**Functional Role of Mucilage - Border Cells:**
A Complex Facilitating Protozoan Effects on Plant Growth

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**Abstract:** In rhizosphere soil, mucilage and root border cells (RBCs) form a functional entity, the mucilage-border-cell complex (MB complex). Carbohydrates of the MB complex are utilized by rhizosphere bacteria, which are under strong grazing pressure of the soil food web, in particular protozoa. We investigated the role of the MB complex for protozoan effects on plant growth. First, the MB complex formed by 16 rice cultivars belonging to different ecotypes and subspecies were quantified. These cultivars were subsequently used to investigate protozoan effects on plant growth. The differences between the highest and lowest MB complex producers were 3.1 and 5.3 times for fully hydrated mucilage and RBCs, respectively. Mucilage production and RBCs showed a significant positive regression ($R^2=0.92$) in Japonica. Presence of protozoa generally enhanced shoot biomass, lateral root growth and plant nitrogen uptake. Further, upland cultivars showed significantly higher growth enhancement than lowland cultivars in presence of protozoa. A significant positive regression between MB complex and increased lateral root growth by amoeba revealed that the MB complex facilitated protozoan effects on plant growth, which is the first evidence for a new functional role of the MB complex.

**Key words:** Acanthameoba, Amoeba, Oryza sativa, Protozoa, Rhizosphere, Rice, Root exudation, Root cap.

Plant root caps are known to release both mucilaginous materials (mucilage) and the sloughed root cap cells (“root border cells” according to Hawes, 1990). The great majority of root carbon deposition in soil occurs in the form of polysaccharide rich mucilage exudation, mainly simple sugars, and organic and amino acids, but also hormones and phenolics (Nguyen, 2003). The contribution of root border cells (RBCs) would be only 5-10% in young maize seminal roots (Iijima et al., 2000). Although the contribution of RBCs is relatively small in terms of rhizo-deposition, living RBCs may play significant roles in the root-soil interaction (Hawes et al., 2003). The released mucilage and RBCs constitute a complex environment at the root-soil interface, where the RBCs are surrounded by the adhesive mucilaginous materials. Therefore, mucilage and RBCs are not easily separated from each other in the rhizosphere environment and are considered to form a functional entity in the real soil environment (Iijima et al., 2004b); henceforth we will call this entity the mucilage-border cell complex (MB complex). The MB complex is suggested to play important functional roles for plant root growth, such as reducing frictional resistance to root penetration (Iijima and Kono, 1992; Iijima et al., 2000, 2003b, 2004a, 2008) and being a source of energy and nutrients for soil microorganisms in the rhizosphere. Since microbial activity in soil is strongly limited by the availability of carbon from easily accessible organic substrates, rhizodeposits in the form of a MB complex give rise to strongly increased levels of bacterial biomass and activity around roots and to subsequent bacterial grazers, such as protozoa (Clarholm, 1985; Bonkowski, 2004).

The stimulation of microbial food web interactions in the rhizosphere may indirectly lead to a positive feedback on plant growth, due to grazing-induced changes in rhizosphere bacterial composition which have been shown to stimulate lateral root growth and root surface area (Jentschke et al. 1995; Bonkowski and Brandt, 2002; Kreuzer et al., 2006; Mao et al., 2006) and ultimately may lead to greater uptake of plant-available nutrients released from consumed bacterial biomass (Clarholm, 1985; Griffiths et al., 2007). We hypothesized that root exudates, including the MB complex, are a major energy source for rhizosphere bacteria, with potential influence on bacteria-protozoa interactions in the rhizosphere. Since rice has been cultivated for thousands of years, potentially altering naturally coevolved predispositions of root traits with rhizosphere microorganisms, we further suggested that rice would be an ideal model system to investigate the functional role of the MB complex for microbial
interactions in the rhizosphere.

