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Legacy of land use history determines reprogramming of plant physiology by soil microbiome

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Legacy of land use history determines reprogramming of plant physiology by soil microbiome

Abstract:

Microorganisms associated with roots are thought to be part of the so-called extended plant phenotypes with roles in acquisition of nutrients, production of growth hormones, and defense against diseases. Since the crops selectively enrich most rhizosphere microbes in the bulk soil, we hypothesized that changes in the composition of bulk soil communities caused by agricultural management affect the extended plant phenotype. In the current study, we performed shotgun metagenome sequencing of the rhizosphere microbiome of the peanut (*Arachis hypogaea*) and metatranscriptome analysis of the roots of peanut plants grown in the soil with different management histories, peanut monocropping and crop rotation. We found that the past planting record had a significant effect on the assembly of microbial community in the peanut rhizosphere, indicating a soil memory effect. Monocropping resulted in a reduction of the rhizosphere microbial diversity, an enrichment of several rare species and a reduced representation of traits related to plant performance, such as nutrients metabolism and phytohormone biosynthesis. Furthermore, peanut plants in monocropped soil exhibited significant reduction in growth coinciding with a down-regulation of genes related to hormone production, mainly auxin and cytokinin, and up-regulation of genes related to the abscisic acid, salicylic acid, jasmonic acid and ethylene

production. These findings suggest that land use history affects on crop rhizosphere microbiomes and plant physiology.

Introduction

Soil microbial communities are key contributors to host nutrition, development, and immunity (Raaijmakers & Mazzola, 2016; Castrillo et al., 2017; Fitzpatrick et al., 2018). However, agricultural practices can drive the composition of plant-associated microbiomes to adapt the plant to biotic and abiotic stresses (Wagg et al., 2014). It has been shown that application of herbicides, pesticides, and tillage practices can lead to shifts in the rhizosphere microbial community compositions (Sessitsch et al., 2004; Griffiths et al., 2007; Lin et al., 2007; Garbeva et al., 2008; Schlemper et al., 2017), with possible consequences for crop performance (Lau and Lennon 2011; Wagg et al., 2014; Smith et al., 2016). In a comparison of plants grown in monocultures and mixtures it was found that the former had the lowest microbial diversity (Zuppinger-Dingley et al., 2014). Yet, the effect of continuous monocropping is not necessarily negative, as not exclusively pathogens but also antagonists of pathogens may become enriched (Santhanam et al., 2015). However, our understanding of how farming practices affect the rhizosphere community assembly remains limited. Therefore, it is essential to have a better understanding of the role of rhizosphere microbiomes in the functioning of crops (Janvier et al., 2007; Expósito et al., 2017).

Plants exude 5–21% of their photosynthetically fixed carbon through the roots (Marschner, 1995). Therefore, the rhizosphere is a hotspot of microbial activity, whereas the surrounding bulk soil is depleted in easily degradable organic matter (Lambers et al., 2009; Bakker et al., 2012; Berendsen et al., 2012; Chaparro et al., 2013; Niu et al., 2017). Different plants species select for different rhizosphere microbial communities and this is largely determined by the composition of rhizodeposits (Bulgarelli et al., 2015; Dawson et al., 2017). For example, addition of *p*-coumaric acid (a root exudate component) to the soil changes the organization and composition of the bacterial rhizosphere communities of cucumber seedlings (Zhou and Wu, 2012). In addition, stable isotope probing studies indicate that carbon fixed by the plant via photosynthesis is directly incorporated by specific bacterial taxa in the rhizosphere (Hernández et al., 2015). Therefore, we hypothesized that repeated planting of the same crop in a field would lead to a gradual enrichment of a species subset in the crop rhizosphere.

The performance of rhizosphere community, e.g., nutrient acquisition, growth hormone productions, and defense against diseases, is crucial to the plant phenotype (Edwards et al., 2015; Vandenkoornhuyse et al., 2015). Detailed investigations of the interactions between plants and microbial strains are performed to determine various plant responses to the rhizosphere microbes (Bais et al., 2004; van Loon et al., 2007; Bordiec et al., 2011; Josefina Poupin et al., 2013; Armada et al., 2018). According to a recent study, root exudate-mediated changes in the rhizosphere community of peanut (*Arachis hypogaea*) seedlings strongly

influence the physiology and further development of peanut plants (Li et al., 2014). In the present study, we aimed to decipher and link the impact of the cropping history on the peanut rhizosphere community and the resulting crop phenotype. Peanut plants were grown in soils with different cropping history (monocropped or crop rotation). The microbiome of the peanut rhizosphere was then assessed by shotgun metagenome analysis, and plant responses were evaluated by transcriptomics.

Materials and methods

Field trial and treatments

Field experiments were performed at a field station of the Chinese Academy of Sciences, Jiangxi Province, China (28°130' N, 116°550' E). Prior to the field experiment, the location had been fallow (from August 2011). In March 2012, the site was split into six plots (6 m × 10 m). The experiment included two cropping systems (treatments): (1) monocropping plots with peanut; and (2) rotation plots with a 2-year rotation of peanut alternated with other crops. Three plots (replicates) of the two cropping treatments were laid out in a randomized block design. For monocropping plots, peanut (*A. hypogaea*) was consecutively grown for four planting seasons (2012–2015) using the same peanut cultivar (Ganhua-5). In the rotation plots, peanut was grown in the first (2012) and third (2014) year, whereas maize (*Zea mays* L.) was grown in the second year (2013) and potato (*Solanum tuberosum*) in the fourth year (2015). In each growing season, the sowing or planting took place in April

and harvesting was done in August. The plots lay fallow after harvest until the following sowing. Commonly used management practices, including tillage, fertilizer application, and weed control, were applied manually. The experimental setup is summarized in Fig. 1, and a detailed description of the field-planting procedure is provided in the supplementary Materials and Methods. The soil in the study area is classified as Udic Ferrosol (FAO 1998 classification), and the physiochemical properties are summarized in Supplementary Table S1.

Peanut seedling cultivation in a pot experiment

On 25 March 2016, before the 2016 planting season, ca. 30 kg of the soil (0–20 cm layer) was randomly collected from each plot, uniformly mixed per plot after removal of visible plant material, and used for pot cultivation experiments in the greenhouse (Fig. 1). For each plot, five pots were filled with 3 kg of the sampled soil respectively and were sown with one surface-disinfect peanut seed (Ganhua-5). Hence, for each plot (six), there were five biological replicate pots, for a total of 30 experimental units (5 pots × 3 field plots × 2 crop systems). After 30-d cultivation, plants were carefully removed from the pots and rhizosphere samples were collected by brushing the soil adhering to the roots. The rhizosphere soil from the five pots corresponding to the same field plot was pooled. Hence, three independent replicates (field plots) of monocropping and rotation were used in subsequent analyses (Fig. 1).

