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Diclofop-methyl affects microbial rhizosphere community and induces systemic acquired resistance in rice

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ABSTRACT

Diclofop-methyl (DM), a widely used herbicide in food crops, may partly contaminate the soil surface of natural ecosystems in agricultural area and exert toxic effects at low dose to nontarget plants. Even though rhizosphere microorganisms strongly interact with root cells, little is known regarding their potential modulating effect on herbicide toxicity in plants. Here we exposed rice seedlings (Xiushui 63) to 100 μg/L DM for 2 to 8 days and studied the effects of DM on rice rhizosphere microorganisms, rice systemic acquired resistance (SAR) and rice-microorganisms interactions. The results of metagenomic 16S rDNA Illumina tags show that DM increases bacterial biomass and affects their community structure in the rice rhizosphere. After DM treatment, the relative abundance of the bacterium genera *Massilia* and *Anderseniella* increased the most relative to the control. In parallel, malate and oxalate exudation by rice roots increased, potentially acting as a carbon source for several rhizosphere bacteria. Transcriptomic analyses suggest that DM induced SAR in rice seedlings through the salicylic acid (but not the jasmonic acid) signal pathway. This response to DM stress conferred resistance to infection by a pathogenic bacterium, but was not influenced by the presence of bacteria in the rhizosphere since SAR transcripts did not change significantly in xenic and axenic plant roots exposed to DM. The present study provides new insights on the response of rice and its associated microorganisms to DM stress.

Introduction

Diclofop-methyl (DM) or 2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate is a phenoxypropanoic acid herbicide commonly applied on food crops around the world (Alban et al., 1994). Through inhibition of acetyl CoA carboxylase (ACCase) activity, DM strongly decreases fatty acid synthesis in graminaceous weeds and inhibits photosynthesis and meristem activity (Alban et al., 1994; Liu et al., 2008). However, a significant fraction of DM may penetrate into the soil surface after application and migrate in nearby ecosystems (Smith et al., 1986; Waite et al., 1992; Grover et al., 1997). Low residual DM and diclofop acid concentrations (at the ppb level) persisting in soils of agricultural areas could have negative effects on the

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metabolism and growth of other non-target plant species (Hoppe and Zacher, 1985; Qian et al., 2011; Ding et al., 2014a). A recent study conducted in our laboratory have showed that DM can be very toxic in rice grown in hydroponics since short-term (48 hr) exposure to 50 μg/L DM already inhibited rice root elongation during the early rice seedling stage (Ding et al., 2014a). In that study, we found that DM also increased citrate exudation by inducing citrate synthase (CS) transcription and activity as well as by increasing the transcription of anionic cell membrane channels (Ding et al., 2014b). This reported induction of citrate exudation upon DM stress is important since it could affect the (bio) chemistry of soil pore water (pH, organic carbon respiration), which could affect the community and abundance of bacteria colonizing the vicinity of plant roots and therefore possibly modulate plant nutrition and DM toxicity.

Plant root exudates sustain a high microbial activity and high microbial density, and the rhizosphere is thereby selectively enriched in some special microorganisms that to use specific plant exudates or to adapted to highly competitive environments and to the utilization of Yergeau et al. (2014). The rhizosphere is of central importance for plant nutrition and metabolism, but also for microorganism-driven carbon sequestration, ecosystem functioning and nutrient cycling in terrestrial ecosystems (Berg and Smalla, 2009). Rhizosphere microorganisms are indeed known to be affected by abiotic factors (such as pesticides) (Prithiviraj et al., 2007; Qian et al., 2015; Sillen et al., 2015; Somenahally et al., 2011) and interact continuously with plant roots. They can induce or delay flowering of *Arabidopsis thaliana* or crucifers (Panke-Buisse et al., 2015) and modulate resistance to plant disease and abiotic (*e.g.*, heat, salt and metal compounds) stresses (Khan, 2005; Rodriguez et al., 2008; Zollaa et al., 2013). The best-studied induced resistance response to abiotic stressors or pathogens in plants is by far systemic acquired resistance (SAR) (Baker et al., 1997; Hunt et al., 1996). Induced defense responses are regulated by a network of interconnecting signal transduction pathways in which the hormonal signals salicylic acid (SA) and jasmonic acid (JA) play a major role (Glazebrook, 2001; Pieterse and Van, 1999; Thomma et al., 2001). Studies in the literature have focused on the effects of pathogenic bacteria, fungus, insects and metal compounds on the induction of SAR (Gao et al., 2000; Weech et al., 2008), but, to our knowledge, the level of induction of SAR signaling pathway in relation to changes in rhizosphere microorganism community in plants exposed to pesticides is currently unknown. Given that pesticides can affect microorganisms in plant rhizosphere (Qian et al., 2015) and that crosstalk between bacteria and plant roots occur (Prithiviraj et al., 2007), more knowledge on the plant-microorganisms-pesticides interactions is needed to deepen our understanding of pesticide toxicity mechanisms in plants. Moreover, knowledge of the interactions among plant, abiotic stress and their microbial communities in the rhizosphere is important for developing sustainable management practices. Our study thus aimed to determine the response of the rhizosphere microbial community in rice to the presence of DM, the DM-induced signaling pathway in rice and the possible interplay between microorganisms, cell signaling and DM. Establishes the perfect mechanism for the effects of DM on plant, meanwhile, provided scientific basis for environmental safety assessment.