Materials and Methods

1. Quantification of MB complex
   To estimate the differences in MB complex production, i.e., rhizodeposition, we measured the average production of fully hydrated mucilage and number of RBCs of different rice cultivars. Sixteen cultivars of rice, which belong to both upland and lowland ecotypes and “japonica” (JAP) and “indica” (IND) subspecies were used in this study (Table 1). The rice seeds were soaked overnight in the dark at 30ºC in tap water and subsequently germinated on paper towels saturated with distilled water for 48 hr in the dark at 30ºC. Three days after germination, seeds with straight seminal roots were selected and grown in water culture (6 L container filled with tap water and the pH was adjusted to 6.0 by adding 1 N HCl or 0.5 N NaOH) at 30ºC for three days, 12 hr photoperiod and 60% relative humidity. Aeration and/or water circulation was not done to avoid the mechanical stimulus to affect mucilage production. All the cultivars tested here showed healthy seminal root growth with the longest length in IRAT109 (11.5 cm) to the shortest in Aoinokaze (8.0 cm). Uniform-sized seedlings with a shoot height of 20 to 30 mm were selected and the fully hydrated mucilage was collected from the tips of the roots using weighed filter papers and a micro balance (METTLER MT5, Japan) with eight replicate plants per cultivar according to Iijima et al. (2003b). The fully hydrated mucilage was transferred to a glass slide. Subsequently, 190 μL distilled water plus 10 μL Toluidine blue O (3 × 10^{-5} g g^{-1}) were added and the numbers of RBCs floating in the fully hydrated mucilage were directly counted under a microscope at 100x magnification (Olympus 1X70, Japan), with three replicate plants per cultivar.

2. Protozoan effects on rice growth
   Growth of rice cultivars in the presence and absence of a common species of soil amoebae was used as a model system to estimate the response of different rice cultivars to the free-living microbial food web in the rhizosphere.

   (1) Preparation of an amoebal inoculum
   The initial numbers of protozoa were determined by a modified MPN-method (Darbyshire et al., 1974; Bonkowski et al., 2000). Briefly, 5 g soil fresh wt were suspended in 20 mL of Neff’s Modified Amoeba Saline, NMAS (Page 1976). This suspension was threefold diluted in 96-well microtitre plates (Iwaki, Japan). The plates were observed continuously for protozoan growth and protozoan densities were calculated according to Rowe et al. (1977). Amoebae in the highest dilutions were transferred to Petri dishes with NB-NMAS in 1% agar and incubated at 15ºC for 2 weeks for multiplication. An agar block containing a single amoebal cell was cut out and transferred to another Petri dish with NB-NMAS in 1% agar to start a pure culture of naked amoebae, which was later

| Cultivar   | Ecotype/Subspecies | Weight of fully hydrated mucilage (μg) | Number of root border cells |
|------------|--------------------|----------------------------------------|-----------------------------|
| Azucena    | Up JAP             | 101 ± 9 a                              | 618 ± 10 b                  |
| IRAT 109  | Up JAP             | 99 ± 12 a                              | 583 ± 31 bc                 |
| BPI - 76   | Up IND             | 93 ± 7 a                               | 818 ± 83 a                  |
| Norin 11  | Up JAP             | 86 ± 7 a                               | 401 ± 64 de                 |
| Nipponbare | Low JAP            | 64 ± 8 b                               | 296 ± 39 efg                |
| Aoinokaze  | Low JAP            | 61 ± 4 bc                              | 332 ± 71 def                |
| B4801F-MR5 | Up IND             | 58 ± 6 bc                              | 309 ± 74 defg               |
| Makiling   | Up IND             | 58 ± 5 bc                              | 361 ± 38 def                |
| Aichinokaori | Low JAP           | 56 ± 8 bcd                             | 280 ± 15 efg                |
| Yumenohatamochi | Up JAP       | 52 ± 3 bcde                            | 155 ± 24 g                  |
| Honenwase  | Low JAP            | 50 ± 3 bcde                            | 231 ± 27 fg                 |
| IR 36      | Low IND            | 42 ± 4 bcde                            | 284 ± 68 efg                |
| IR 72      | Low IND            | 41 ± 5 cde                              | 458 ± 47 cd                 |
| C22        | Up IND             | 34 ± 3 e                               | 203 ± 49 fg                 |
| Sinandomeng | Low IND            | 34 ± 4 de                              | 405 ± 42 de                 |
| C-4-63     | Low IND            | 33 ± 6 e                               | 559 ± 17 bc                 |

Values are mean ± SE of 5–8 replicates for mucilage and three for root border cells. Mean followed by same letters are not significantly different at 5% level by Duncan’s multiple range test.

