Research Achievement Report

TAIKICHIRO MORI MEMORIAL RESEARCH FUND FOR THE ACADEMIC YEAR 2021

DIVERSITY OF THE WILD STRAWBERRY ROOT MICROBIOME

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ABSTRACT

Plant health is heavily dependent on the root microbiome, microorganisms living inside (endophytic) and the outside (soil rhizosphere) of the plant roots. The root microbiome includes both beneficial and pathogenic microorganisms such as bacteria, fungi, archaea and viruses. The beneficial microbes such as mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), and endophytic fungi/bacteria boost plant growth and plant immunity. The wild strawberry *Fragaria vesca* is the perfect model for research on perennial-specific traits and development and ripening of fleshy fruit because unlike the model organism Arabidopsis thaliana, it is polycarpic (flowers several times a year) and perennial (persists through the winter), while still retaining the advantage of a relatively small (240Mb), fully sequenced, diploid genome (2n=2x=14). My study aims to 1) identify the strawberry-specific root microbiome by sampling lab-grown and wild-grown wild strawberries and 2) identify and confirm the molecular pathways acted upon by the root microbiome involved in promoting *F. vesca* growth and immunity.

RESEARCH ACHIEVEMENT OF ACADEMIC YEAR 2021

1. **Goal 1: Identifying the root-microbiome of the wild strawberry *Fragaira vesca***

To optimize the DNA extraction method and PCR conditions for wild strawberries, *Fragaria vesca* seeds were planted in June 2021. After six months of growing in a controlled environment, the leaves of the lab-grown strawberry were used for the experiment. The CTAB extraction resulted in successful PCR on the ITS marker, and sequencing confirmed the species as *F. vesca*. A DNA sample collected from a wild strawberry found growing on our campus was identified as *Fragaria x*

ananassa, and comparison between previously published marker sequences of the genus Fragaria suggested that the ITS marker was suitable to distinguish between species of wild strawberry.

The only place in Japan where the wild strawberry *F. vesca* can be found outdoors is in Hokkaido. Approximately 30 leaf samples of wild strawberries and 20 soil samples of their habitat were collected from Sapporo, Hokkaido in October 2021, with authorization from respective localities. To confirm the species, DNA was extracted from the leaf by CTAB extraction method (Ganbino *et al*., 2007) and the ITS region was amplified by PCR using the p5 and u4 plant-specific and universal primers previously published by Tao Cheng *et al*. in 2016. The PCR products of 25 samples shown as in *Figure 1* were purified and sequenced by the Sanger method. The data analysis is ongoing.

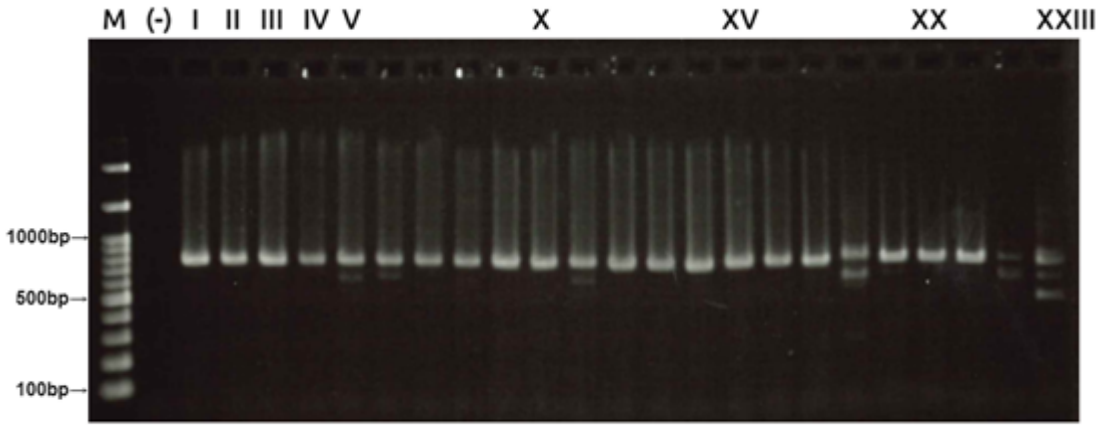

Figure 1. Agarose gel electrophoresis of the PCR products. M. DNA marker; (-). No DNA negative control, I-XXIII. ITS region from the samples 1 to 23 (Sapporo, Hokkaido).