1. Materials and methods

1.1. Plant materials

Rice seeds (*Oryza sativa L. japonica* cv. Xiushui 63) were sterilized with 75% ethanol for 3 min and 0.1% HgCl for 15 min. The seeds were then thoroughly washed with sterile water. The sterile seeds were subsequently put on agar in sterile culture dish and heated at 30°C for 2 days for germination. Uniform rice seedlings were cultivated in Murashige and Skoog (MS) nutrient solution and exposed to 100 μg/L DM or 136 mg/L SA solution for 2 to 8 days. The plants were grown in a xenic (non-sterile) glass tubes for virtually all experiments. However, for the experiments aiming to determine the effect of rhizosphere microbes on SAR response to DM, we grow axenic cultures of rice seedlings in glass tubes (containing the Johnson medium) that have been autoclaved and covered with a 0.45-μm membrane filter. Rice cultures were performed at 25°C under a 12 hr light/12 hr dark cycle at a light intensity of 5000 Lux.

1.2. Extracellular oxalate and malate analyses by HPLC

After DM exposures, the root tissues of seven rice seedlings were rinsed 5 times with distilled water. The culture medium was collected to purify dissolved organic acids exuded by rice roots. The medium was then passed through a cation-exchange column (16 mm × 20 cm) filled with 5 g of Amberlite IR-120B resin (H^+ form) and an anion-exchange column (16 mm × 20 cm) filled with 2 g of Dowex 1 × 8 (100–200 mesh, for mate form). Organic acid anions were retained on the anion-exchange resin and eluted with 2 mol/L HCl. The eluate was then dried in a 40°C water bath. Purified organic acids were dissolved in 1 mL of Milli-Q water. Determination of dissolved oxalate and malate exuded in the culture medium was performed by HPLC according to the method described in Ding et al. (2014b).

1.3. Fluorescence staining of the rhizosphere microbial community and observation by confocal laser scanning microscopy

SYTO®13 (Sangon, Shanghai, China; excitation wavelength of 525 nm; emission wavelength of 595 nm), a cell permeable fluorescent nucleic acid dye, was used to stain root cells and rhizosphere bacterial cells. SYTO®13 was dissolved in 0.1% dimethyl sulfoxide. The 2 mL of SYTO®13 solution was added to plant root cells and root-associated bacteria, which were harvested on a blotting paper that was soaked into the culture medium at the surface of rice root tips. Plant cells and associated bacterial cells were let stained for 20 min in the dark at room temperature, and then washed with phosphate-buffered saline (PBS) before being observed under a confocal laser scanning microscope (Nikon, TE300, Japan).

1.4. RNA extraction of rice roots and real-time quantitative polymerase chain reaction (qRT-PCR) of key genes involved in SAR

Rice seedlings were frozen in liquid nitrogen, and total ribonucleic acid (RNA) was extracted in 1 mL of RNAiso

reagent according to the manufacturer's instructions (TaKaRa Company, Dalian, China). Total RNA was treated with DNase. The 500 ng RNA, which was measured by ultraviolet spectrophotometery (Bio Teke ND5000, China), was then reverse transcripted in cDNA using a M-MLV reverse transcript kit (TaKaRa Company, Dalian, China). The transcription level of 6 key genes involved in SAR (see Fig. S1 for a scheme of all genes involved in SAR) was measured by real-time quantitative RT-PCR (qRT-PCR) which was carried out on an Eppendorf Mastercycler ep Realplex4 (WesselingBerzdorf, Germany) using specific primers (Table S1). qRT-PCR was performed in 10-μL aliquots, containing 1 μL of cDNA, 0.2 μL of each primer (10 μmol/L, 5 μL of 2× mix buffer (Master mix, TOYOBO, Japan), and 3.8 mL of sterile distilled water. The samples were first heated at 95°C for 1 min and were then submitted to 40 cycles of heating at 95°C for 15 sec, and cooling at 60°C for 1 min as described in Qian et al. (2015). Gene transcription normalized to that of 25S rDNA was analyzed by the $2^{-\Delta\Delta Ct}$ method, where *Ct* is the cycle number at which the fluorescent signal rises statistically above the background.