The shoots and roots of peanut plants were separated, and washed with ddH_2O. The roots of all plants were scored for disease symptoms, snap-frozen in liquid

nitrogen, and stored at −80 °C until total RNA extraction. The same pooling strategy as that used for the rhizosphere soil was employed for peanut root samples, so that three replicates per cropping treatment were used for the peanut plant metatranscriptome analysis. Hereafter, we used the terms “monocropped peanut” and “monocropped peanut rhizosphere”, in reference to pot experiments with the soil from monocropped field plots; and “rotation peanut” and “rotation peanut rhizosphere”, in reference to pot experiments with the soil of rotation plots.

Determination of plant growth responses to bacteria extracted from the field soils

To assess plant growth responses to the microbial soil community, bacterial suspensions were first prepared. Briefly, for each field plot, soil equivalent to 5 g dry mass soil and 50 mL of sterile water were mixed on a rotary shaker (200 rpm) for 1 h, followed by 1-minute sonification at 47 kHz twice and shaking for another 0.5 h (Hol et al., 2015). Next, the suspensions were filtered through a 5-μm filter to remove a large proportion of fungal propagules (de Boer et al., 2015). In total, six bacterial suspensions were prepared, with three independent replicates per cropping system.

Peanut seedling cultivation under sterile condition was performed with slight modification of the method used by Li et al. (2009). First, peanut seeds were surface-disinfected as described in the supplementary Materials and Methods. Then, a well-grown and uncontaminated seedling was planted in a 200-mL beaker containing sterile vermiculite and 50 mL of sterile Hoagland’s nutrient solution (1⁄4 strength). Four 200-mL beakers were then placed in a 5-L beaker, covered with four

159 layers of sterile gauze to prevent microbial contamination (Figure 4b), and incubated
160 in a plant growth chamber (30 °C, 70% relative humidity, light intensity 500 μ Mm^{-2}
161 s^{-1}).

162 After 7 d of cultivation, 5 mL of bacterial suspensions from the monocropped or
163 rotation soils were added to the vermiculite in a 200-mL beaker; the same amount of
164 sterile water was used in controls. Each independent suspension representing a field
165 plot was used to treat four seedlings. After 20 d of incubation, the plant growth
166 status, i.e., plant height, fresh weight, and root length and weight were determined.
167 The data from four seedlings per field plot (bacterial suspension) were pooled,
168 resulting in three independent replicates per the original planting regime that were
169 used in statistical analyses.

170

171 **Metagenomic DNA analyses of the peanut rhizosphere community**

172 To obtain sufficient metagenomic DNA (2 μg per sample), 4–6 extractions per
173 rhizosphere sample were performed using the FastDNA SPIN kit for the soil (MP
174 Biomedicals, Santa Ana, CA, USA), and pooled. DNA concentration was determined
175 using NanoDrop spectrophotometer (Thermo Scientific, USA) and DNA integtity was
176 assessed by agarose gel electrophoresis. DNA libraries of ca. 300-bp fragments were
177 prepared using Covaris M220 (Thermo Scientific, USA), and were sequenced using
178 the Illumina Hiseq 4000 instrument (Illumina, USA). This yielded 30 Gb of data, 282M
179 reads in total, with an average read length of 151 bp (Supplementary Table S2). The
180 3'-end of each read was trimmed with FASTX using Sickle

(https://github.com/najoshi/sickle) at a quality threshold of 20. Read pairs with reads shorter than 50 bp were removed. The resultant set of high-quality reads (>97.1% of raw reads) was used in further analyses.

The assembly of metagenomes and protein-coding genes was performed as described previously (Rho et al., 2010; Luo et al., 2012). All genes in the catalogue were translated to amino acid sequences and aligned with data in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database v 59 using USEARCH (E<1×10^{-5}). Each protein was assigned a KEGG ortholog based on the best-hit gene in the KEGG database. The abundance of any KEGG ortholog was calculated as a sum of the abundances of genes annotated to the specific feature. The relative abundances of microbial taxa in the metagenome were estimated based on the best matching amino acid sequences using the MG-RAST server (Meyer et al., 2008).

Peanut plant metatranscriptome analysis

Plant RNA was isolated from the peanut roots using the Trizol® reagent (Invitrogen, Carlsbad, USA) method, following the manufacturer's instructions. The average sample RNA integrity number (RIN) was 8.1, as determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) (Supplementary Fig. S1). Poly(A) mRNA was then separated from the total RNA using oligo(dT) magnetic beads (Invitrogen) and fragmented into ca. 200-bp pieces using a fragmentation solution (Ambion, USA). These mRNA fragments were used as templates in a random

hexamer-primed cDNA synthesis reaction performed using reverse transcriptase (Invitrogen). Double-stranded cDNA was synthesized using the SuperScript Double-Stranded cDNA synthesis kit (Invitrogen). cDNA was then purified using the QIAquick PCR extraction kit (Qiagen, Germany) and, following end-repair and poly(A)-processing, ligated with sequencing adaptors. The libraries were prepared for sequencing on an Illumina HiSeq 4000 platform (Illumina, USA), following manufacturer's protocols.

Low-quality raw reads were discarded and the clean reads from each library were assembled using the *Arachis ipaensis* genomic sequence in SOAPdenovo (v1.05, http://soap.genomics.org.cn/soapdenovo.html). The *A. ipaensis* genome data were downloaded from the NCBI databases (http://www.ncbi.nlm.nih.gov/genome/35711, http://www.ncbi.nlm.nih.gov/genome/12052). The distribution of reads for reference genes was calculated and coverage analysis was performed using the alignment data. Gene expression levels were determined by RNA sequencing (RNA-seq) as reads per kb of exon model per M mapped reads (RPKM) using the Cuffdiff (http://cole-trapnell-lab.github.io/cufflinks/) (Mortazavi et al., 2008). Differentially expressed genes and the corresponding *p*-values were determined using the Cuffdiff algorithm. Fold-changes (as Log_2 Ratio) in expression were determined based on the normalized gene expression in each sample. Threshold value of false discovery rate (FDR) >0.001 and the absolute value of Log_2 Ratio>3 were used to determine the significance of the differences in gene expression between treatment conditions. To identify pathways that were significantly differentially expressed in peanuts from the

monocropped and crop rotation soils, KEGG enrichment analysis was performed. In that analysis, a *q*-value threshold of <0.05 was used to demonstrate significant enrichment of gene sets.

Statistical analysis

Statistical analysis of data was performed using the STAMP software (Parks et al., 2014), to identify differences in the taxonomical composition of bacteria from the moncropped and rotation peanut rhizosphere. Statistical significance for the relative abundances of microbial rhizosphere composition and the reporter pathways were determined using the Welch's *t*-test ($p<0.05$). The confidence interval was estimated using the Newcombe-Wilson method. We determined Shannon diversity indices with the 'vegan' package (Dixon, 2003) in R (The R Foundation for Statistical Computing). Principal coordinates analysis (PCoA) matrices were used to visualize the community structure of samples, using the generated taxonomic and functional abundance matrices. The PCoA plots were generated from the Bray–Curtis similarity index matrices of all samples and created using the PAST software program (Hammer et al., 2001). One-way PERMANOVA analysis was performed to test the effects of soil type on microbial composition and functional diversity.

