

Establishment of an alert system for *Fusarium oxysporum* f. sp. *cubense*, the banana and plantain wilt pathogen, and mitigation strategy of the disease

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Bananas (banana and plantain) are native to Southeast Asia, including Indo-Malaya. It is a monocotyledonous herbaceous plant of the genus *Musa*, cultivated in more than 130 countries, mainly in the so-called "banana belt" between 30° latitudes. In 2022, global banana fruit production reached 1.35 billion tons for fresh-eating and cooking. One of the most devastating threats to banana production is soilborne wilt (FW; or Panama disease), which is caused by *Fusarium oxysporum* f. sp. *cubense* (*Focb*). *Focb* is classified into three races (1, 2, and 4) based on their pathogenicity to banana cultivars (Nitani 2018). In the 1950s, exports of *M. acuminata* "Gros Michel" (AAA triploid genotype) from Central America were dramatically reduced by the outbreak of the *Focb* race 1. Race 1 causes FW not only in "Gros Michel" but also in "Silk" (AAB) and "Pome" (AAB). Race 2 is endemic to Central America and causes FW in some cooking banana varieties, such as "Bluggoe" (ABB). To avoid damage caused by *Focb* races 1 and 2, "Cavendish" (AAA), which is resistant to the races, was selected, and now "Cavendish" occupies about 99% of the trading bananas. However, in the 1960s, race 4, which is also pathogenic to "Cavendish", was reported in subtropical regions, such as Taiwan, the Philippines, and the Canary Islands, and is now called subtropical race 4 (race SR4). Furthermore, since the late 1980s, devastating damage caused by FW to 'Cavendish' in tropical Asia has been reported as the "new Panama disease." The causal *Focb* of this serious damage is genetically distinct from race SR4 and is called tropical race 4 (race TR4). Currently, TR4 is the most devastating *Focb* race, and due to its pandemic, it has spread worldwide, including to Asia, including the Philippines, India, Australia, East Africa, Colombia (2019), Peru (2021), and Venezuela (2023), causing serious damage to banana plantations in various locations.

With these backgrounds, we are currently working on five sub-themes in the SATREPS project titled above, with the goal of suppressing the occurrence of FW in the Tingo Maria region, Peru, and preventing the invasion of race TR4, thereby protecting banana cultivation in the region, improving the quality of the bananas produced, improving farmers' income and living standards, and training young researchers and engineers involved.

1. Diagnosis and alert system for FW occurrence at the macro and micro levels

Banana fields in the Tingo Maria region are distributed from riverbanks to mountain slopes, making it difficult to regularly approach banana individuals and conduct health examinations. Therefore, we aimed to first grasp individuals or populations suspected of FW from a macro perspective by drone image analysis of fields and then apply micro-level technology to diagnose the pathogen of each FW-suspected individual. To date, we have identified genes characteristic of each *Focb* race by comparing whole genome data. Based on this, we have developed LAMP primer sets for specific identification of *Focb* and its races, which are now sold by Nippon Gene Co. (<https://www.nippongene.com/kensa/products/lamp-primer/banana/foc.html>). We are also working to disseminate this technology by conducting training courses at Universidad Nacional Agraria La Molina

(UNALM), Universidad Nacional Agraria de la Selva (UNAS), and other collaborative research partners in Peru. In addition, using these methods, we found that most *Focb* occurring in the Tingo Maria region were of race SR4, and a small number were of race 1. We also confirmed that race TR4 has not yet invaded the region.

2. Creation of new banana varieties resistant to FW by inducing mutations through heavy ion beam irradiation, etc.

Eating bananas are triploid and do not form seeds; thus, breeding for disease resistance through crossbreeding is difficult. Therefore, mutation induction is being performed using heavy ion beams in Japan and gamma rays in Peru in an attempt to develop new varieties resistant to FW. Bananas, including “Cavendish”, have been irradiated with heavy ion beams several times at the RIKEN Nishina Center, and a screening system based on FW resistance is currently being established.

3. Publicizing the importance of using pathogen-free seedlings and establishing a supply system for them

The *Focb* race TR4 isolates collected from around the world are monophyletic in molecular phylogeny. This indicates that *Focb* race TR4, which originated in tropical Asia, has spread worldwide. *Focb* can be transmitted by seedlings, agricultural equipment, water, human feet and etc. Farmers can plant seedlings from the base of banana parent plants grown in the field at no cost, but there is a possibility that latent *Focb* may be transmitted by the seedlings. Therefore, farmers should understand the importance of using *Focb*-free seedlings generated from mericlones, and Peruvian institutions are working together to supply *Focb*-free seedlings to farmers. The LAMP primer sets developed in “1” can also be used to test whether the seedlings are *Focb*-free.

4. Analysis of the microbial flora of FW-suppressive soil

Soil that is unlikely to cause disease despite the presence of pathogens is called "disease-suppressive soil." We plan to collect soil from fields in the Tingo Maria region where FW has not occurred and to clarify the microbial flora thought to be involved in disease suppression through metagenomic analysis. We are currently testing disease suppressiveness and conducting metagenomic analysis of soil microbial flora (fungi and bacteria) using soil imported from Peru.

5. Establishment of environmentally friendly FW control technology such as biopesticides

There is a global demand for environmentally friendly plant disease control technology. Banana cultivation in the Tingo Maria region is generally organics. Therefore, it is necessary to control FW using technology with low environmental impact. Currently, we are considering the application of nonpathogenic *Fusarium* spp. (Saito 2021) and of plant activators, such as Validamycin A (Ishikawa 2005), to induce resistance in bananas to control FW. In the future, we would like to propose new biopesticides using SynCom technology, which artificially reconstructs the microbial flora involved in disease suppression based on the results of 4.

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References T Nitani et al. (2018) J Gen Plant Pathol 84:165–168; H Saito et al. (2021) Appl Environ Microbiol 87:e01959-20; R Ishikawa et al. (2005) Phytopathology 95:1209–1216