Attractancy of bacterivorous nematodes to root-adhering soils differs according to rice cultivars
Mahafaka Ranoarisoa, Eric Blanchart, Kirsten Vom Brocke, Alain Ramanantsoanirina, Mathilde Sester, Claude Plassard, Laurent Cournac, Jean Trap

To cite this version:
Mahafaka Ranoarisoa, Eric Blanchart, Kirsten Vom Brocke, Alain Ramanantsoanirina, Mathilde Sester, et al.. Attractancy of bacterivorous nematodes to root-adhering soils differs according to rice cultivars. Rhizosphere, 2017, 3 (1), pp.128-131. 10.1016/j.rhisph.2017.04.001. hal-01605972

HAL Id: hal-01605972
https://hal.science/hal-01605972
Submitted on 25 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License
Attractancy of bacterivorous nematodes to root-adhering soils differs according to rice cultivars

Mahafaka Patricia Ranoarisoa¹,², Eric Blanchart¹,², Kirsten vom Brocke³, Alain Ramanantsoanirina⁴, Mathilde Sester⁵, Claude Plassard⁶, Laurent Cournac⁷, Jean Trap¹,²*

¹Laboratoire des Radio Isotopes, BP 3383, Route d’Andraisoro, Antananarivo 101, Madagascar
²IRD, UMR Eco&Sols, Laboratoire des Radio Isotopes, BP 3383, 101 Antananarivo, Madagascar
³CIRAD, UR AGAP, SRR FOFIFA, BP 230, 110 Antsirabe, Madagascar
⁴SRR FOFIFA, BP 230, 110 Antsirabe, Madagascar
⁵CIRAD, UR AIDA, TA-B-115/02, Avenue Agropolis, 34398 Montpellier, France
⁶INRA, UMR Eco&Sols, 2 place Viala, 34060 Montpellier Cedex 2, France
⁷IRD, UMR Eco&Sols, LMI IESOL, Centre IRD/ISRA de Bel Air, BP 1386, Dakar, Senegal

*Corresponding author. Jean Trap, IRD, UMR Eco&Sols, Laboratoire des Radio Isotopes, BP 3383, 101 Antananarivo, Madagascar, Tél : +261 (0)32.47.126.74, Email : jean.trap@ird.fr

Abstract
We tested the ability of root-adhering soils (RAS) from eight rice cultivars to attract a bacterivorous nematode (*Acrobeloides* sp.) living in a poor-nutrient soil in Madagascar. We showed that the ability of rice to attract bacterivorous nematodes in its rhizosphere is variable among cultivars. FOFIFA 172 was the cultivar with the highest attraction value, the lowest root biomass (RB) and the highest RAS:RB ratio and RAS respiration. In contrast, nematodes exhibited strong repellent response to W880 RAS. Also, nematode attractancy was not related to rice resistance against pests (blast rice disease and *Striga asiatica*) neither to cultivar ability to grow at specific altitude. Our results suggest a trade-off in carbon allocation between plant biomass and root exudation driving nematode attractancy and raise questions about the impact of plant breeding on cultivar ability to interact with soil mutualists such as bacterivorous nematodes. Further investigations are required into (i) variation in root exudate quantity and composition among the eight cultivars and (ii) the response of rice cultivars to bacterivorous nematode inoculation.

**Keywords:** upland rice cultivar, *Acrobeloides* sp., nematode attractancy, ferrallitic soil, Madagascar.
Main text

Bacterivorous nematodes are key plant mutualists (Irshad et al. 2011; Mao et al. 2006; Trap et al. 2016). Their grazing activity stimulates bacterial growth, especially in the rhizosphere of plants where bacteria proliferate, which enhances the availability of nutrients for plant roots (Anderson et al. 1978; Bonkowski and Clarholm 2012; Irshad et al. 2012). In nutrient-poor soils, the presence of bacterivorous nematodes in the vicinity of roots can significantly increase the competitive advantage of plants over bacteria regarding nutrients (Bjornlund et al. 2012; Trap et al. 2016).

