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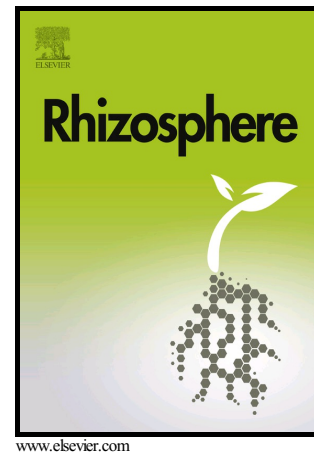
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Attractancy of bacterivorous nematodes to root-adhering soils differs according to rice cultivars

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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 *Acrobeloides* 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 farmers. (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 $\mu\text{M.m}^2.\text{s}^{-1}$ 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 μm 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 CO₂ 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_{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;

Trap et al. 2016). 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 ($R^2 = 0.79$, 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 *Acrobelloides* 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 *Acrobelloides* 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.

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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).

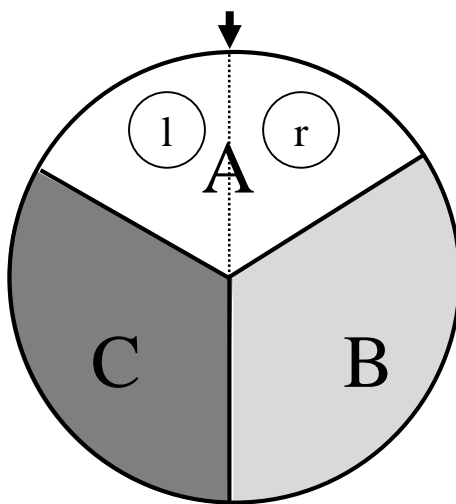


Fig. 2. Attractancy of nematodes to root-adhering soils (RAS) from eight upland rice cultivars (CD, F154, W880, F182, F152, N4, F172 and B22). The horizontal bars correspond to standard deviation. NS: not significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ according to a binomial test (5 replicates).