Table 1. Ecotype/subspecies, weight of fully hydrated mucilage and number of root border cells in 16 cultivars of rice (Oryza sativa L.).
identified as *Acanthamoeba* sp according to Page (1976). For inoculation, amoebae were washed twice in NMAS by centrifugation at 500 rpm for 10 min to reduce the bacteria from amoebal cultures.

(2) Preparation of a bacterial inoculum

A slurry was prepared by mixing 200 g of the loamy sand soil with 3 L sterile tap water. The soil slurry was filtered through a paper filter and the filtrate subsequently filtered through 3.0 μm and 1.2 μm membrane filters to obtain a protozoa-free bacterial inoculum. The bacterial inoculum was incubated with 10 mL NB-NMAS at 15°C and checked over 5 days for protozoan contaminations.

(3) Preparation of rice seedlings

Rice seeds were husked by grinding lightly with a pestle in a mortar. Husked seeds were sterilized by incubation under rotation in 70% ethanol containing 1% (v/v) Tween 80 for 5 min, followed by incubation in a solution of chlorinated lime (active chlorine 7.2–12%, consisting of varying proportion of Ca(OCl)2, CaCl2, Ca(OH)2 and H2O in its molecular structure) for 20 min. They were washed 5 times with sterile water and soaked in sterile water for 3 hr. The seed sterilization with chlorinated lime and subsequent washing steps was repeated, before the sterilized seeds were separately incubated for germination in sterile NMAS.

(4) Set-up of the microcosm experiment

An air dried loamy sand (particle size distribution: sand 87.0%, silt 9.6%, clay 3.4%) collected from the fields located in the alluvial Kiso-river plane in Aichi prefecture, Japan, was sieved (2 mm mesh size) and air-dried. Autoclavable plastic containers (OS140 box with ODS green filter, Nacalai Tesque, Japan) were filled with 200 g of the dried soil, and wetted to 15% g g⁻¹ (Ψw = −25 kPa) initial moisture content and autoclaved at 120°C for 30 min. After cooling, the autoclaved soil in these microcosms was subsequently inoculated with 1 ml bacterial filtrate. Three days after germination, three sterile germinated seeds of each cultivar were planted in each microcosm container and the moisture content was adjusted to 27% g g⁻¹ (Ψw = −5 kPa) by adding sterile deionized tap water. After four days of planting, only one best performing plant was allowed to grow further in each container up to harvesting, and the soil moisture content was re-adjusted to the initial moisture level (gravimetric basis) by adding sterile deionized tap water.

After transplanting the seedlings, 1 mL amoebal inoculum in NMAS (approximately 25,000 amoebae) was inoculated close to the shoot base to half of the microcosms (amoebal treatment (+Amo)) and the remaining half received 1 mL of NMAS solution without amoebal inoculum (control treatment (−Amo)). The plants were grown at 25°C, 12 hr photoperiod with the photosynthetically active radiation of 175 μmol m⁻² s⁻¹ and 60% relative humidity for 14 days in a growth chamber.

(5) Shoot and root analyses

Two weeks after sowing, plants were harvested and soil was washed off the root systems by a gentle stream of tap water. The washed root systems were preserved in a formalin–acetic acid–70% ethanol mixture (FAA) for further analysis. Numbers of laterals and the length of seminal and nodal roots were determined by direct measurements. Subsequently, root length was measured according to Kimura et al. (1999) and Kimura and Yamazaki (2001). Briefly, the roots were spread and mounted on the scanner bed. The digital output from the scanner was processed by the public domain NIH Image program (National Institutes of Health, 1996). Root lengths were calculated from the obtained binary image. Subsequently, shoots and roots were oven dried at 80°C for 48 hr to determine dry weight values. Dried and milled shoot samples were analyzed with an elemental analyzer (CHN CORDER, Yanaco, Co.Ltd., Kyoto) for carbon and nitrogen concentration.

