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Title: Root system architecture analysis of root-knot nematode resistant rice cultivars

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Applicant: Mst Zobaida Lahari, Ghent University, Ghent (BE)

Host: Aska Goverse, Wageningen UR, Wageningen (NL)

Background:

The rice root-knot nematode *Meloidogyne graminicola* (Golden and Birchfield, 1965) is one of the most important pests of Asian rice (*Oryza sativa* L.) (De Waele and Elsen, 2007; Kyndt *et al.*, 2012; Ji *et al.*, 2013; Jones *et al.*, 2013). It can cause substantial yield losses to upland, lowland, and deepwater rice as well as in rice nurseries (Bridge *et al.*, 2005; Pokharel *et al.*, 2010; Win *et al.*, 2011). It is now considered as one of the major biotic causal agents of yield failure in tropical aerobic rice (Kreye *et al.*, 2009; De Waele *et al.*, 2013).

The infective second-stage juveniles (J2) of *M. graminicola* invade rice roots just behind the root tips and migrate intercellularly towards the central cylinder. At this site, the J2 puncture selected vascular cells with their stylet and inject pharyngeal secretions, which ultimately leads to the reorganization of the plant cells into typical feeding structures called giant cells. From these giant cells the nematode feeds throughout its life cycle of 2-3 weeks (Gheysen G and Mitchum MG, 2011). This life cycle is repeated for few generations during the whole growing season of rice causing formation of typical hook-like gall at the root tip, yellowing, stunted growth, seedling mortality, reduction of tillering, reduction of root proliferation, delayed maturation, and eventually 17% to 32% grain yield losses in rice (Bridge *et al.*, 2005; Kyndt *et al.*, 2014).

At the moment, the options to manage *M. graminicola* population densities below damage threshold levels are limited. Crop rotation, flooding, and the use of nematicides are often used to manage plant-parasitic nematodes in the infested fields (Bridge *et al.*, 2005) but each of these practices has a number of drawbacks (De Waele *et al.*, 2013). In this context, the identification and use of rice genotypes resistant to *M. graminicola* offers an excellent and durable alternative to limit the yield losses caused by this nematode species.

Recently for the first time we have analysed and confirmed two *O. sativa* accessions (LD 24 and Khao Pahk Maw) that have complete resistance to *M. graminicola* (Dimkpa *et al.*, 2015). These two genotypes appear ideal donors for breeding to generate rice cultivars resistant to root-knot nematodes. Nevertheless we know nothing about the gene (s) underlying this resistance. Recently a cross between rice accessions Vialone and LD24 has been done. LD24 has a high level of resistance to *M. graminicola*, while Vialone is highly susceptible (Dimpka *et al.*, 2015). An F2 population has been developed and this segregating F2 population is being analysing for resistance against *M. graminicola* and for SNPs cosegregating with this trait. The final goal is map based cloning of the resistance gene(s).

For in-depth characterization of these resistant rice accessions we have done a detailed analysis of root system architecture (RSA) in comparison with some *M. graminicola* susceptible reference rice genotypes. RSA is one of the factors influencing plant susceptibility to nematodes and also water and nutrients uptake under drought. Therefore these data not only provide valuable information to fully characterize the resistance but also for the development of breeding strategies. We do not have the facilities to study RSA in our lab. Dr. Aska Goverse and her research group at the laboratory of Nematology, Wageningen University is studying many aspects of plant-nematode interactions. In her lab, Rhizoscanner (WinRHIZO) is being used to analyse plant root system architecture (RSA). So I went to the Laboratory of Nematology to use the facilities of Rhizoscanner (WinRHIZO) for RSA analysis of root-knot nematode resistant rice cultivars.

Objectives/ Purpose of the visit:

- Analysis of root system architecture (RSA) of two *M. graminicola* resistant *O. sativa* accessions (LD 24 and Khao Pahk Maw) in comparison with *M. graminicola* susceptible reference rice accessions CO39, T65, Nipponbare and Vialone.

Description of the work

WP1: Preparation of plant material

The root-knot nematode *M. graminicola* resistant rice (*O. sativa* L.) accessions *viz*. LD24 (ssp. *indica* from Sri Lanka), Khao Pahk Maw (ssp. *aus* from Thailand), and susceptible reference rice accessions CO39 (ssp. *indica*), Nipponbare (ssp. *japonica*), T65 (ssp. *japonica*) and Vialone (ssp. *japonica*) were used to analyse RSA. The rice plants were grown in SAP medium (Reversat *et al.,* 1999) at 27^0C under 12h/12h light regime with 70-75% relative humidity for 2 weeks in a climate chamber, Ghent University. Then the plant samples were carried from Ghent University to the Laboratory of Nematology, Wageningen University.

WP2: Root system architecture measurement

The roots were washed with water to free SAP particles and cut at the root shoot junction to separate the plant root system from the shoot system. The root system was placed on **Rhizoscanner** to digitalize the root system. From this digitalized root system various root parameters like length, surface area, average diameter, volume, number of tips were calculated using the image analysis software **WinRHIZO**. The host Laboratory provided the support to this work package.

WP3: Discussion with researchers at the nematology department

I discussed especially with Sonja Warmerdam, a PhD student, who is doing research on “Identification of susceptibility factors in Arabidopsis and tomato to nematode infections” as well as with other members of the Laboratory of Nematology to share knowledge about plant-nematode interactions and underlying resistance mechanisms of plants against nematodes.