Nematode behaviour is influenced by a wide variety of compounds (ions, amino acids, volatile organic compounds, vitamins, CO₂), as well as pH gradients and bacterial food (Anderson and Coleman 1981; Grewal and Wright 1992; Rasmann et al. 2012; Young et al. 1996). Bacterivorous nematodes move along a signal gradient towards the bacteria (Hallem et al. 2011; Rengarajan and Hallem 2016). Such signals are found in the rhizosphere where root facilitates nematode movement (Trap et al. 2015) and root exudates promote bacterial proliferation (el Zahar Haichar et al. 2014) and subsequently attract nematodes (Bais et al. 2006; Horiuchi et al. 2005). In view of the fact that the amount and composition of root exudates varies according to the rice cultivar (Aulakh et al. 2001; Bhattacharyya et al. 2013), we hypothesized that the ability of rice to attract bacterivorous nematodes in its rhizosphere also varies according to the cultivar. We tested this hypothesis using an original three-compartment Petri dish experiment with eight upland rice cultivars growing in a poor-nutrient tropical soil in Madagascar and Acroboloides sp. as a bacterivorous nematode model species.

Pot experiment

We sampled the first 10 cm of a Ferrallitic soil under natural savanna from Lazaina (18°46'55 59° S, 47°32'46 3°N, 1274 m altitude, Madagascar) in October 2016. The soil (sandy-clay
texture, pH 5.5, C:N 16, Olsen P 4.7 mg.kg\(^{-1}\)) was air-dried until constant weight (7 days) and sieved at 2 mm. Eight upland rice cultivars (Oryza sativa L.) were selected in order to be representative of the large panel of rice cultivars used by famers. (Table 1): B22, FOFIFA 182, FOFIFA 172, FOFIFA 152, FOFIFA 154, NERICA 4, Chhromrong Dhan and WAB 880-1-32-1-1-P2-HB-1. Forty-five pots containing 1 kg of dry soil were prepared. For each cultivar, three seeds were planted in each pot and five replicates were used. Pots without seeds were used as control. The soil moisture content was maintained at 80% of water-holding capacity. After 32 days of growth in a greenhouse (mean temperature 28.1°C; mean and max PAR light 522 and 2378 \(\mu\)M.m\(^2\).s\(^{-1}\) between 6:00 am and 6:00 pm), the plants were gently removed from the pot together with their soil. The plants were then held by hand at the shoot collar and dropped 30 cm high on a plastic tray before being lifted from the tray and placed on a 200 \(\mu\)m sieve. The soil remaining on the tray was removed. The root-adhering soil (RAS) was carefully separated from the roots by washing over the sieve with sterile deionized water (constant volume). RAS was air-dried over 7 days before the Petri dish experiment. RAS sampling was carried out by the same person to ensure protocol reproducibility. The plant shoot and root were separated, dried at 60°C for 48 hours and weighted. RAS respiration was measured by weighting 20 g of dry RAS, moistened at 80% of holding capacity and incubating over 7 days at 28°C in the dark within 150 ml hermetic glass flasks (three replicates). At the end of the incubation, the total atmospheric CO\(_2\) concentration was measured in all flasks using a micro-CPG (CP-4900, Varian, Middelburg, The Netherlands). Flasks without soil were used as control. RAS pH\(_{\text{water}}\) was measured after 30 mn of stirring in distilled water (1:2.5 soil:water ratio).

**Petri dish experiment**

Monoxenic populations in sterile Petri dishes of Acrobeloides sp. on Escherichia coli were obtained from Lazaina and maintained at 25°C in the laboratory following the procedure
described in Irshad et al. (2011). *Acrobeloides* is a common ubiquitous genus that dominates bacterivorous nematodes in upland rice systems in the Malagasy highlands (Djigal et al. 2012; Villenave et al. 2009). Before use in the Petri dish experiment, nematodes were removed from the breeding TSA plates (by washing the surface with sterile water) and transferred to a fine cellulose wadding paper placed in a Petri plate (90 mm diameter) with water during 48 h. Living nematodes will move from the filter paper into the water without agar pollution. Tri-compartment Petri dishes (90 mm diameter) were used to test the nematode attractancy of RAS (Fig. 1). Compartment A of the Petri dishes was filled with 7 ml of sterile agarose (10 g.L\(^{-1}\)) and communicated with the two other compartments by open gates. Compartments B and C were not connected and were filled with either 10 g of dry RAS or bulk soil from the five control pots, then moistened with 4.8 or 4.4 ml of distilled water for RAS and bulk soil, respectively. Compartment A was moistened with 200 µl of sterile distilled water to favour nematode migration. Around 100 food-deprived nematodes were inoculated on the top of the middle line (dark dashed line in Fig. 1). Petri dishes were incubated in the dark at 25°C. After 16 hours, the nematodes were counted in the right (r) and left (l) zones of each side of the middle line of the compartment A. For each cultivar, we replicated the experiment five times so as to match the five replicates from the pot experiment. The attractancy was expressed in percentage.