1.5. Bacterial virulence assay and determination of bacterial growth on plant leaves

The pathogenic bacterium *Xanthonmonas oryzae* pv. Oryzae was grown in modified Watanabe nutrition medium. The bacteria were grown at 28°C with shaking at 200 r/min for 16 hr (initial pH 6.4–6.7). After 5 days of culture of the rice seedlings in presence (or absence) of 100 μg/L DM, we increased relative humidity to 100% and *X. oryzae* in the logarithmic growth phase (bacterial concentration in the inoculum of 3×10^8 CFU/mL) was injected into rice leaves with needleless syringes following the method described in Shen et al. (2012). Lesion lengths in leaves were measured after 5 days of *X. oryzae* infection.

1.6. Analyses of the community structure and abundance of microorganisms in rice rhizosphere

Illumina-pyro sequencing, a mainstream technology in microbial ecology, was used to analyze the community structure of microorganisms in the rice rhizosphere (Qian et al., 2015). Note that the definition we use here for the rhizosphere is that of Chaparro et al. (2004). Here the rhizosphere consists of three zones: the endorhizosphere (root tissue area), the rhizoplane (root surface with epidermis) and the ectorhizosphere (soil directly surrounding the root).

Plant rhizosphere genomics DNA was first extracted from the root material using the One-Tube DNA Out Kit (Sangon, Shanghai, China). The V3-V4 region of the 16S rRNA gene in the microbial genomic DNA was PCR-amplified using primers (forward primer, 5′-ACTCCTACGGGAGGCAGCAG-3′; reverse primer, 5′-GGACTACHVGGGTWTCTAAT-3′). The PCR products were second detected and quantified using dual-indexing amplification and sequencing approach on the IlluminaMiSeq platform and then the diversity of the rhizosphere microorganisms was analyzed using the QIIME software (version 1.6.0). Moreover, the abundance of bacteria in the rhizosphere was inferred by measuring the number of copies of 16S rDNA genes per mg root fresh weight (fw) using the primer pair of 16s-F and 16s-R (Table S1) and qRT-PCR.

1.7. Statistical analysis

Data were presented as mean ± standard error of the mean (SEM) and statistical significance was analyzed using the Origin 6.0 software (Microcal Software, Northampton, MA). Values were considered significantly different when the probability (p) was less than 0.05. All experiments were performed in triplicates.

2. Results

2.1. The release of organic acids from rice roots after DM treatment

Dissolved malate and oxalate (normalized per gram of root fw) in rice cultures exposed for 5 days to 100 μg/L DM increased by approximately 2.1- ($p < 0.001$) and 3.4-fold ($p < 0.001$) relative to the control, respectively. After 10 days of DM exposure, dissolved malate and oxalate concentrations were still significantly higher by 1.6- ($p < 0.01$) and 1.9-fold ($p < 0.01$) relative to those of the control, respectively (Fig. 1).

2.2. The effect of DM on microbial biomass and diversity in the rice rhizosphere

Results in Fig. 2a and b showed that the intensity of green fluorescence (*i.e.*, microorganism nucleic acids) surrounding the roots after 5 or 10 days of DM treatment was higher than that of the control, but the stimulatory effect of DM was weaker after 10 days than after 5 days. Similarly, the abundance of 16S rDNA genes in the rhizosphere strongly increased from 1.1×10^8 copies/mg root fw in the control sample to 1.01×10^{12} copies/mg root fw after 5 days of exposure to DM. However, the abundance of 16S rDNA genes did not increase significantly after 10 days of DM exposure when compared to the control.

The Illumina sequencing analyses show that DM not only influence the number of microorganisms in the rice rhizosphere, but also affect their diversity. A total of 6849 and 9115 operational taxonomic units (OTUs) were identified from the control and DM-treated group (for 5 days), respectively; and 7542 and 8536 OTUs were obtained from the control and DM-treated group (for 10 days), respectively. The results in Table 1 showed that the number of phylum, class, order, family, genus was 10, 18, 32, 48, 71 in the control group, respectively, but decreased to 6, 9, 18, 24, 32 after 5 days of DM exposure, respectively. A 10-day DM exposure also decreases the number of phylum, class, order, family and genus in the rhizosphere, but more weakly than after 5 days of DM exposure. Furthermore, richness and Shannon indexes also decreased after 5 or 10 days of DM exposure, but this decrease was more pronounced after 5 days than after 10 days of exposure (Fig. 3). The changes in the microbial community (at the genus level) of the rice rhizosphere due to DM treatment are shown in Fig. 3c. The genera *Massilia* and *Anderseniella* were affected the most by DM. Indeed, the proportion of