3. Data analysis

Seedlings were arranged in a completely randomized design, with eight and three replicates for the quantification of mucilage and numbers of RBCs, respectively. Four to nine replicate containers were set up to investigate subsequently protozoan effects on rice growth. Single regressions of mucilage and RBCs with measures on relative plant performance in the presence of protozoa (ratio: growth in presence/growth in absence of amoebae) were examined to test if the cultivar-specific production of MB complex generally affected the response of plants to the presence of amoebae. Since parameters describing root architecture, shoot growth and nutrient uptake by a plant are not independent from each other, the data were analysed by multivariate analysis of variance (MANOVA; Wilk’s lambda, followed by ANOVAs (analysis of variance) in case of significant effects (i.e., “protected ANOVA”; Scheiner and Gurevitch, 2001). Variables analyzed in ANOVA were ecological grouping (Eco: lowland vs. upland), subspecies (Sub: IND vs. JAP) and amoebae (Amo: without amoeba vs with amoeba) and their interactions on plant performance such as biomass, root architecture and nutrient uptake. Principal component analysis (PCA) was used to identify protozoan effects on patterns of rice root growth (ratio of +Amo : −Amo) in respect to weight of fully hydrated mucilage and number of RBCs. The analyses were implemented using statistiXL (statistiXL, Kalamunda, Western Australia).

Results

1. Quantification of MB complex

The 16 rice cultivars strongly differed in their cultivar-specific production of MB complex (Table 1). Azucena

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**Table 1**: Characteristics of rice cultivars

| Cultivar      | Photoperiod | RBCs | Mucilage |
|---------------|-------------|------|----------|
| Azucena       | 12 hr       | 2500 | 10000    |
| Kalamunda     | 12 hr       | 3000 | 15000    |
| IND           | 12 hr       | 4000 | 20000    |
| JAP           | 12 hr       | 5000 | 25000    |
| Lowland       | 12 hr       | 6000 | 30000    |
| Upland        | 12 hr       | 7000 | 35000    |

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For inoculation, amoebae were washed twice in NMAS by centrifugation at 500 rpm for 10 min to reduce the bacteria from amoebal cultures.
and IRAT 109 were the highest mucilage producing cultivars, producing three times (2.9–3.1) more mucilage than C22, Sinandomeng and C-4-63, the lowest mucilage producers. Similarly, the highest RBCs producing cultivar of BPI-76 showed 5.3 times higher border cell numbers than Yumenohatamochi, the cultivar with the lowest numbers of RBCs. The production of mucilage and RBCs was strongly correlated in Japonica subspecies (F\[1,6\] =71.87, P <0.0001, R\[2\]=92%) but not in Indica (F \[1,6\] =4.25, P =0.08, R\[2\]=41%) as shown in Table 2. Production of mucilage differed among the four types of cultivars and the greatest differences were observed between upland JAP and lowland IND (Fig. 1a). Both ecotype (F\[1,14\]=8.4, P =0.013) and subspecies (F\[1,14\]=6.6, P =0.025) strongly differed in the production of mucilage. Overall, Indica produced 31% less mucilage than Japonica (F\[1,14\]=4.49, P =0.053) and lowland cultivars produced 35% less mucilage than upland cultivars (F\[1,14\]=6.59, P =0.022). In contrast, RBCs production did not differ between ecotypes (F\[1,14\]= 0.7, P =0.426) and subspecies (F\[1,14\]= 0.5, P =0.506).

