【Workshop 5】Perspectives of Metagenomics in Agricultural Research

A novel Detection Method for Plant Parasitic Nematodes with Different Life Stages Using Soil Compaction and Real-time PCR

Keita Goto, Erika Sato, Yu Yu Min and Koki Toyota
Tokyo University of Agriculture and Technology
(kokit@cc.tuat.ac.jp)

Plant-parasitic nematodes cause significant economic losses to a wide variety of crops. Since there is a significant correlation between an initial population density of plant-parasitic nematodes in soil and the degree of damage on the host, reliable and rapid identification and counts of the casual agents are critically important for successful management of the nematode pests. Firstly, we developed different real-time PCR primers for the quantitative detection of the major plant-parasitic nematodes, the soybean cyst nematode (SCN) *Heterodera glycines*, the potato cyst nematode (PCN) *Globodera rostochiensis*, the root-knot nematode (RKN) *Meloidogyne incognita* and the root-lesion nematode (RLN) *Pratylenchus penetran*. Secondly, we developed a DNA extraction method from soil inhabited by nematodes, in which soil was put into a 100-cc core and compacted using an apparatus in order to destroy nematodes with different forms, such as vermiforms and cysts. Then, DNA was extracted from the compacted soil and used as templates for real-time PCR. *Ct* values were the lowest in the soil most compacted (1.4 g cm^{-3}), the maximum physical compaction of the soil used (andosoil) in the compactor, and the difference in the *Ct* values between compacted and non-compacted soil was four cycles at the largest, suggesting that 16 times more DNA derived from SCN was detected by the compaction. Different numbers (10 to 3000) of SCN eggs were added to 20 g of a non-infested andosoil and the soils were compacted to 1.4 g cm^{-3}. There was a significant correlation (r^2 = 0.8615, P < 0.001) between the *Ct* values and the number of eggs added. Similar calibration curves were also prepared for PCN, RKN and RLN by adding different numbers of eggs or vermiforms to non-infested soils. These results demonstrated that the present method using a combination of soil compaction and real-time PCR enabled rapid and sensitive quantification of plant-parasitic nematodes in soil.