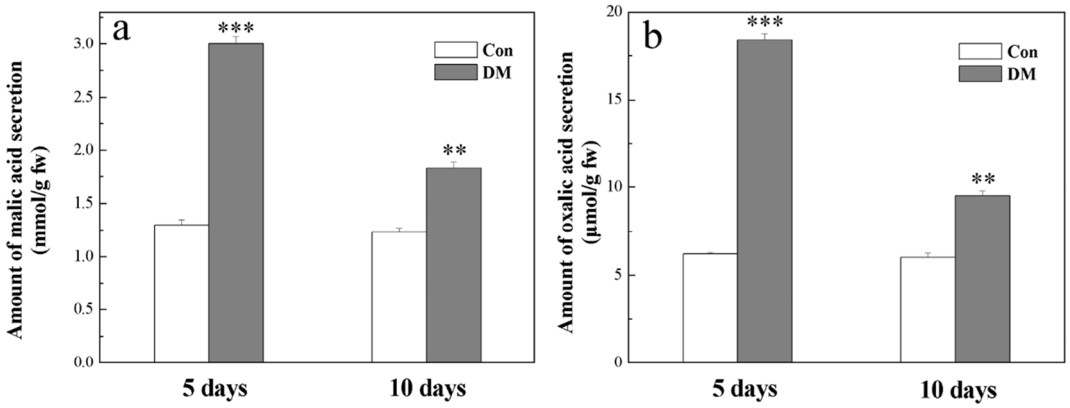

Fig. 1 – Dissolvedmalic acid (a) and oxalic acid (b) measured in the culture medium after 5 and 10 days of exposure to 100 μg/L DM (diclofop-methyl). The concentrations are normalized to root fresh weight. and *** represent statistically significant differences relative to controls at $p < 0.01$ and at $p < 0.001$, respectively. Error bars represent the standard errors of three replicates (Con: control).**

Massilia relative to all other rhizosphere microorganisms sharply increased from 15.1% in the control to 79.9% after 5 days of DM treatment ($p < 0.001$), and increased from 11.8% in the control to 78.5% after 10 days of DM treatment ($p < 0.001$). The number of *Anderseniella* strongly decreased from 46.7% in the control to 5.7% after 5 days of DM treatment ($p < 0.001$), and also decreased from 36% in the control to 6.1% after 10 days of DM treatment ($p < 0.001$). Also, the proportion of *Sphingomonas* increased from 0.47% in the control to 1.1% after 5 days of DM treatment ($p < 0.001$). The biomass of other microorganisms, such as *Candidatus*, *Odyssella, Sediminibacterium*, *Prochlorococcus*, also significantly increased upon DM treatment, whereas *Rhizobium*, *Stenotrophomonas*, *Methylophilus*, *Bradyrhizobium*, *Bacillus*, *Nevskia*, and *Mesorhizobium* significantly decreased after 5 and 10 days of DM exposure.

2.3. Effects of DM on the transcript of genes involved in SAR and on the resistance to infection by a pathogenic bacterium

We first analyzed the transcript of a key SAR gene, *NH1* (an *Arabidopsis* NPR1 ortholog (Non-expressor of *PR1*), a key regulator of the SA and JA-mediated disease resistance of *Arabidopsis* (Dong, 2004), and found that the abundance of *NH1* increased to approximately 3.62- ($p < 0.05$) and 2.71-fold ($p < 0.05$) of that in the control after a 2 days exposure to 136 mg/L SA or 100 μg/L DM, respectively. After 5 days of exposure; transcription of *NH1* also increased by 1.8 ($p < 0.05$) and 1.7-fold ($p < 0.05$) relative to that of the control, respectively, while it almost recovered to the base level in all treated groups after 8 days of exposure (Fig. 4a). The transcription level of *PR1a*, a defense-responsive gene involved in SA-dependent pathway, followed a similar trend than that found for *NH1* (Fig. 4b). The transcript of *PR1a* in SA- and DM-treated groups significantly increased by 1.7- to 2.9-fold relative to that of the control after 2 and 5 days of exposure, and then recovered to base level in the two treated groups (Fig. 4b).

We also analyzed the transcription of *EDS1* and *PAD4* genes, which participate in a defense amplification loop in response to SA and reactive oxygen intermediate-derived signals (Rusterucci et al., 2001). The transcription of *EDS1* and *PAD4* (Fig. 5a, b) after 2 and 5 days of exposure to SA or DM (no measurements were done after 8 days) followed a similar trend than that found for *NH1* and *PR1a*. Indeed, the 2 days SA