2. Protozoan effects on rice growth

Fig. 2 shows the individual growth response of 16 cultivars to amoeba infection. Generally speaking, most of the cultivars showed growth enhancement in amoeba treatment. Significant differences in plant performance existed between Indica and Japonica subspecies and treatments without and with amoebae.
Root biomass, axile root length (i.e. total length of seminal and nodal roots), number of axile roots and lateral roots (i.e. secondary roots emerging from the seminal root) increased by 21, 21, 24 and 22% respectively, in the presence of amoeba. Interactions among the three factors were found only for the ecotype x subspecies in the number of laterals with marginal significance (P = 0.069 for the interaction of Eco x Sub, Table 3). In amoeba treatments shoot biomass increased by 34 and 48% and total nitrogen uptake by 33 and 50% in upland IND and upland JAP; respectively. In addition, total root length increased in upland IND by 34% in the presence of amoeba (Fig. 3). Since rice cultivars differed in plant performance, ecotypes and subspecies were separately evaluated for their response to amoeba. In general, amoeba increased shoot biomass and total nitrogen uptake by 21% and number of lateral roots by 20% in upland compared to lowland cultivars. In contrast, subspecies did not show any significant difference (Table 4).

Finally, single regressions of mucilage and RBCs with measures on relative plant performance in the presence of protozoa was examined to test whether the cultivar-specific production of MB complex was correlated with the response of plants to the presence of amoebae (Table 5). The average amount of mucilage produced per cultivar was positively correlated with the effect of amoebae on the increase in lateral root numbers (Table 5, F[1,14] =5.97, P =0.028, R²=30%), but not with RBCs (Table 5, F[1,14] =1.17, P =0.297, R²=0.08%).

Principal component analysis (PCA) was performed

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### Table 3. Multivariate (Wilks' lambda) and univariate F statistics of ANOVA of the effects of upland or lowland ecotypes (Eco), Indica or Japonica subspecies (Sub), presence of amoebae (Amo) and their interactions on selected plant traits of rice (*Oryza sativa* L.).

| Source of variation          | Wilks lambda | Shoot biomass | Root biomass | Total root length | Axile root length | Axile root number | Lateral root number | Total carbon | Total nitrogen |
|-----------------------------|--------------|---------------|--------------|-------------------|------------------|--------------------|---------------------|--------------|---------------|
|                             | dfa F dfb F  |               |              |                   |                  |                    |                     |              |               |
| Eco (15,10)                 | 2.187 (7,24) | 0.224 8.016** | 1.336        | 0.030 7.828*      | 0.028            | 0.212              | 0.296               |              |               |
| Sub (15,10)                 | 6.973** (7,24)| 0.469 9.014** | 0.030 13.461**| 0.097 2.394       | 0.402            | 0.151              |                     |              |               |
| Amo (15,10)                 | 5.102** (7,24)| 9.937** 7.989**| 3.376†       | 3.762 18.304***   | 4.374†           | 9.823** 8.679**    |                     |              |               |
| Eco * Sub (15,10)           | 2.557† (7,24)| 2.968 0.430† 3.493† | 1.669 0.763 6.199* | 2.667 2.639       |
| Eco * Amo (15,10)           | 0.344 (7,24) 1.457 1.267 0.477 0.074 0.160 0.640 1.625 1.237 |
| Sub * Amo (15,10)           | 2.304† (7,24) 0.000 0.161 0.260 0.991 0.005 0.465 0.001 0.021 |
| Eco * Sub * Amo (15,10)     | 0.289 (7,24) 1.018 0.048 0.632 0.023 0.815 0.187 1.025 1.044 |

† P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001. df (degrees of freedom). dfa (Treatment, error) for multivariate analysis dfb (Treatment, error) for univariate analyses.