2. Goal 2. Identify and confirm the molecular pathways acted upon by the root microbiome involved in promoting *F. vesca* growth and immunity

The first step to achieve knowledge about molecular pathways by root microbiome on *F. vesca* is RNA extraction of both the soil and the plant in their natural habitat. While part of my study will be done in the laboratory, where adequate equipment for RNA extraction and storage is available, part of the study will be done in the field, where the equipment and storage options are limited. In 2020, I had optimized protocols for DNA and RNA extraction from soil and another protocol for plant RNA extraction with only portable equipment that can be run on an electricity generator, compatible with fieldwork.

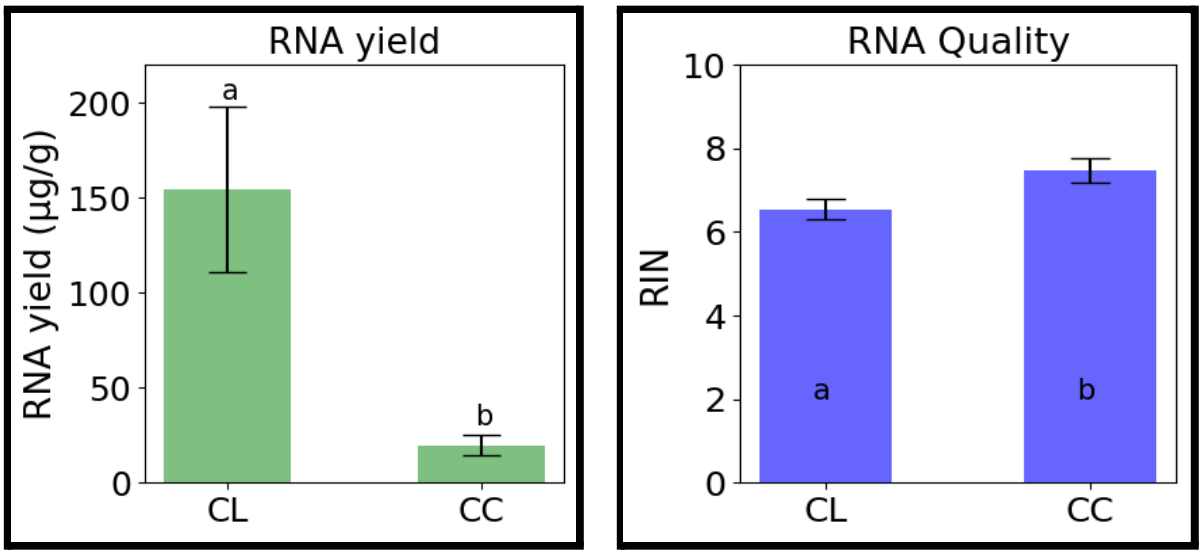

Figure 2. RNA yield and RNA Quality of CL: Original laboratory method, CC: field RNA extraction method

However, the plant RNA extraction protocol was developed using the Japanese hyacinth *Heloniopsis orientalis*, and did not work with *F. vesca,* possibly due to richness of polyphenols. Therefore, in 2021, I optimized the RNA extraction to make it work for strawberries. *Figure 2* shows the best results I got during this academic year.

Even though the RNA quality of the field method was significantly better than the original method, the RNA yield was lower than expected. This will be further optimized in the future.

In conclusion, the grant allowed me to make the necessary protocol optimizations and initial fieldwork sampling to enable the study of the root microbiome of wild strawberry.

PRESENTATIONS OF ACADEMIC YEAR 2021

1. Date: 7th – 9th July 2021
   Type of presentation: Poster presentation
   Title: Extraction of High-Quality RNA from Non-Model Plants and Soil in Remote Areas using Portable Equipment
   Conference: The 22nd Annual Meeting of the RNA Society of Japan (第 22 回日本 RNA 学会年会)

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