Statistical analyses were performed with R (Team 2016). One-way ANOVA and Tukey HSD post-hoc tests were performed on shoot and root biomass (RB), RAS mass, RAS:RB ratio and RAS respiration. Data normality was checked using the Wilk-Shapiro test. Significance in RAS nematode attractancy was assessed with a binomial test. Pearson correlations were used to test for linear relationships between nematode attractancy and RAS mass.

Bacterivorous nematodes graze on dense bacterial populations that mineralize root exudates, increasing bacterial turnover and nutrient cycling in the rhizosphere of plants (Griffiths 1986;
We formulated the hypothesis that the translocation of plant carbon to the soil may be a mechanism by which rice attracts mutualistic nematodes, resulting in a higher respiration. In agreement with this assumption, F172 was the cultivar that exhibited the highest attraction value (61%) (Fig. 2) and RAS respiration, i.e. 1.5 times more than the bulk soil (Table 2). This hypothesis is also supported by the significant correlation between RAS:RB ratio and nematode attractancy when W880 was excluded from the data set (R² = 0.79, P-value 0.006). F152 and F182 attracted fewer nematodes (56% average) and had lower RAS respiration than F172. Finally, the abilities of rice roots to aggregate the soil and to attract *Acrobeloides* sp. are thus most likely mediated by high amounts of root exudation (el Zahar Haichar et al. 2014). F172 had also the lowest root biomass (RB) and the highest RAS:RB ratio among cultivars (Table 2), supposing a trade-off between carbon allocation in root biomass and exudates.

Interestingly, we observed that *Acrobeloides* sp. individuals were significantly more attracted to the bulk soil than the RAS produced by W880 (Fig. 2). W880 did not show lower respiration values than the bulk soil, suggesting that nematode repulsion by W880 RAS was not related to a low rhizodeposition. Likewise, W880 did not alter soil pH as N4 did (Table 2), rejecting the hypothesis that W880 repulsed nematodes by changing soil pH (Jairajpuri and Azmi 1978). Knowing that nematode movement is also influenced by ions or volatile compounds (Le Saux and Queneherve 2002; Rasmann et al. 2012), it is possible that W880 (i) altered its rhizospheric nutrient concentrations or (ii) produced volatile compounds or favored production of bacterial metabolites acting as repellents (Dusenbery 1983).

To conclude, we showed that plant breeding, already known to affect interactions between rice and plant-parasitic nematodes (Dimkpa et al. 2015), impact the ability of rice to interact with mutualistic bacterivorous nematodes. Increasing production of root exudates by rice cultivars may thus favour nematode recruitment, with possible positive feedbacks for bacterial
colonization of rhizosphere and nutrient release (Knox et al. 2003). However, the role and involvement of selection criteria in the ability of rice to attract nematodes remains unclear. For instance, nematode attraction did not seem related to the resistance or tolerance against pests (blast rice disease and Striga asiatica), nor to the cultivar ability to grow at specific altitude (Table 1). Screening a larger panel of rice cultivars would be necessary to better link agronomic performance with nematode attractancy. Also, characterizing root exudation of the eight rice cultivars, as well as their nutrient response to bacterivorous nematode inoculation, would constitute useful further investigations to better understand interactions between upland rice genotype diversity, mutualist nematodes and the agronomic outputs.

Acknowledgements

This work received support from the French state (Labex Agro: ANR-10-LabX-001-01), generated by the National Research Agency under the program “Future Investments”. We are grateful to the “Institut de Recherche pour le Développement” (IRD) for having provided a PhD grant to P. Ranoarisoa through the “Allocation de Recherche pour une Thèse au Sud” (ARTS) program. We want to thank K. Rahajaharilaza and H.M. Ralalason for their assistance during root-adhering soil collection and Dr. C. Villenave (ELISOL Environnement, Nime, France) for nematode identification.
References

Anderson RV, Coleman DC (1981) Population development and interactions between 2 species of bacteriophagic nematodes Nematologica 27:6-19

Anderson RV, Elliott ET, McClellan JF, Coleman DC, Cole CV, Hunt HW (1978) Trophic interactions in soils as they affect energy and nutrient dynamics. 3. Biotic interactions of bacteria, amebas, and nematodes Microb Ecol 4:361-371

Aulakh M, Wassmann R, Bueno C, Kreuzwieser J, Rennenberg H (2001) Characterization of root exudates at different growth stages of ten rice (Oryza sativa L.) cultivars Plant Biology 3:139-148