For the functional analysis using KEGG orthologs, Wilcoxon rank sum test was used to test for differential abundances between groups, and *p*-values were corrected for multiple testing as previously described (Qin et al., 2012). The KEGG grouping of orthologs into pathways was used as input to the reporter feature

algorithm and for calculating reporter pathways with differentially abundant KEGG orthologs. Each pathway was then scored based on the contributing *p*-values of KEGG orthologs and direction by fold-changes in expression to calculate the global *p*-value for each pathway.

The annotated genes were inspected to identify ones involved in plant growth promotion, i.e., the production of indole acetic acid (IAA); solubilization of phosphate; synthesis of siderophores, acetoin, and 2,3-butanediol; suppression of pathogenic fungi; resistance to oxidative stress; and nitrogen and sulfur metabolism (as summarized in Supplementary Table S4). For the KEGG pathway analysis of the peanut transcriptome, all differentially expressed genes in the pathways were examined to uncover common expression patterns by KOBAS (http://kobas.cbi.pku.edu.cn/home.do). A heatmap of the clustered genes and samples was generated by complete linkage.

Accession numbers

The metagenome raw sequence data of peanut rhizosphere community and RNA-Seq reads were deposited in the Sequence Read Archive (SRA) service of the GenBank database under the accession numbers SUB4375926 and SUB4426379, respectively.

Results

Differential assemblage of rhizosphere microbial communities in monocropped and

rotation soils

We analyzed the rhizosphere metagenome of peanuts planted in soils from two cropping systems. The community composition and functions were first compared (the Bray-Curtis distance). The monocropped peanut rhizosphere harbored microbes whose phylogenetic and functional composition were distinct from those in the rotation rhizosphere (Supplementary Fig. S2a, Fig. 2a). Furthermore, the microbial community diversity in the monocropped peanut rhizosphere, as estimated by the Shannon indices, was lower than that of the rotation peanut rhizosphere (p<0.05, Fig. 2b).

Proteobacteria dominated the rhizosphere bacterial communities with 62.3–74.3% of all reads (Supplementary Fig. S3a). Gammaproteobacteria ($F_{1,5}$=49.7, p=0.002) and Betaproteobacteria ($F_{1,5}$=40.3, p=0.003) were significantly less abundant in the monocropped peanut rhizosphere than in the crop rotation peanut rhizosphere. By contrast, a slight increase (1-fold) was seen in the relative abundance of Deltaproteobacteria in the monocropped peanut rhizosphere ($F_{1,5}$=325.6, p<0.001). Acidobacteria were significantly less abundant in the monocropped peanut rhizosphere than in the rotation peanut rhizosphere ($F_{1,5}$=482.9, p<0.001), whereas Actinobacteria ($F_{1,5}$=73.5, p=0.001), Bacteroidetes ($F_{1,5}$=145.3, p<0.001), Firmicutes ($F_{1,5}$=535.7, p<0.001), Chloroflexi ($F_{1,5}$=1218.5, p<0.001), and Verrucomicrobia ($F_{1,5}$=691.3, p<0.001) showed the opposite pattern (Supplementary Fig. S3a). Significant differences in abundance were also observed for the two most abundant fungal phyla, Ascomycota (comprising 76% of all fungal

291 sequences) and Basidiomycota (13% of all fungal sequences) (Supplementary Fig.
292 S3b). The representatives of Archaea were significantly more abundant in the
293 monocropped peanut rhizosphere, in which Thaumarchaeota appeared to be
294 enriched (Supplementary Fig. 3c), than in the rotation peanut rhizosphere.

295 In-depth analyses were then performed at genus levels, and the dominant (>1%),
296 common (0.1–0.1%), and rare (<0.1%) genera were classified based on the relative
297 abundance of the respective sequences within the community sequences (Lynch and
298 Neufeld, 2015). The analysis revealed that the effect of the cropping system on most
299 of the dominant bacterial genera in the peanut rhizosphere was not pronounced
300 (fold-change <1), but the genera *Bordetella* ($F_{1,5}$=129.5, p<0.001) and *Burkholderi*a
301 ($F_{1,5}$=208.0, p<0.001) were significantly enriched in the monocropped peanut
302 rhizosphere (Fig. 2c). Among the common genera, *Ktedonobacter* ($F_{1,5}$=575.8,
303 p<0.001) was enriched more than 10-fold in the monocropped peanut rhizosphere,
304 whereas other genera did not vary appreciably with the cropping system
305 (fold-changes <1). Thirty-five rare genera were highly enriched in the monocropped
306 peanut rhizosphere (>5-fold increase in abundance) and over 150 genera were
307 somewhat enriched therein (>2-fold increase in abundance) (Fig. 2c). Notably, in the
308 monocropped peanut rhizosphere, some operational taxonomic units (OTUs)
309 annotated as *Ktedonobacter racemifer*, *Opitutus terrae*, *Thermomicrobium roseum*,
310 *Chloroflexus aggregans*, *Thermosediminibacter oceani*, and *Dehalogenimonas*
311 *lykanthroporepellens*were were overrepresented (>10-fold increase in abundance) as
312 compared to the rotation peanut rhizosphere.

A significant overrepresentation of the genera *Colletotrichum*, *Rhizoctonia*, *Rhizophagus*, and *Dactylellina* was observed in the monocropped peanut rhizosphere. By contrast, the relative abundance of *Penicillium*, *Aspergillus*, *Fusarium*, and *Trichosporon* genera was significantly higher in the rotation peanut rhizosphere than in the monocropped peanut rhizosphere (overall, $p<0.05$).

Differences in abundances of metabolic functions in the rhizosphere metagenomes of monocropped and rotation soils

Several metabolic pathways were differentially abundant in the rhizosphere metagenome of monocropped soil compared to those of rotation soil (Supplementary Table S5). The pathways that were enriched the most in the monocropped peanut rhizosphere included KEGG orthologs for bacterial chemotaxis ($F_{1,5}=114.3$, $p<0.001$), sphingolipid metabolism ($F_{1,5}=72.0$, $p=0.001$), inositol phosphate metabolism ($F_{1,5}=98.0$, $p=0.001$), starch and sucrose metabolism ($F_{1,5}=283.5$, $p<0.001$), nucleotide excision repair ($F_{1,5}=588.0$, $p<0.001$), phenylpropanoid biosynthesis ($F_{1,5}=60.5$, $p=0.001$), glycan degradation ($F_{1,5}=36.1$, $p=0.004$), and fructose and mannose metabolism ($F_{1,5}=108.0$, $p<0.001$) (Supplementary Fig. S2b). By contrast, a significant decrease of lipopolysaccharide biosynthesis ($F_{1,5}=288.0$, $p<0.001$), ABC transporter ($F_{1,5}=42.9$, $p=0.003$), and riboflavin metabolism ($F_{1,5}=4050.0$, $p<0.001$) functions was noted for monocropped rhizosphere samples.