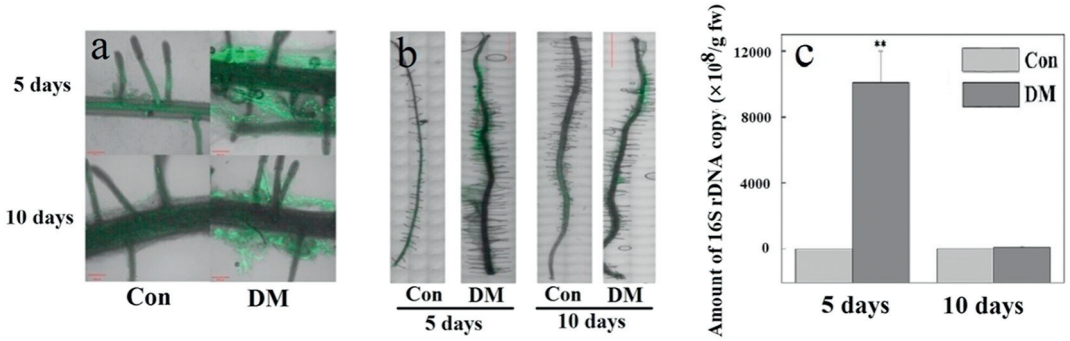

Fig. 2 – Microbial biomass in the rice rhizosphere inferred from fluorescence and 16S rDNA analyses after 5 and 10 days of exposure to 100 μg/L DM and in the controls. (a) Fluorescence signals obtained by confocal laser scanning microscope in the elongation zone; (b) Fluorescence signals obtained by confocal laser scanning microscopy for the 3 first cm of the root cap; (c) 16S rDNA (**) represent statistically significant differences relative to controls at $p < 0.01$.

Table 1 – The classification of microbial rhizosphere community after DM treatment.

Classification	Control 5 days	DM 5 days	Control 10 days	DM 10 days
Phylum	10	6	9	7
Class	18	9	16	11
Order	32	18	30	19
Family	48	24	47	29
Genus	71	32	71	40

and DM treatments increased the transcript of *EDS1* by 4.14- and 3.68-fold relative to that of the control ($p < 0.05$), respectively (Fig. 5a). The 5 days of SA and DM treatments also induced the transcription of *EDS1* by approximately 3-fold relative to that measured in the control ($p < 0.05$) (Fig. 5a). After 2 or 5 days of SA and DM treatment, the transcription of *PAD4* was also induced to more than 2-fold of the control transcription level (Fig. 5b). The transcription levels of *EDS1*, *PAD4*, *NH1* and *PR1a* increased to the same extent in sterile or non-sterile rice roots upon DM exposure for 2 and 5 days (Fig. S2).

By contrast to the genes involved in SA signaling, the transcripts of two genes (*AOS2* and *LOX*) of the JA signaling pathway were not significantly affected by the SA and DM treatments (Fig. 5c, d). Regarding the effect of DM on rice resistance to a pathogenic bacterium, we found that DM exposure triggered resistance in rice to *X. oryzae* infection. Indeed, the average lesion length in rice leaves due to *X. oryzae* infection was 2.7 cm in the control group (without DM), but only 0.9 cm and 1.1 cm in DM non-sterile and DM-sterile groups, respectively (Fig. S3).

3. Discussion

Residual DM concentrations affect cell wall composition, citrate metabolism and exudation, and modulate the activity of antioxidant enzymes through induction of oxidative stress in rice roots (Ding et al., 2014b). Here we found that exposure