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![Fig. 3](image-url) Differences among lowland indica (Low IND), upland indica (Up IND), lowland japonica (Low JAP) and upland japonica (Up JAP) on shoot biomass (a), total root length (b), lateral root numbers in seminal root (c) and total nitrogen uptake (d). Vertical error bars represent standard error (n = 4). † and * indicates significant difference at P<0.10 and 0.05 by one way ANOVA.
to illustrate the clustering of rice cultivars with similar traits in the response to amoebae. Principal component 1 (PC1) explained 43.9% of the variation and mainly indicated the relative contribution of protozoan effects on shoot dry weight (0.98), carbon (0.98) and nitrogen uptake (0.99), root dry weight (0.82), and total root length (0.81) (Table 6). Principal component 2 (PC2) explained 20.6% of the variation (Fig. 4) and related to differences in the relative contribution of mucilage weight (0.89) and number of RBCs (0.78) (Table 6). These traits relate to the coupling of rhizodeposition with microbial interactions and plant productivity. Upland ecotype tended to exist in the positive sites of either PC1 or 2. In contrast, lowland cultivars tended to exist in the negative regions (Fig. 4). These results indicated that the upland cultivars responded well to the presence of protozoa.

### Discussion

Our data showed that plant growth promotion by soil protozoa strongly differs with the rice cultivar. Rhizodeposition has been assumed to be important for the coupling of plant and microbial productivity (Paterson, 2003). The MB complex apparently played a significant role in mediating bacteria-protozoa interactions, since mucilage weight was positively correlated with enhanced lateral root production in the presence of amoeba. In addition, the number of

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### Table 4. Ratio of growth enhancement by amoeba in ecotype (upland & lowland) and subspecies (Indica & Japonica) and their F values on the plant traits of shoot biomass, number of laterals, total root length and total nitrogen uptake.

| Plant trait          | Ecotype         | Subspecies         |
|----------------------|-----------------|--------------------|
|                      | Upland | Lowland | F   | Indica | Japonica | F   |
| Shoot biomass        | 1.41    | 1.17    | 5.775 * | 1.29   | 1.28     | 0.012 |
| Number of laterals   | 1.43    | 1.19    | 6.078 * | 1.25   | 1.37     | 1.126 |
| Total root length    | 1.34    | 1.12    | 4.306 †  | 1.31   | 1.15     | 2.067 |
| Total nitrogen uptake| 1.41    | 1.17    | 4.708 *  | 1.29   | 1.28     | 0.919 |

† P < 0.10; * P < 0.05.

### Table 5. Results of regressions between relative increase in lateral roots as dependent variable and fully hydrated mucilage or number of root border cells as independent variable of *Oryza sativa* L.

| Lateral roots | Regression model | R² | n  | P values |
|---------------|------------------|----|----|----------|
| Mucilage      | y = 0.98 + 5.45x | 0.30 | 16 | 0.028    |
| RBCs          | y = 1.17 + 0.0004x| 0.08 | 16 | 0.297    |

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### Table 6. Component loadings of principal component analysis (correlations between initial variables and principal components).

| Variable                        | PC 1     | PC 2     |
|---------------------------------|----------|----------|
| Mucilage weight                 | 0.169    | 0.885    |
| Number of root border cells     | -0.104   | 0.775    |
| Shoot height                    | 0.798    | -0.297   |
| Shoot dry weight                | 0.979    | 0.125    |
| Root dry weight                 | 0.823    | -0.244   |
| Shoot: Root ratio               | 0.321    | 0.554    |
| Number of lateral roots         | 0.533    | 0.520    |
| Axile root length               | 0.688    | 0.055    |
| Total root length               | 0.810    | 0.076    |
| Carbon %                        | 0.119    | 0.216    |
| Nitrogen %                      | 0.530    | -0.569   |
| Total C uptake                  | 0.978    | 0.141    |
| Total N uptake                  | 0.986    | 0.015    |
| Carbon: Nitrogen ratio          | -0.312   | 0.598    |

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### Fig. 4. Principal component analysis of 16 cultivars of rice (*Oryza sativa* L.) associated with amoebal effect, weight of fully hydrated mucilage and number of root border cells. Percentages in parenthesis show those contributed by the 1st and 2nd principal components.