With respect to the nutrient cycles, pathways involved in nitrogen metabolism

($F_{1,5}$=784.0, p<0.001), sulfur metabolism ($F_{1,5}$=72.0, p=0.001), and oxidative phosphorylation ($F_{1,5}$=19.4, p=0.012) were significantly underrepresented in the monocropped peanut rhizosphere (Supplementary Fig. S2b). Functions related to oxidative stress, peroxisome ($F_{1,5}$=60.5, p=0.001), and cysteine and methionine metabolism ($F_{1,5}$=216.0, p<0.001) were also underrepresented therein (Supplementary Fig. S2b).

Underrepresentation of genes involved in plant growth promotion in the rhizosphere of monocropped soil

Genes that were potentially involved in plant growth promotion were next identified among the annotated KO genes of KEGG orthologs (Supplementary Table S4). With respect to nitrogen cycling, the genes encoding nitronate monooxygenase [EC:1.13.12.16], nitrite reductase [EC:1.7.2.1], and nitric oxide reductase [EC:1.7.2.4] involved in dissimilatory and assimilatory nitrate reduction were less abundant in the monocropped peanut rhizosphere than in the rotation peanut rhizosphere (Fig. 3). The relative abundance of the n*ifU* gene encoding a nitrogen fixation protein was significantly reduced in the monocropped peanut rhizosphere (Fig. 3). Many genes encoding nonspecific phosphatases, such as phosphotransferase [EC:2.7.3.9], phosphoserine phosphatase [EC:2.6.1.52], 3 -deoxy -manno -octulosonate -8 -phosphatase [EC:3.1.3.45], phosphoglycolate phosphatase [EC:4.2.1.12], and inositol-phosphate phosphatase [EC:3.1.3.25], were identified whose abundance was significantly reduced in the monocropped peanut rhizosphere (Fig. 3). These encoded

357 enzymes catalyze the conversion of organic phosphorus into plant-available forms of
358 this element, thereby facilitating plant growth. In addition, the number of genes
359 involved in hydrogen sulfide (H_2S) production and sulfite biosynthesis was
360 significantly reduced in the monocropped peanut rhizosphere (Fig. 3). The number of
361 genes involved in the production of siderophores, such as genes encoding
362 acyl-homoserine-lactone acylase [EC:3.5.1.97] and diaminobutyrate-2-oxoglutarate
363 transaminase [EC:2.6.1.76], was also reduced therein, as was the number of genes
364 encoding 4-hydroxybenzoate 3-monooxygenase [EC:1.14.13.2] and diaminobutyrate
365 -2 -oxoglutarate transaminase [EC:2.6.1.76].

366 Plant hormones, e.g., the auxin IAA, are synthesized from tryptophan via three
367 alternative pathways: indolepyruvate, tryptamine, or indole-3-acetamide pathways
368 (Spaepen et al., 2007). The relative abundance of some genes encoding aldehyde
369 dehydrogenase [EC:1.2.1.5], nitrilase [EC:3.5.5.1], tryptophan 2,3-dioxygenase
370 [EC:1.13.11.11], and indolepyruvate ferredoxin oxidoreductase [EC:1.2.7.8], i.e.,
371 proteins that are involved in the indole-3-acetamide and indolepyruvate pathways,
372 was significantly reduced in the monocropped peanut rhizosphere (Fig. 3).
373 Underrepresentation of some Trp cluster genes, e.g., anthranilate synthase
374 [EC:4.1.3.27] and tryptophan synthase [EC:4.2.1.20], involved in the biosynthesis of
375 tryptophan, the precursor of IAA biosynthesis, was also observed. The recently
376 described volatile compounds acetoin and 2,3- butanediol directly affect plant
377 growth by stimulating root formation. Interestingly, genes encoding pyruvate
378 dehydrogenase [EC:1.2.5.1], alcohol dehydrogenase [EC:1.1.1.2], diacetyl reductase

379 [EC:1.1.1.4 1.1.1.- 1.1.1.303], *S*-(hydroxymethyl) glutathione dehydrogenase
380 [EC:1.1.1.284 1.1.1.1], and 4-hydroxyphenylpyruvate dioxygenase [EC:1.13.11.27], all
381 of which are involved in acetoin production, were underrepresented in the
382 monocropped peanut rhizosphere. The same was observed for the (*S*,*S*)-butanediol
383 dehydrogenase gene [EC:1.1.1.- 1.1.1.76 1.1.1.304], encoding a protein responsible
384 for the conversion of acetoin to 2,3-butanediol (Fig. 3).
385
386 **Lower plant performance in the monocropped than in rotation soils**
387 Monocropped peanuts were significantly smaller than those planted in rotation soils,
388 with a significant reduction of plant height, root length, and shoot and root weights,
389 but no root disease symptoms were observed (Fig. 4a). Similar observations were in
390 an independent experiment where the peanut plants were grown on vermiculite
391 inoculated with bacterial suspensions obtained from the soils from the two cropping
392 systems (Fig. 4b).
393
394 **Comparative transcriptome analyses of peanut roots**
395 Heatmap analysis revealed distinct patterns of genes expression in peanuts
396 cultivated in monocropped and crop rotation soils (Fig. 5a). Plant hormones are not
397 only essential for plant growth and development, but also play crucial roles in the
398 host-microbe interactions (Vleesschauwer et al., 2013; Alazem and Lin, 2015).
399 Consequently, the expression of plant genes involved in the synthesis of auxin,

cytokinin, abscisic acid (AA), salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) was evaluated (Fig. 5b). The transcriptome data revealed that in the auxin production pathway, the genes encoding auxin-resistant1 (AUX1), AUX/IAA, auxin response factor (ARF), and small auxin-up RNA (SAUR) were down-regulated in the monocropped peanut (Fig. 5b). In the cytokinin pathway, A-ARR and B-BRR transcription factor genes were also down-regulated, whereas genes encoding GID1 and transcription factors involved in gibberellin signal transduction were up-regulated. By contrast, most genes from the SA, JA, and ET signaling pathways were up-regulated, as also was the *ABF* gene that encodes a transcriptional repressor of AA synthesis (Fig. 5b).

Furthermore, many genes involved in plant responses to bacterial factors, including flagellin and EF-Tu, were up-regulated in the monocropped peanut (Supplementary Fig. S5). However, the expression of most genes involved in responses to fungal pathogens remained apparently unchanged. Some genes (e.g., *GLU2*) involved in glutamate synthesis for nitrogen metabolism were down-regulated in the monocropped peanut (Supplementary Fig. S5), while many genes involved in isoflavonoid biosynthesis and phenylpropanoid biosynthesis were up-regulated (Supplementary Table S5).

Discussion

The role of microbial rhizosphere communities in plant growth and health is widely investigated, with most studies focusing on the effects of beneficial bacteria (Mendes

et al., 2011; Berendsen et al., 2012; Philippot et al., 2013; Armada et al., 2018). However, understanding of how agricultural land practices manipulate rhizosphere community's assembly and thus influence plant productivity is needed (Chaparro et al., 2012). In the current study, we used the metagenome sequencing approach to characterize the composition and potential function of the microbial community in the rhizosphere of peanut cultivated in soils sampled from field plots with a history of continuous monocropping or crop rotation. The analyses revealed that the typically rare taxa, rather than dominant and common taxa, were highly enriched in the monocropped peanut rhizosphere, implying that colonization of the peanut rhizosphere by some species increased after continuous peanut culturing. To gain insight into the physiological mechanism underpinning the performance of microbial communities, we performed functional characterization of the metagenomes in conjunction with gene expression profiles of peanut plants. These analyses indicated that the microbial communities assembled in the peanut plant rhizosphere in the monocropped soil might be involved in reducing plant hormone signal transduction in the peanut.