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms Annu Rev Plant Biol 57:233-266

Bhattacharyya P, Das S, Adhya T (2013) Root exudates of rice cultivars affect rhizospheric phosphorus dynamics in soils with different phosphorus statuses Communications in soil science and plant analysis 44:1643-1658

Bjornlund L, Liu MQ, Rønn R, Christensen S, Ekelund F (2012) Nematodes and protozoa affect plants differently, depending on soil nutrient status European Journal of Soil Biology 50:28-31

Bonkowski M, Clarholm M (2012) Stimulation of plant growth through interactions of bacteria and protozoa: Testing the auxiliary microbial loop hypothesis Acta Protozoologica 51:237-247

Dimkpa SO, Lahari Z, Shrestha R, Douglas A, Gheysen G, Price AH (2015) A genome-wide association study of a global rice panel reveals resistance in Oryza sativa to root-knot nematodes Journal of experimental botany:erw470
Djigal D, Saj S, Rabary B, Blanchart E, Villenave C (2012) Mulch type affects soil biological functioning and crop yield of conservation agriculture systems in a long-term experiment in Madagascar Soil and tillage research 118:11-21

Dusenbery DB (1983) Chemotactic behavior of nematodes J Nematol 15:168

el Zahar Haichar F, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated interactions belowground Soil Biology and Biochemistry 77:69-80

Grewal P, Wright D (1992) Migration of Caenorhabditis elegans (Nematoda: Rhabditidae) larvae towards bacteria and the nature of the bacterial stimulus Fundamental and applied nematology 15:159-166

Griffiths BS (1986) Mineralization of nitrogen and phosphorus by mixed cultures of the Ciliate protozoan Colpoda steinii, the nematode Rhabditis sp and the bacterium Pseudomonas fluorescens Soil Biology & Biochemistry 18:637-641

Hallem EA, Dillman AR, Hong AV, Zhang Y, Yano JM, DeMarco SF, Sternberg PW (2011) A sensory code for host seeking in parasitic nematodes Current Biology 21:377-383

Horiuchi J-i, Prithiviraj B, Bais HP, Kimball BA, Vivanco JM (2005) Soil nematodes mediate positive interactions between legume plants and rhizobium bacteria Planta 222:848-857

Irshad U, Brauman A, Villenave C, Plassard C (2012) Phosphorus acquisition from phytate depends on efficient bacterial grazing, irrespective of the mycorrhizal status of Pinus pinaster Plant and Soil 358:148-161

Irshad U, Villenave C, Brauman A, Plassard C (2011) Grazing by nematodes on rhizosphere bacteria enhances nitrate and phosphorus availability to Pinus pinaster seedlings Soil Biology & Biochemistry 43:2121-2126

Jairajpuri M, Azmi M (1978) Aggregation and repulsion of nematodes at pH gradients Nematologia mediterranea 6:107-112
Knox OGG, Killham K, Mullins CE, Wilson MJ (2003) Nematode-enhanced microbial colonization of the wheat rhizosphere Fems Microbiology Letters 225:227-233
doi:10.1016/s0378-1097(03)00517-2

Le Saux R, Queneherve P (2002) Differential chemotactic responses of two plant-parasitic nematodes, Meloidogyne incognita and Rotylenchulus reniformis, to some inorganic ions Nematology 4:99-105 doi:10.1163/156854102760082258

Mao X, Hu F, Griffiths B, Li H (2006) Bacterial-feeding nematodes enhance root growth of tomato seedlings Soil Biology & Biochemistry 38:1615-1622

Raboin L-M et al. (2013) Création variétale pour la riziculture pluviale d’altitude à Madagascar: bilan de 25 années de sélection Cahiers Agricultures 22:450-458

Raboin L-M, Randriambololona T, Radanielina T, Ramanantsoanirina A, Ahmadi N, Dusserre J (2014) Upland rice varieties for smallholder farming in the cold conditions in Madagascar's tropical highlands Field Crops Research 169:11-20

Rasmann S, Ali JG, Helder J, van der Putten WH (2012) Ecology and evolution of soil nematode chemotaxis J Chem Ecol 38:615-628

Rengarajan S, Hallem EA (2016) Olfactory circuits and behaviors of nematodes Current Opinion in Neurobiology 41:136-148

Team R (2016) RStudio: Integrated Development for R.