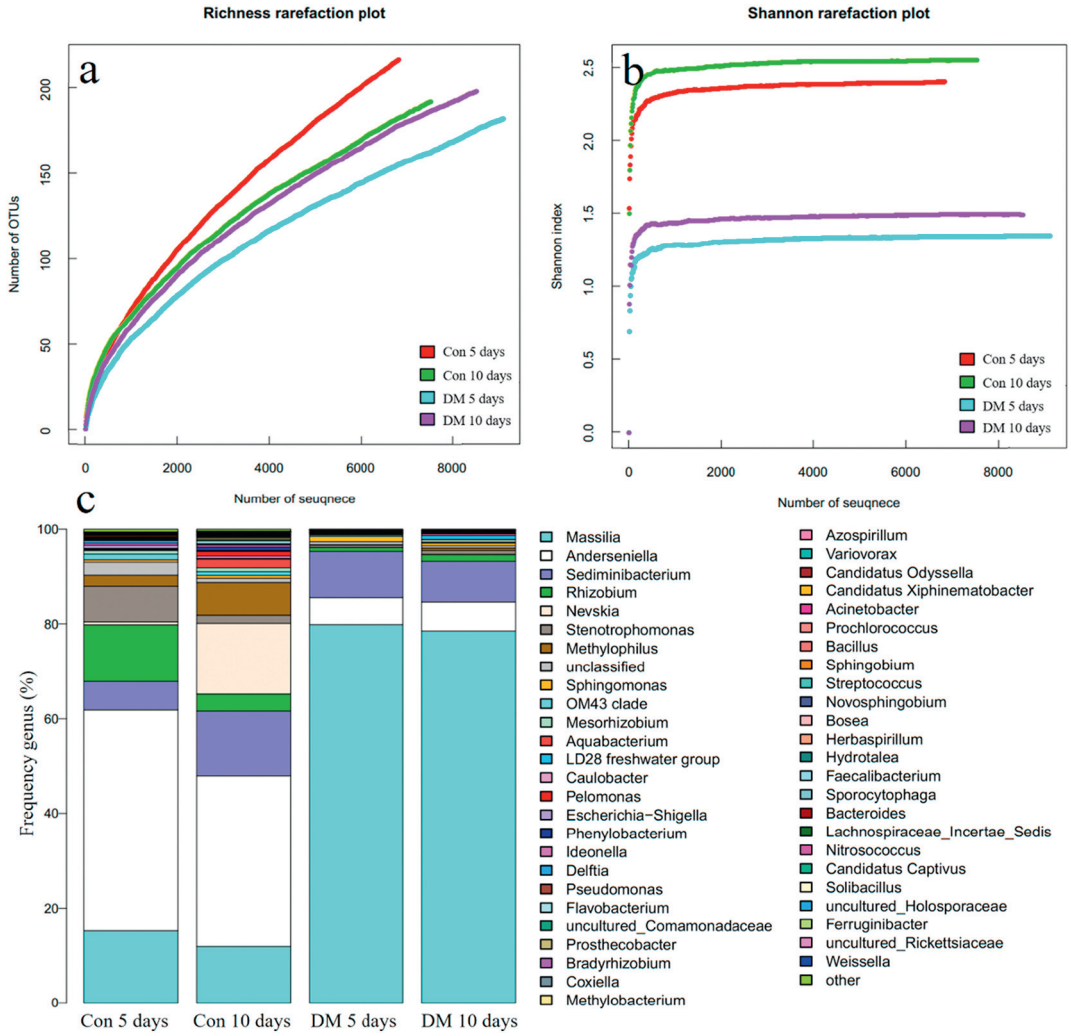

Fig. 3 – Analysis of the bacterial diversity in the rhizosphere in the controls and after 5 or 10 days of exposure to 100 μg/L DM. (a) Richness index; (b) Shannon index; (c) Relative abundance of all bacteria genera measured by miSeq-pyrosequencing of the 16S rDNA gene.

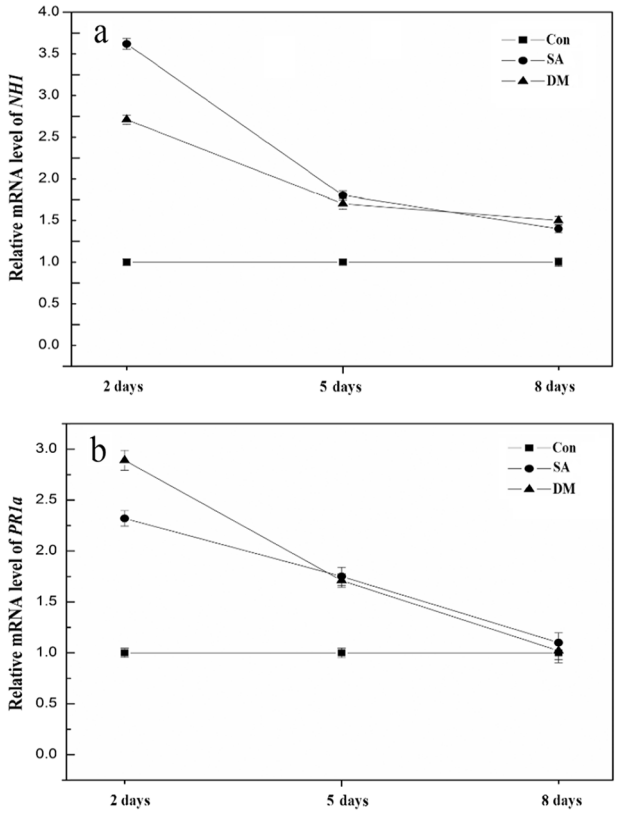

Fig. 4 – The effect of a 100 μg/L DM or a 1 mmol/L SA (salicylic acid) exposure for 2, 5, and 8 days on the transcription level of the *NH1* (a) and *PR1a* (b) gene. Transcription levels of each gene were normalized to that of the gene coding for 25S rDNA. Error bars are the standard errors of three replicates.