Plants drive and shape the selection of rhizosphere microbes by secreting specific compounds in root exudates that can be utilized by microbes (Bakker et al., 2012; Berendsen et al., 2012; Chaparro et al., 2012; Mendes et al., 2013). The current study revealed that the cropping history affects the rhizosphere communities of subsequently grown peanut plants. This may coincide with land use history, i.e., planting of different crop species, in agreement with many studies that show that

444 soil microbial communities are affected by agricultural management practices (Xu et
445 al., 2009; Sugiyama et al., 2010; Hartmann et al., 2015). Since the host plants
446 alternate with other crop species during crop rotation, low abundance of certain
447 microbial species in the newly assembled rhizosphere microbes may be associated
448 with the selective effect of the preceding crop (Lennon and Jones, 2011; Dawson et
449 al., 2017; Niu et al., 2017). In monocropped systems, the same types of root exudates
450 are repeatedly released into the soil, which would stimulate the colonization of the
451 rhizosphere by certain microbial species. Several bacterial species, such as *K.*
452 *racemifer*, *Burkholderia* spp., and *O. terrae*, that are highly abundant in the
453 monocropped peanut rhizosphere, preferentially utilize specific root exudates (van
454 Passel et al., 2011; Zúñiga et al., 2013; Li et al., 2014), suggesting that the ability to
455 catabolize plant-supplied resources impacts microbial rhizosphere populations
456 (Lennon and Jones, 2011). However, an increased relative abundance of certain
457 bacteria would involve competition for resources and space, as in a typical
458 rhizosphere (Cottee-Jones and Whittaker, 2012; Jousset et al., 2014; Wei et al., 2015).
459 In the current study, we found a high relative abundance of energy consumption
460 pathways in the monocropped peanut rhizosphere, e.g., the inositol phosphate
461 metabolism, starch and sucrose metabolism, and various sugar degradation
462 pathways (Supplementary Fig. S2a). Importantly, enrichment of several functions,
463 e.g., bacterial chemotaxis and nucleotide excision repair, was observed; these
464 functions were shown to be involved in the rhizosphere competence of cultivated
465 model organisms (Taghavi et al., 2010; Fibach-Paldi et al., 2012; Centler and Thullner,

2015).

We extracted the rhizosphere community functions relevant to plant traits/growth development from the rhizosphere metagenome to associate them with the composition of the microbial assembly in the peanut rhizosphere (Supplementary Table S4). Overall, the relative abundance of specific genes was lower in the monocropped peanut rhizosphere than in the rotation peanut rhizosphere (Fig. 3), which may explain the observed reduced plant growth in the former. Recent studies of the functional attributes of *Arabidopsis thaliana* and the soybean rhizosphere point to the importance of mineral nutrient metabolism and iron acquisition for plant growth (Ofek-Lalzar et al., 2014; Mendes et al., 2014; Panke-Buisse et al., 2014). In the acidic soil used in the current study, limited quantities of soluble phosphate and the available nitrogen would restrict plant growth. We observed reduced abundance of the nitrogen metabolism genes, as well as phosphate solubilization and sulfur cycle pathways in the monocropped peanut rhizosphere. Another striking reduction in rhizosphere functions concerned the production of phytohormones, including IAA, and acetoin and 2, 3-butanediol synthesis. These compounds all promote plant growth by stimulating root branching and elongation (Overvoorde et al., 2011; Lavenus et al., 2013).

Furthermore, we observed that plant growth was significantly reduced after planting in the monocropped soil. The reduced plant growth-promotion ability, combined with the differences in the assembled rhizosphere communities uncovered in the current study, may therefore indicate that the microbial

488 community in the rhizosphere acts as mediator between the soil management and
489 plant performance, similarly to what has been recently determined for root
490 microbiome of diverse plant species (Fitzpatrick et al., 2018). Allelochemical
491 metabolites that accumulate in the soil as result of monocropping may also
492 contribute to the reduced peanut growth, however, this may not be the case in the
493 current study. First, the soils sampled for pot cultivation experiments have been
494 already fallowed for almost 8 months (August to the following April) after the
495 planting season. Therefore, the levels of allelochemical metabolites would be
496 generally below the phytotoxic dose, since they are easily degraded by the soil
497 microbes (Perry et al., 2007; Huang et al., 2013; Li et al., 2014). For instance, even
498 the highest levels of the so-called autotoxins detected in soil samples after
499 continuous cropping are far below the previously reported of allelopathic potential
500 (Blair et al., 2006; Perry et al., 2007). Second, the controlled experiments with
501 microbial suspensions extracted from the monocropped plot soils, reinforced the
502 roles of rhizosphere communities in reducing plant performance; in these
503 experiments, only limited amounts of allelochemicals would have been transferred
504 to the culture solutions had they co-extracted of allelochemicals with water. In fact,
505 these observations supported our hypothesis that the type of species-specific plant
506 rhizodeposits especially allelochemical, would lead to a different rhizosphere
507 community assembled in a subsequent plant and, consequently, plant phenotype.
508 However, more effort should be dedicated in the future to account for the possible
509 synergistic effects of microbes and allelochemicals in the soil in associated with plant

510 performance (Chaparro et al., 2012).

511 We then used high-throughput mRNA sequencing to compare the global gene
512 expression of peanut plants grown in monocropped and crop rotation soils. The
513 analysis indicated that plant hormone pathways are involved in the interactions
514 between the rhizosphere community and plants *in vivo*. Regulation of genes involved
515 in auxin, cytokinin, AA, SA, JA, and ET synthesis pathways might explain reduced plant
516 growth in the monocropped soil. For instance, the expression of many genes related
517 to the production of hormones, such as auxin, and cytokinin, was down-regulated in
518 plants grown in the monocropped soil. Meanwhile, the expression of genes related
519 to flavonoid biosynthesis was elevated in peanuts cultivated in the monocropped soil,
520 which may be linked to the reduced plant hormones levels (Deng et al., 2012;
521 Ferreyra et al., 2012; Son and Oh, 2013). Intriguingly, it is known that
522 over-production of such compounds as AA, SA, JA, and ET (suggested by the present
523 study in the monocropped soil) reduces plant growth (Yadav et al., 2005; Vicente and
524 Plasencia, 2011; Iqbal et al., 2017).