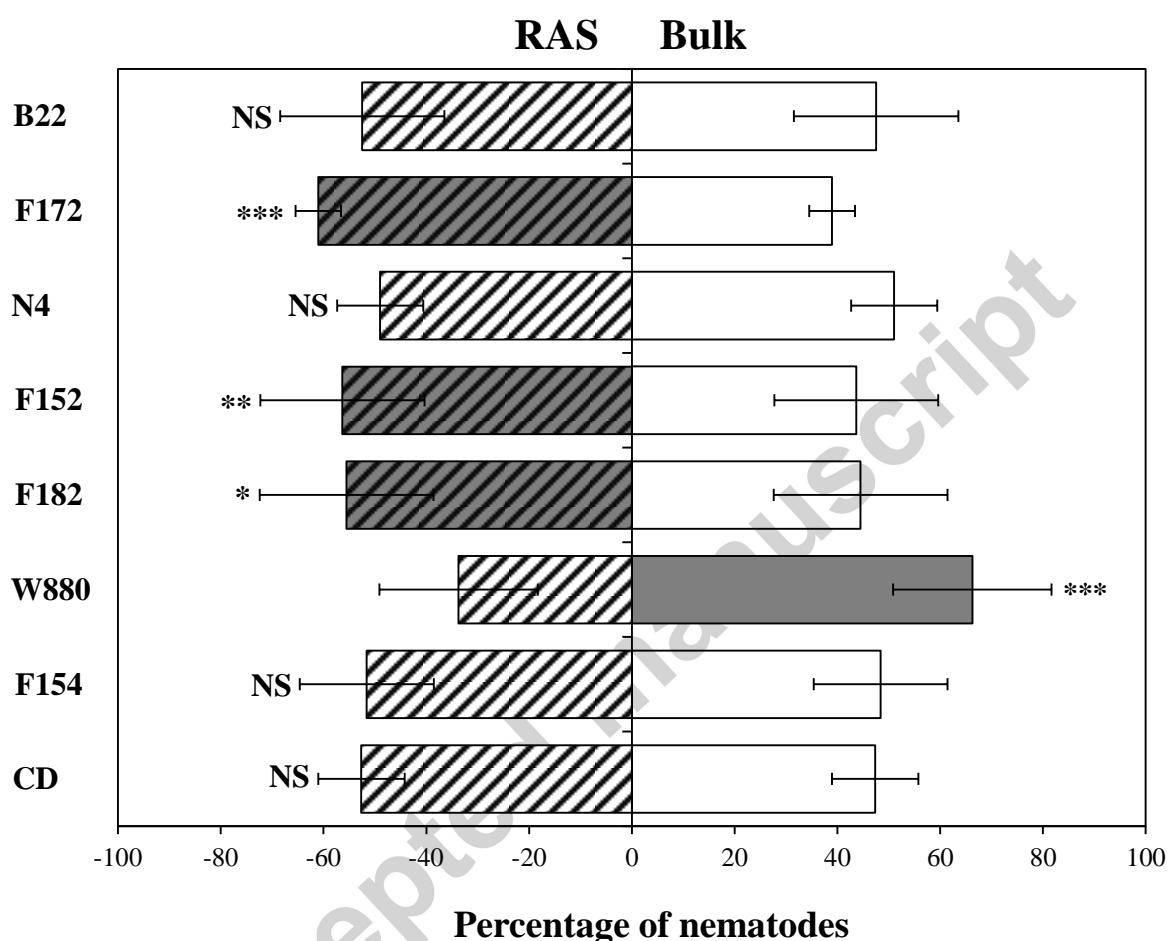


Table 1. Agronomic criteria used in the selection of the eight upland rice cultivars

Rice cultivars	Selection criteria	Optimal altitude for cropping	Adoption by farmers ^{\$}
B22	Cultivar susceptible to root parasites, Striga (<i>Striga asiatica</i>) and susceptible to rice blast disease	700 to 1200 m	Highly in some districts

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 (<i>Striga asiatica</i>)	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

^s see Raboin et al. 2013; Raboin et al. 2014.

Table 2. The effect of rice cultivar on root (RB), shoot and root-adhering soil (RAS) dry mass, RAS:RB ratio, RAS respiration and pH_{water} after 32 days of growth in a poor-nutrient Malagasy Ferrasol.

Rice cultivars	Root biomass	Shoot biomass	RAS	RAS:RB ratio	RAS respiration	RAS pH _{water}
	(mg)	(mg)	(g)	(kg.g ⁻¹)	(µg C-CO ₂ . dry g ⁻¹ .d ⁻¹)	
B22	62.7 (11.6) ab	63.7 (9.1) 62.4 (0.9) b	abc	3.3 (1.1) bc	62.4 (0.9) b	5.38 (0.05) ab
F182	60.9 (8.3) ab	64.5 (4.3) 56.5 (1.0) c	abc	3.8 (0.4) bc	56.5 (1.0) c	5.27 (0.09) b
F172	41.9 (13.8) b	53.2 (3.8) 68.4 (1.1) a	ab	6.5 (2.0) a	68.4 (1.1) a	5.39 (0.11) ab
F152	73.0 (9.7) ab	77.0 (7.7) 62.7 (1.8) b	abc	2.7 (0.8) bc	62.7 (1.8) b	5.38 (0.03) ab
F154	80.4 (20.4) a	71.7 (6.4) 44.2 (1.3) d	bcd	2.4 (1.0) c	44.2 (1.3) d	5.18 (0.17) b
N4	69.4 (18.2) ab	48.2 (5.9) 59.7 (1.5) bc	d	1.7 (0.3) c	59.7 (1.5) bc	5.65 (0.20) a
CD	69.9 (33.7) ab	56.2 (14.9) 44.2 (3.3) d	cd	3.0 (2.2) bc	44.2 (3.3) d	5.39 (0.09) ab
WAB880	53.6 (15.2) ab	56.8 (8.9) 59.8 (2.6) bc	a	5.2 (1.3) ab	59.8 (2.6) bc	5.43 (0.05) ab

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 $P < 0.05$.