of rice seedlings to 100 μg/L DM for 5 days and also for 10 days (although to a smaller extent) increased dissolved malate and oxalate concentrations per unit of root fresh weight (Fig. 1). This increase in dissolved organic acid concentration measured after 5 or 10 days of DM exposure combined with an increase in the absolute or relative abundance of rhizosphere microorganisms that can potentially use these carbon sources for growth (for a more detailed interpretation of the abundance and diversity of rhizosphere microorganisms, see the two next sections of the discussion) suggest that the release of malate and oxalate by rice roots increased under DM exposure. As an ACCase inhibitor, DM could decrease acetyl-CoA utilization for fatty acid synthesis, which could in turn stimulate organic acid (*e.g.*, citrate, malate, and oxaloacetate) synthesis through the TCA cycle. Oxaloacetate can then be converted in oxalate from an oxaloacetase. Oxalate, malate, and other organic acids produced in excess in root cells exposed to DM could be excreted by specific anion channels as demonstrated for citrate in rice roots (Ding et al., 2014a). Another possibility would be that the protonated organic acid species passively diffuse across the plasma lemma of root cells. Further studies are needed to unravel the specific mechanisms explaining the measured efflux of organic acids in rice roots.

We measured an increase in the biomass of microorganisms in the rhizosphere of rice exposed to DM using fluorescence staining and qRT-PCR assays of 16S rDNA (Fig. 2). Most of rhizosphere microbes accumulated in the root elongation zone, likely because root exudates are mainly released in the top part of the root elongation region (Warrant and Gunther, 1999) and root exudates promote microbial accumulation (Saeki et al., 1996). Organic molecules secreted by rice roots are an important energy source and carbon skeletons for the growth of rhizosphere microorganisms and hence the diversity and abundance of microbes in the rhizosphere depends on the concentration and the specific root exudates present in the rhizosphere (Grayston et al., 1997). An increase in microorganisms density in the vicinity of the roots of the plant *A. thaliania* exposed to another herbicide, imazethapyr (IM), has also been recently demonstrated by Qian et al. (2015).

It is now recognized that rhizosphere microorganisms play an essential role in plant health and productivity and are often referred as the plant's second genome (Berendsen et al., 2002; Chaparro et al., 2004). The plant rhizosphere is a site of organic matter/nutrient circulation and energy flow in plant-microbe-environment. Carbon, nitrogen, phosphorus, sulfur, as well as decomposed organic matter can cycle repeatedly in this active zone of the soil (Schimel et al., 2000; Preston-Mafham et al., 2002). Specific bacteria species can be attracted by plant exudates and gather around plant roots and it has been recently shown that the herbicide imazethapyr (IM) can influence the diversity of rhizosphere microorganisms in *A. thaliania*. IM notably promoted the growth of acidophilic species, which were potentially attracted by organic acids released by plant roots (Qian et al., 2015). Here we show that a 5 days exposure to the herbicide DM also affects the diversity (richness, evenness) of microorganisms in the rice rhizosphere (Fig. 3). The marked increase in the density of the bacterial genus *Massilia* in the rhizosphere of rice exposed to DM (Fig. 3c) could be due to DM-induced changes in root exudation of organic matter. Indeed, *Massilia* belongs to Oxalobacteraceae (La et al., 1998), and the majority of Oxalobacteraceae can utilize oxalic acid, which was preferentially excreted under DM exposure (Fig. 1), as a carbon source for growth. Furthermore, *Massiliatimonae*, a common species of *Massilia*, has an important role in the plant defense since it can degrade oligosaccharides from chitin oligosaccharides and use that carbon source for growth (Gomez-Ariza et al., 2007). The increase in the proportion of *Sphingomonas* in the rhizosphere by around 2-fold (Fig. 3) is also interesting in the context of plant–bacteria–pesticide interactions. Since *Sphingomonas* an degrade a wide range of environmental pollutants (Fang et al., 2007), the presence of DM may have promoted the growth of pesticide-degrading bacteria belonging to this genus. In all, these changes may have a indirect effect on plant defense responses and the reduction of the soil contaminated.

Our results clearly show that rice roots did not respond to DM stress via the JA pathway of SAR since the transcription of *LOX* (lipoxygenase) and *AOS2* (allene oxide synthase 2), two major genes involved in the JA signaling pathway (Duan et al., 2014), were unaffected by DM. *EDS1* and *PAD4* are the signal genera located in upstream of SA-signaling pathway (Jirage