525 Peanut is a legume. Hence, the observed different genetic and physiological
526 responses of peanut roots to land use history would affect root nodule formation. For
527 example, according to many studies, changes in auxin balance in the host plant are a
528 prerequisite for nodule organogenesis (Spaepen et al., 2007; Peng et al., 2017).
529 Reduced expression of such auxin-responsive genes as GH3 and AUX1 in peanut roots
530 cultivated in monocropped soils observed in the current study may influence root
531 nodule formation of peanut during rhizobium–plant symbiosis, since the expression

of these genes is required for nodule initiation (Mathesius et al., 1998; de Billy, 2001; Takanashi et al., 2011). Moreover, several studies reported negative effects of SA, ET, JA, and AA signaling on the rate and intensity of rhizobial infection and nodulation (Van Spronsen et al., 2003; Stacey et al., 2006; Sun et al., 2006; Penmetsa et al., 2008; Breakspear et al., 2014). Therefore, plant hormone signal transduction induced by the assembled rhizosphere communities could explain the decreased nodules number in the roots of peanuts planted in monocropped peanut soils, a common phenomenon observed during legume monoculture (Zhang et al., 2018). On the other hand, changes in hormone signal transduction may reduce rhizobial colonization efficiency (Badri et al., 2008; Khan et al., 2012), since nodulation is an energy-consuming process and tightly depends on plant carbohydrate availability (Baier et al., 2007). Overall, these findings provide some clues about the possible mechanisms that regulate adaptive host-rhizobium symbiosis. Future studies are therefore required to unravel the genetic pathways that underlie the effect of peanut monocropping on root nodules formation, as well as rhizobium symbiotic behaviors (e.g., Nod factors).

In addition, microbial-associated molecular patterns recognized by the plant roots are essential for rhizomicrobial colonization but do not necessarily play a role in pathogenicity (Martin and Kamoun, 2012; Newman et al., 2012). Well-known examples are bacterial flagellin and EF-Tu (the major structural component of bacterial elongation factor and motility). Indeed, in the current study, we observed the up-regulation of genes in monocropped peanuts that are known to be responsive

554 to bacterial factors, including flagellin, EF-Tu, and bacterial secretion system, but not
555 to fungal factors. This suggested that certain bacteria exert higher pressure on the
556 root surfaces of monocropped peanut than on that of rotated peanut (Ofek et al.,
557 2014).
558
559 **Conclusions**
560 Genomic analyses of host-associated microbial communities elucidated the
561 functional importance of rhizosphere microbial associates. Our study revealed the
562 important effects of the agricultural cultivating history on the rhizosphere microbiota
563 associated with the current crops, and that the rhizosphere microbiome assembly is
564 tightly associated with the plants phenotype. The species that became enriched in
565 the crop rhizosphere after continuous monoculture may lead to a decline in
566 community function of the crop rhizosphere in cultivated soils. This may involve the
567 regulation of plant hormone signal transduction, with possible consequences on crop
568 performance. Overall, the presented results provide insight into the effect of land
569 use history on plant phenotype exerted via the selection of specific rhizosphere taxa,
570 and will serve to guide future plant microbiome research for improved plant
571 performance.
572
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586

587 **Author contributions**

588 X.G. and X.X. conceived the project and designed the experiments; X.G, W.D. and V.J.
589 analyzed the results with assistance from A.J., E.K. and X.X.; X.G. wrote the first draft
590 of the manuscript, and W.D., E.E., and T.L. contributed substantially to revisions.

591

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Figure legends

Fig. 1. Flow diagram of the key experimental arrangements in the current study. (A) Experimental plots were established on a representative upland field. (B) From March 2012, the experimental plots were managed under two cropping systems (treatments): a) peanut monocropping, b) 2-year rotation of peanut alternated with other crops. (C) At the start of the 2016 planting season, soil samples (0–20 cm layer) were randomly collected from each of the six plots and were used in pot cultivation experiments. For each field plot soil was transferred to five pots in which peanut plants were grown. (D) At the harvest the plants and rhizosphere soil samples from five pots per field plot were pooled. This resulted in three independent replicates for each field cropping system in the subsequent (E) analyses.

Fig. 2. a) Based on the lineage-specific weighted UniFrac analysis, the first (PC1) and second (PC2) principal coordinates explain the significant variations (p<0.05) in bacterial community of peanut rhizosphere cultivated in the monocropped and rotation soils. MP: peanut rhizosphere of the monocropped soil, RP: peanut rhizosphere of the rotated soil. **b)** Comparison of community diversity revealed significant lower in peanut rhizosphere cultivated in the monocropping soils than that in the rotation soils. “-1”, “-2”, and “-3” are replicate plot samples. Asterisk indicates significant differences of variable means between the monocropped and rotated soils (p<0.05). **c)** Fold-changes in the relative abundance of bacterial genera in the peanut rhizosphere cultivated in the monocropped soil, compared to that

cultivated in the rotation soil. Fold change is defined as (MP-RP)/MP, in which MP is the relative abundance of bacterial genera in the monocropped soil, and RP is the relative abundance of bacterial genera in the rotation soil. Red, fold changes>2. Dominant: >1%, common: 0.1–1%, rare: <0.1%.

Fig. 3. Main differentially abundant genes associated with plant growth promotion functions. Fold changes are defined as (MP-RP)/MP, in which MP is gene expression level in the monocropped soils, and RP is gene expression level in the rotation soil. Green, gene down-regulated in peanut rhizosphere of the monocropped soil; red, gene up-regulated in peanut rhizosphere of the monocropped soil. All genes associated with plant growth promotion functions are listed in Supplementary Table S5.

Fig. 4. a) Pots cultivation of peanuts. The experiment demonstrated that peanut growth (length indicated by the right y-axis and weight by left y-axis) was significantly lower in the monocropped soil than in the rotation soil. **b**) Sterile vermiculite cultivation of peanuts. The experiment revealed that peanut growth (length indicated by the right y-axis and weight by left y-axis) was also reduced upon exposure to bacterial suspensions extracted from the monocropped soils. MP, peanut grown in the monocropped soil; RP, peanut grown in the crop rotation soil. The mean values and standard deviations of three replicates are presented. Asterisk indicates significant differences of the variable means between the monocropped

872 and crop rotation soil samples (p<0.05).

873

874 **Fig. 5. a)** Heatmap showing the expression patterns of different genes of peanut
875 cultivated in the monocropped and rotation soils. The color bar represents the $\log_{10}$
876 (RPKM) value, ranging from green (-4.0) to red (4.0). Top, gene tree; right, sample
877 tree. MP, peanut grown in the monocropped soil; RP, peanut grown in the crop
878 rotation soil. “-1”, “-2”, and “-3” are samples from replicate plots. **b)** Analysis of the
879 expression of genes of the plant hormone signaling pathways in peanut. Colored
880 boxes indicate the expression of individual genes, and the heatmap in the chart plots
881 on the right indicates the expression levels of pathway genes in peanuts from the
882 monocropped soil relative to those in peanuts from a crop rotation soil. Green boxes,
883 down-regulated genes; red boxes, up-regulated genes.

A) Previous 2012

A dryland field

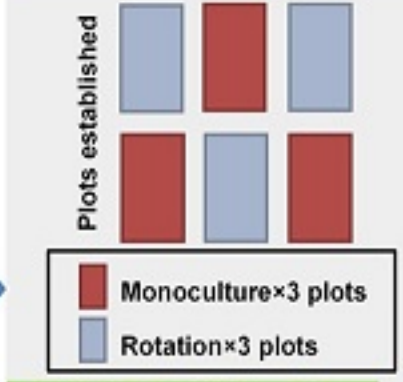
Cultivar history: Peanut, corn, melon, cereal etc.

