple non-coding RNAs, show the reproductive phenomena.

2-2 Site-specific RNA silencing in stamen developments

The development of imaging technology and RNA profiling has clarified the spatiotemporal regulation of these reproductive small RNAs, and subsequently the different functions of 21-nucleotide (nt) and 24-nt small RNAs during male organ development.

I reviewed the biogenesis, diversification, spatiotemporal expression pattern and function of these small RNAs in plants (**Komiya 2021 *Genes & Genetic Systems*; Fig.1**).

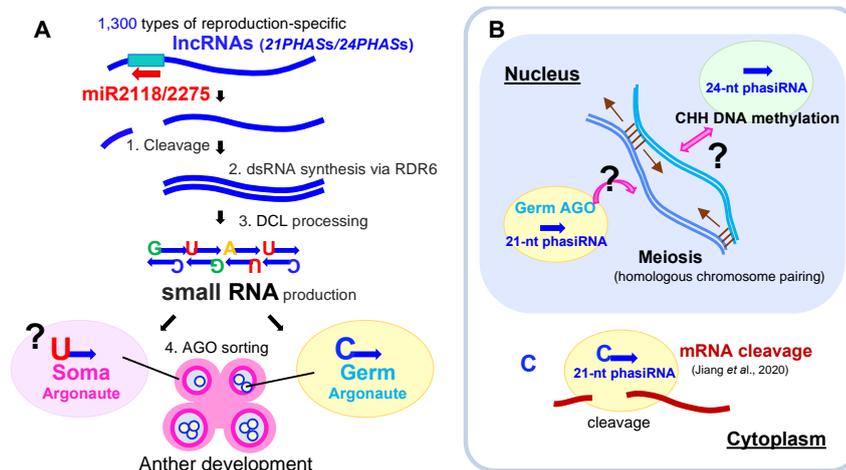


Fig. 1. Model for cell-specific phasiRNA biogenesis and roles during rice reproduction.

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2-3 Male specific RNA silencing in rice

Small RNAs associated with RNA binding proteins form the RNA-induced silencing complex (RISC), the core machinery in silencing systems that are conserved in many organisms.

To elucidate the function of reproductive small RNAs, we performed RNA profiling and mass spectrometry and identified the interactor of these reproductive small RNAs. Furthermore, we generated the mutants of these interactors by genome editing and clarified the role of male RISC during reproduction. We are preparing the publication about male RISCs in rice (**Tamotsu *et al.*, in preparation**)

2-4 3D imaging of stamen structure

We succeeded in the 3D-imaging of stamen, a male reproductive organ, to obtain the overall structure of rice stamen (Fig.2).

This protocol is published in *Methods in Molecular Biology* as follows.

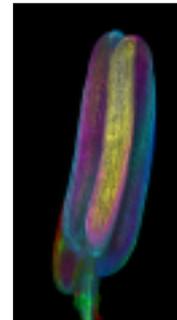


Fig. 2. Imaging of rice stamen using Lightsheet microscope.

3 My group members (by FORESTO research)

1. Ms. Saori Araki, Technician
2. Ms. Hinako Tamotsu, Technician

4 Collaborations

1. Dr. Koji Koizumi, OIST Science imaging section
2. Dr. Ayako Yokoi, Institute of Aerobiological Sciences, National Agriculture and Food Research Organization

5 Publications (†:Corresponding author)

1. **Komiya, R.†**. Spatiotemporal regulation and roles of reproductive phasiRNAs in plants. *Genes & Genetic Systems*. 96, 1-7 (2021).
2. Koizumi, K. and **Komiya, R.†**. 3D imaging and *in situ* hybridization for uncovering the functions of microRNA in rice anther. *Methods in Molecular Biology* (*in press*).

6 External Funding

1. Japan Science and Technology Agency (JST) FORESTO research
PI: **Komiya R.** FY2021 ~ FY2023 (~Max FY2026).
2. The Naito Foundation
PI: **Komiya R.** December 2020 ~ March 2024.