Main results:

The *M. graminicola* resistant rice accession LD24, Khao Pahk Maw developed significantly longer roots than *M. graminicola* susceptible reference rice accessions T65, Nipponbare. But the root length was comparable between resistant rice accession Khao Pahk Maw and susceptible rice accession Vialone. The resistant rice accession LD24 had significantly greater root surface area than susceptible reference rice accessions T65, Nipponbare but lower than Vialone. On the other hand Khao Pahk Maw had also significantly greater root surface area than CO39**,** T65, Nipponbare but similar with Vialone. The resistant rice accession LD24 and Khao Pahk Maw had significantly lower average root diameter than Nipponbare and Vialone, but comparable with CO39**,** T65. The resistant rice accession LD24 had significantly greater root volume than susceptible reference rice accessions T65, Nipponbare but lower than Vialone. The other resistant rice accession Khao Pahk Maw had also significantly greater root volume than CO39**,** T65, Nipponbare but similar with Vialone. The number of root tips was significantly higher in resistant rice accession Khao Pahk Maw than all susceptible reference rice accessions CO39**,** T65, Nipponbare and Vialone. On the other hand, the number of root tips was comparable between resistant rice accession LD24 and susceptible CO39 and T65 but significantly greater than Nipponbare and less than Vialone.

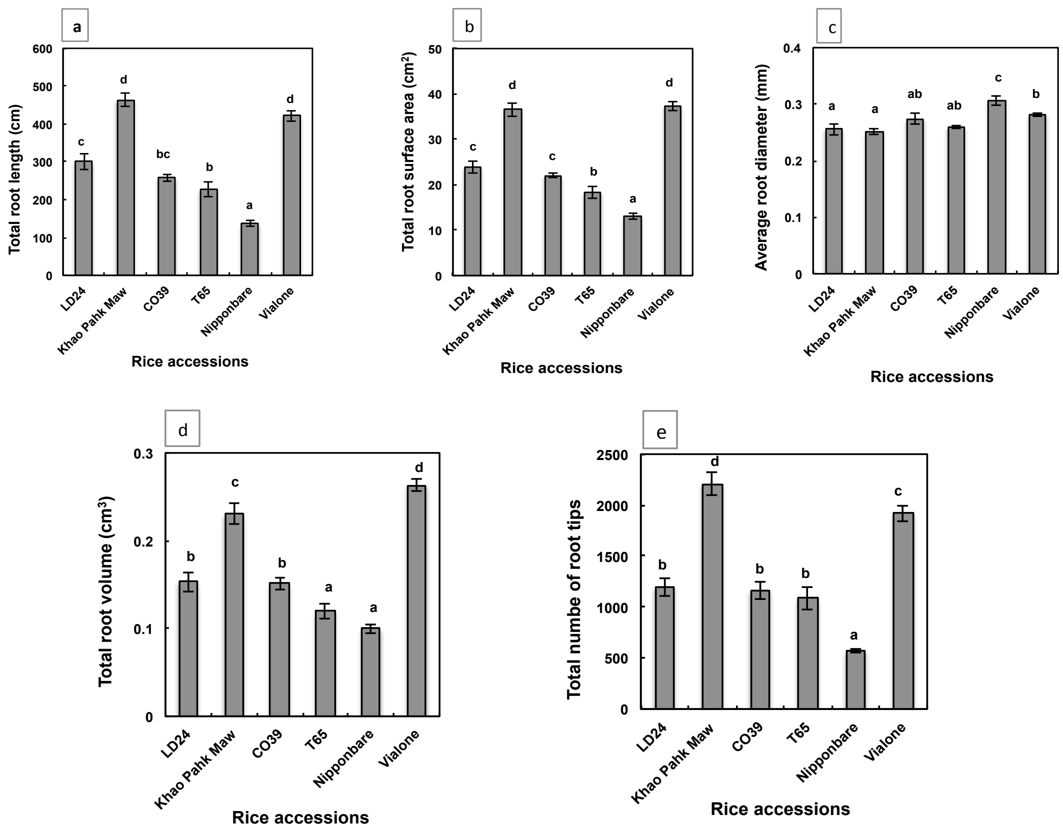

Figure1: Different root parameters of 2-week-old *M. graminicola* resistant rice accessions LD24, Khao Pahk Maw, and *M. graminicola* susceptible reference rice accessions CO39, Nipponbare, T65 and Vialone grown in Sand Absorbent Polymer (SAP) medium. (a) Total root length, (b) total root surface area, (C) average root diameter, (d) total root volume and (e) total number of root tips. Different letters indicate significant differences at $P<0.05$ level. Bars are the mean ± SE of eight plants (n=8).

Future collaboration with the host institution (if applicable):

It was a very useful trip. I really acknowledge the supervision of Dr. Aska Goverse, Laboratory of Nematology during my stay at Wageningen University. In the future we will continue the collaboration with her research group in the field of plant-nematode interactions. Moreover, as another part of my research project I have found Strigolactones (SLs), a new group of plant hormones, to be involved in rice-root knot nematode (RKN) interactions. I am interested to analyse the level of SLs in some rice cultivars during RKN infections, but we do not have the facilities to analyse SLs in our lab. In the host institution, professor dr. Harro Bouwmeester and his research group, Laboratory of Plant Physiology, have very good expertise to analyse the level of SLs in different rice genotypes. So during my stay at Wageningen University, I also took the opportunity to discuss with prof. Bouwmeester and we agreed to establish a collaboration to analyse the level of SLs in some rice cultivars during RKN infections. For this purpose I am planning to go back to Wageningen University next year.

Projected publications/articles related to or resulting from the STSM:

The outputs of this project will be included in a future publication.

Other comments (if any):