Fallow history: From August 2011

Size: ca. 0.3 ha

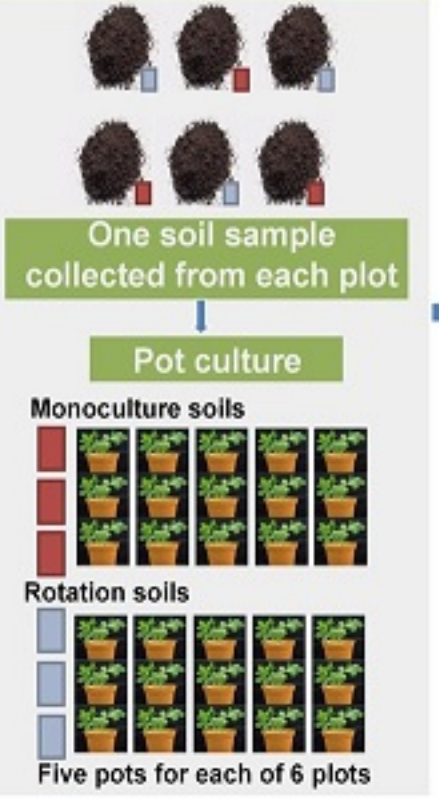
B) 2012 to 2015

Field Experiment



C) March 2016

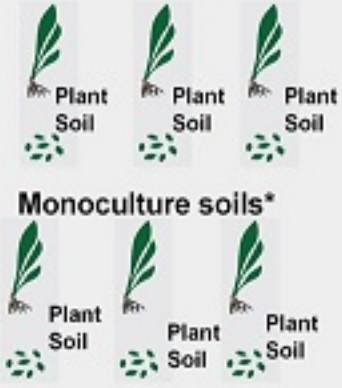
Pot Experiment



D) 2016

Samples collected

30 rhizosphere and plant samples



Notes: * material from replicate pots for each plot (five pots) was pooled, for three independent replicates per cropping system