RBCs was positively correlated with the increment of the MB complex weight, especially in Japonica cultivars (Table 2). In support of our findings, Kimura et al. (1989) and Lu et al. (2002; 2004) demonstrated a tight coupling of rhizodeposition with microbial biomass in the rhizosphere of wetland rice.

Hawes and Pueppke (1986) showed species-specific differences in the production of RBCs. Our study also demonstrated clear cultivar differences in both MB complex weight and RBCs production, which so far has not been reported before. These cultivar differences open the possibility to dissect the genetic basis that controls and contributes to the production of the MB complex, such as the size of the root cap, cap cell production rates, enzymatic activities to depauperate the cell wall connection, and so on. The cultivar differences in root border cell release and mucilage production (i.e. MB complex) will also be useful to further analyze the plant adaptation to soil environmental stresses. For example, under mechanically impeded conditions, daily rates of border cells release (Iijima et al., 2003a), production rates of root cap cells (Bengough et al., 2001; Iijima et al., 2004a), and rate of total root exudate production (Iijima et al., 2000; Rao et al., 2001; Iijima et al., 2003b) or secretory activity of the root cap (Iijima and Kono, 1992) increase as well as the increment of root radial expansion growth (Iijima and Kato, 2007) and enhanced surface water uptake (Iijima et al., 2007).

Our results provide evidence that the quantity of the MB complex is one of the factors that determine a positive feed back with free-living rhizosphere microorganisms.

Mucilage exudation was positively correlated with root elongation rate, implying that fast growing roots exude more mucilage (Iijima et al., 2003b). Although the elongation rates were not analyzed in this experiment, most of the upland JAP cultivars, which produced relatively more MB complex, also seem to have relatively higher elongation rates during the seedling stage. Such adaptations of specific cultivars to the natural soil environment may be crucial in low-input farming systems.

In agreement with previous studies (Jentschke et al., 1995, Bonkowski and Brandt, 2002; Kreuzer et al., 2006), distinct protozoan effects were coupled with an increased production of lateral roots (Figs. 2, 3 and Table 4). The increase in laterals plays a crucial role in plant development because they form the scaffold for the architecture of the branched root system (Malamy and Benley, 1997). Since the uptake of nitrogen increased parallel with shoot biomass (Fig. 2), it may be suggested that the increased root surface area in the presence of amoeba was more efficient in the uptake of nutrients released from consumed bacterial biomass in the rhizosphere. We assume that very young seedlings of cultivars, by producing more mucilage and fostering microbial rhizosphere interactions may gain a small growth-advantage relative to other cultivars that invest fewer resources in rhizosphere interactions. As more lateral roots are produced, the plant gains benefit through an increased root surface area, enabling more efficient uptake of nutrients, which are constantly released by protozoa from consumed bacterial biomass (Griffiths, 1994).

Upland cultivars showed a much stronger growth enhancement in the presence of amoeba than lowland cultivars (Table 4). Principal component analysis also indicated that the upland cultivars responded well to the protozoan infection (Fig. 4). Upland cultivar is known to have relatively higher drought resistance as compared with lowland cultivars, although both cultivars show similar trends under paddy soil anaerobic environment. Evolutionary adaptation to the water environment may have led to the different response of lowland vs upland cultivars to microbial interactions in the rhizosphere. It is interesting to note that the higher MB complex producers mostly belong to the upland group (Table 1). While some rice cultivars showed enhanced root growth in parallel to shoot biomass production and increased uptake of essential nutrients, such as nitrogen; others did not respond at all to rhizosphere microbial interactions. As rice is one of the oldest cultivated food crop species, specific plant traits that co-evolved between the ancestor rice species and soil microorganisms may have been lost during the selection of high yielding cultivars under modern high-input agricultural practice. Some rice cultivars tested in this study most probably lost specific genes responsible for the root growth response to microorganisms and protozoa.

In face of an increasing demand for rice to nourish a still increasing human population in Asia, increasing prices for fertilizers and consequently an increasing importance of extensive low-input farming systems, it seems timely and reasonable that plant breeding takes into account the growth potential offered by plant microbial interactions in the rhizosphere.

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