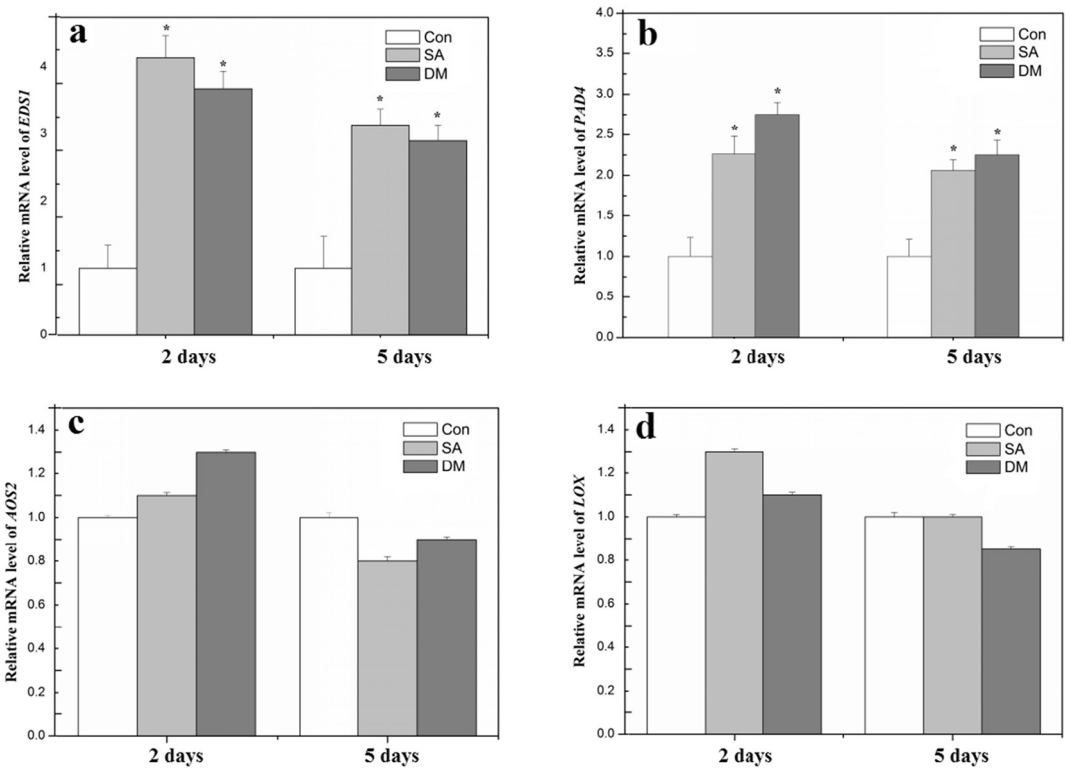

Fig. 5 – The effect of a 100 μg/L DM and 136 mg/L SA exposure for 2 and 5 days on the transcription level of the *EDS1* (a), *PAD4* (b), *AOS2* (c) and *LOX* (d) gene. Transcription levels of each gene were normalized to that of the gene coding for *25S rDNA*. Error bars are the standard errors of three replicates.

et al., 2001). In addition, overexpression of the *NH1* in rice also dramatically enhanced the disease resistance and constitutively activated defense-related genes (Fig. S1). Rather, we demonstrate that rice responds to DM stress via the SA pathway of SAR since all genes involved in the SA pathway of SAR were up regulated after 2 or 5 days of DM exposure and the up-regulation pattern measured for DM or SA exposed rice was very similar for all genes examined (*NH1*, *PR1a*, *EDS1*, and *PAD4*).

We further demonstrate that the DM-induced SAR response was not mediated by changes in bacteria abundance in the rhizosphere since SAR transcript did not change significantly in xenic and axenic plant roots exposed to DM. Moreover, the protection to infection by pathogenic bacteria afforded by DM-induced SAR was not affected by the presence of bacteria in the vicinity of rice roots. Our transcriptomic results, suggest that the SA-mediated SAR response to DM does not involve crosstalk between bacteria and root cells. Nevertheless, our results clearly show that DM exposure can extensively modulate the abundance and diversity of microorganisms in the rice rhizosphere. Even though several rhizobacteria bacteria are able to promote SAR response in plants and can help exclude pathogen bacteria (Maurhofer et al., 1998), it appears that the changes in the diversity and abundance of rhizosphere microorganisms in rice, at least under a short-term DM exposure (5 to 10 days), cannot influence the DM-induced SAR response in rice. Therefore, our results strongly suggest that DM-induced root exudates stimulated SAR against bacterial pathogens. This DM response may have several biogeochemical implications as suggested for the release of phenolic-related compounds in *Arabidopsis*, which can not only deter pathogens, but can also increase nitrogen and phosphorus uptake in conditions of growth limitation (Bednarek, 2002; Millet et al., 2010). These results highlight the multifaceted nature of the interactions between plant, stress and the rhizosphere microbiome.

4. Conclusions

This study brings new insights into the stress response of rice in the presence of DM. Our results suggest that exposure to a low DM concentration stimulates organic acid exudation by rice roots, which potentially affects microorganisms abundance and diversity in the rhizosphere. Rice roots respond to DM stress by inducing the SA-mediated pathway of SAR. This stress response decreases the susceptibility of rice to disease associated to pathogenic bacteria. However, crosstalk between bacteria associated to rice roots did not influence the DM-induced SAR response as monitored via transcriptomic analyses.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.06.027.

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