Trap J, Bernard L, Brauman A, Pablo A-L, Plassard C, Ranoarisoa MP, Blanchart E (2015) Plant roots increase bacterivorous nematode dispersion through nonuniform glass-bead media J Nematol 47:296

Trap J, Bonkowski M, Plassard C, Villenave C, Blanchart E (2016) Ecological importance of soil bacterivores for ecosystem functions Plant and Soil 398:1-24
Villenave C, Rabary B, Chotte J-L, Blanchart E, Djigal D (2009) Impact of direct seeding mulch-based cropping systems on soil nematodes in a long-term experiment in Madagascar Pesquisa Agropecuaria Brasileira 44:949-953

Young I, Griffiths B, Robertson W (1996) Continuous foraging by bacterial-feeding nematodes Nematologica 42:378-382
Fig. 1. Experimental setup made by three-compartment Petri dish used to assess nematode attractancy to root-adhering soils (RAS) from eight rice cultivars. Compartment A (white area), filled with sterile agarose, was connected to the two others by open gates.

Compartments B (light grey area) and C (dark grey area) were filled with RAS or bulk soil. The black dashed line represents an imaginary line splitting the compartment A in two zones (r and l). Nematodes were inoculated in the top of the imaginary black dashed line (black arrow).
Fig. 2. Attractancy of nematodes to root-adhering soils (RAS) from eight upland rice cultivars (CD, F154, W880, F182, F152, N4, F172 and B22). The horizontal bars correspond to standard deviation. NS: not significant, *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ according to a binomial test (5 replicates).

Table 1. Agronomic criteria used in the selection of the eight upland rice cultivars

| Rice cultivars | Selection criteria                                                                 | Optimal altitude for cropping | Adoption by farmers$^s$ |
|----------------|-------------------------------------------------------------------------------------|------------------------------|------------------------|
| B22            | Cultivar susceptible to root parasites, *Striga (Striga asiatica)* and susceptible to rice blast disease | 700 to 1200 m                | Highly in some districts |
| Cultivar Code | Description | Altitude | Status |
|---------------|-------------|----------|--------|
| FOFIFA 182 (F182) | Cultivar tolerant to blast rice disease | 700 to 1200 m | Not yet distributed |
| FOFIFA 172 (F172) | Cultivar resistant to blast rice disease | 1200 to 1650 m | Poorly |
| FOFIFA 152 (F152) | Cultivar susceptible to blast rice disease | 1200 to 1650 m | Abandoned |
| FOFIFA 154 (F154) | Cultivar susceptible to blast rice disease | 1200 to 1650 m | Abandoned |
| NERICA 4 (N4) | Cultivar tolerant to root parasites, *Striga* (*Striga asiatica*) | 700 to 1200 m | Highly in some districts |
| Chhromrong Dhan (CD) | Cultivar tolerant to rice blast disease, optimal culture at an altitude of 1650 to 1900 m. | 1200 to 1900 m | Very highly |
| WAB 880-1-32-1-1-P2-HB-1 (W880) | Cultivar tolerant to blast rice disease | 700 to 1200 m | Not yet distributed |

\* see Raboin et al. 2013; Raboin et al. 2014.
The effect of rice cultivar on root (RB), shoot and root-adhering soil (RAS) dry mass, RAS:RB ratio, RAS respilation and pH after 32 days of growth in a poor-nutrient Malagasy Ferrasol.

| Rice cultivars | Root biomass (mg) | Shoot biomass (mg) | RAS (g) | RAS:RB ratio | RAS respiration (µg CO₂·kg⁻¹·d⁻¹) | RAS pH |
|----------------|-------------------|--------------------|---------|--------------|----------------------------------|-------|
| B22            | 62.7              | 63.7               | 62.4    | 3.3          | 62.4                             |       |
| F182           | 60.9              | 64.5               | 56.5    | 3.8          | 64.5                             |       |
| F172           | 41.9              | 53.2               | 68.4    | 6.5          | 6.5                              |       |
| F152           | 73.0              | 77.0               | 62.7    | 1.8          | 62.7                             |       |
| F154           | 80.4              | 77.0               | 44.2    | 1.7          | 44.2                             |       |
| N4             | 69.4              | 74.0               | 69.7    | 1.8          | 69.7                             |       |
| CD             | 69.9              | 74.0               | 62.7    | 1.8          | 62.7                             |       |
| AB             | 69.4              | 74.0               | 62.7    | 1.8          | 62.7                             |       |

Means (standard deviation) with n=5. Different letters (a, b, c and d) indicate significant changes according to One-way ANOVA and Tukey HSD post hoc tests at P < 0.05.