E) 2016/17

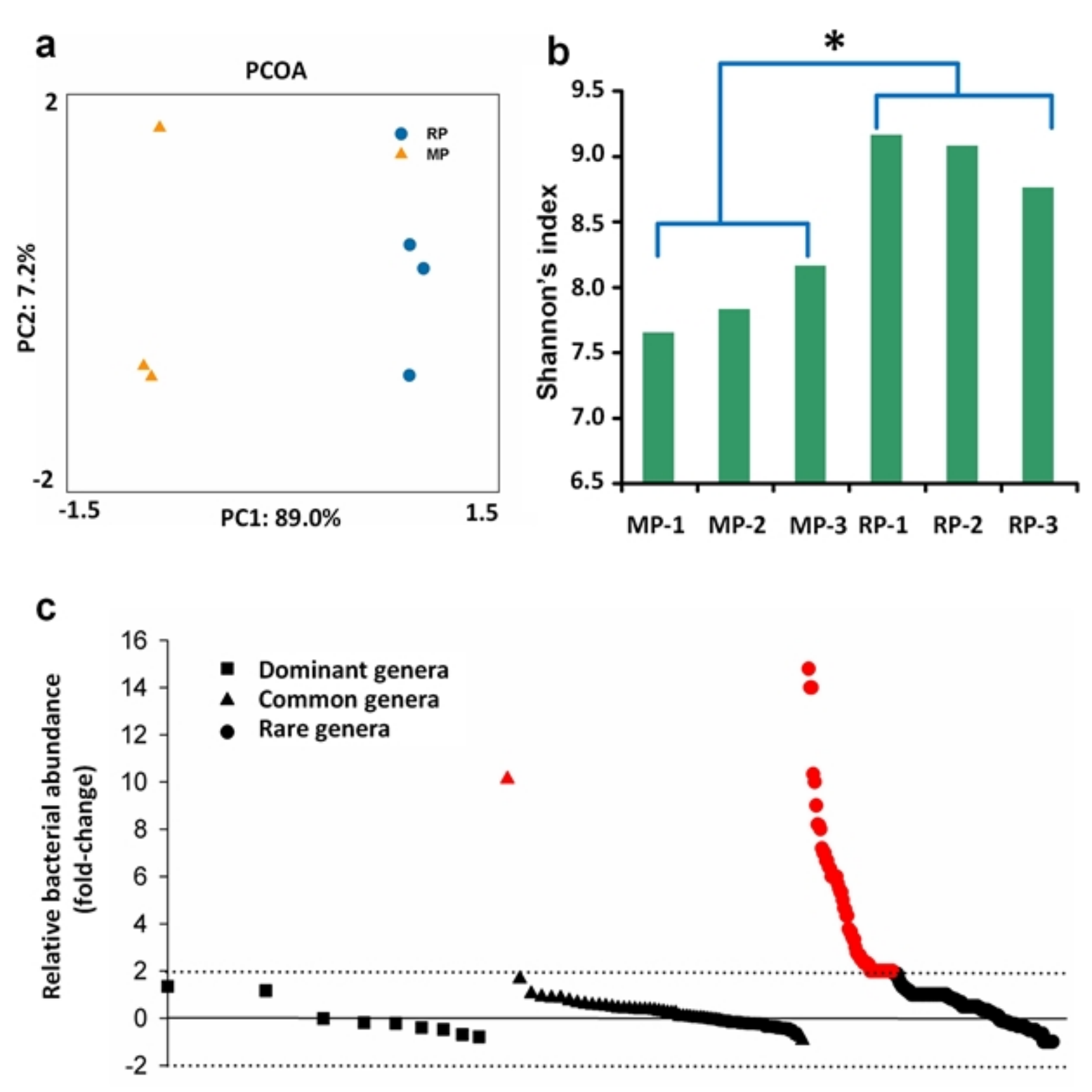
Pipelines analysis

Rhizosphere soils

1. Metagenomic DNA analyses of soils
2. Microbial community
3. Gene functions
4. PGP traits

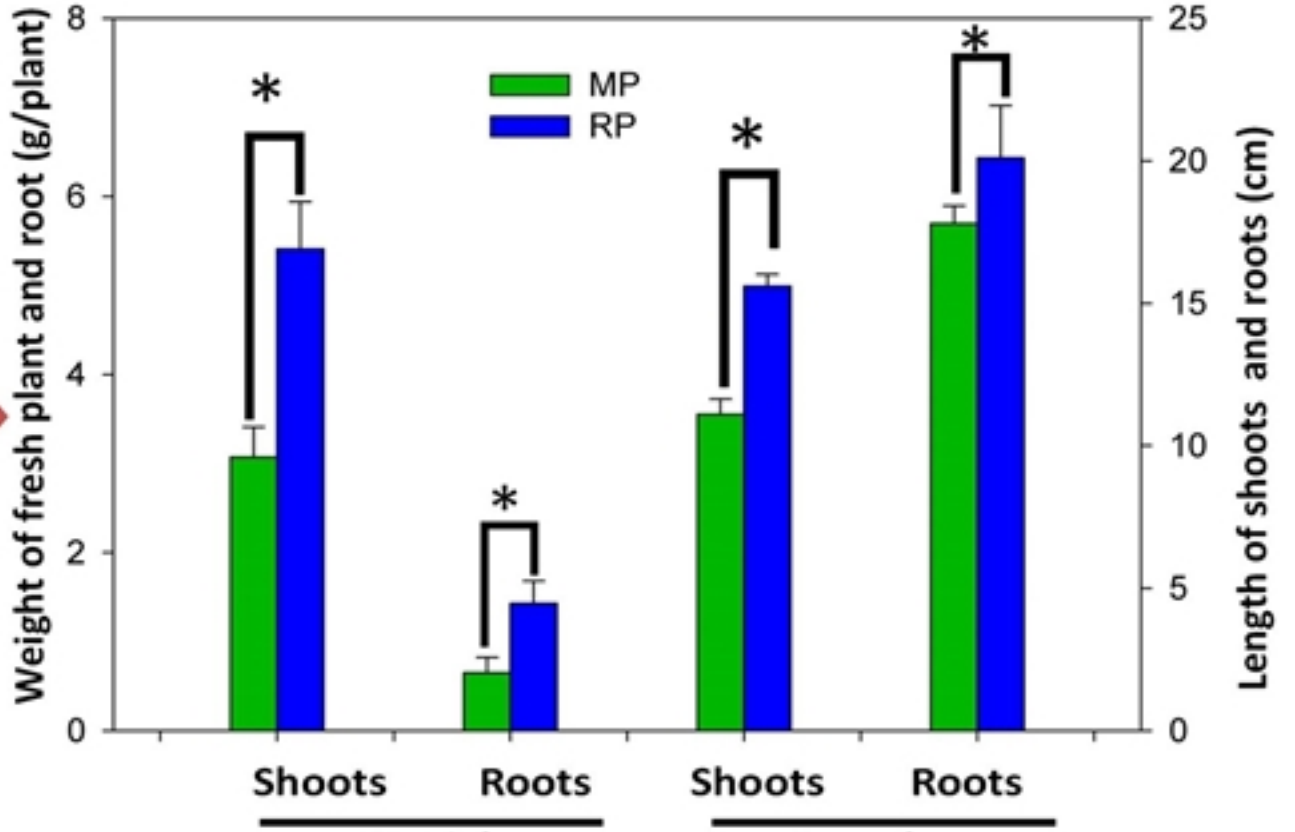
Plants samples

1.Metatranscriptome analysis of roots
2. Gene expression
3. KEGG enrichments
4. Plant growth


a
PCOA
2
-2
-1.5
1.5
PC2: 7.2%
PC1: 89.0%
RP
MP
b
*
9.5
9.0
8.5
8.0
7.5
7.0
6.5
Shannon's index
MP-1
MP-2
MP-3
RP-1
RP-2
RP-3
c
16
14
12
10
8
6
4
2
0
-2
Relative bacterial abundance
(fold-change)
Dominant genera
Common genera
Rare genera

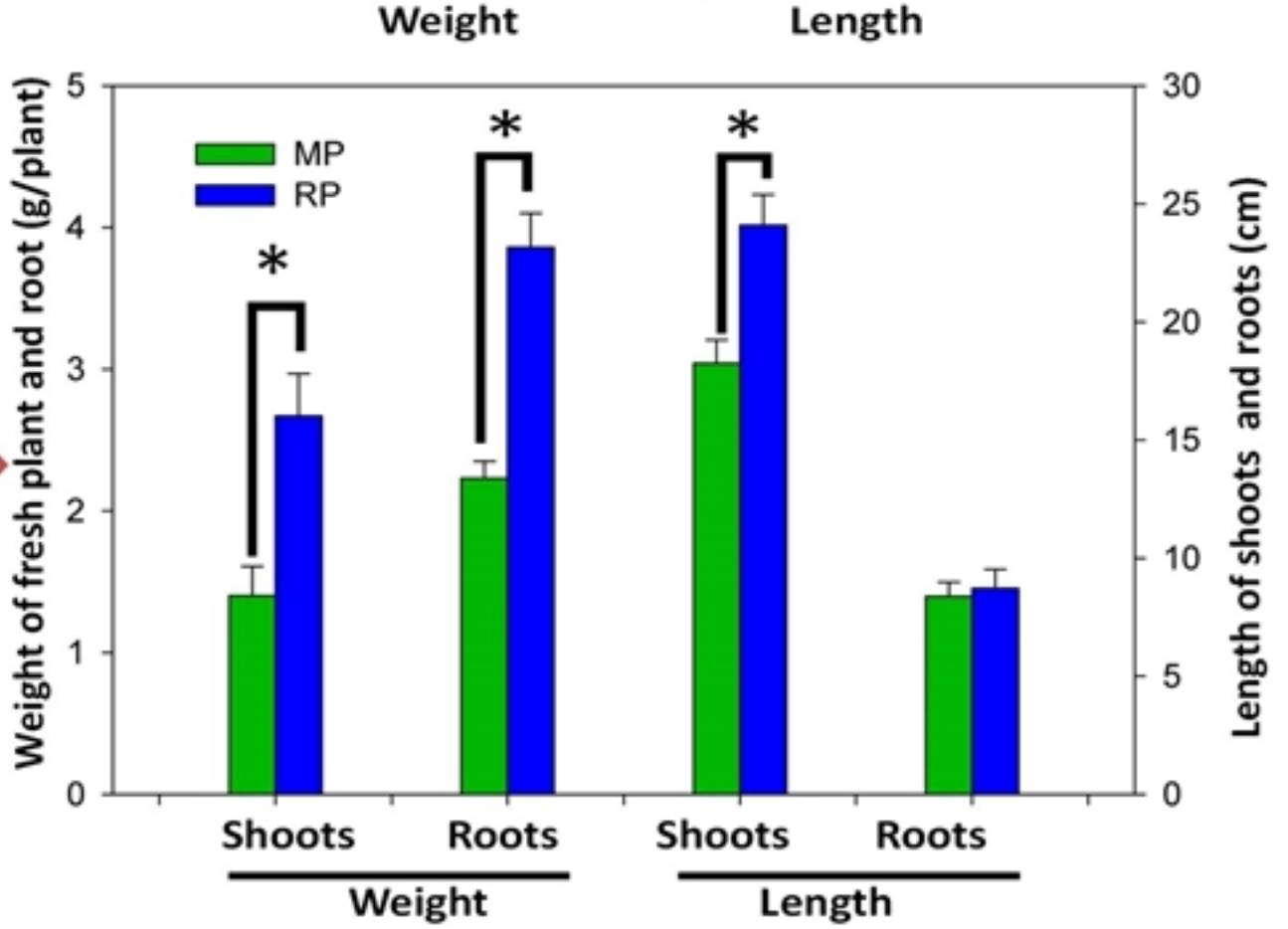
Pot experiment

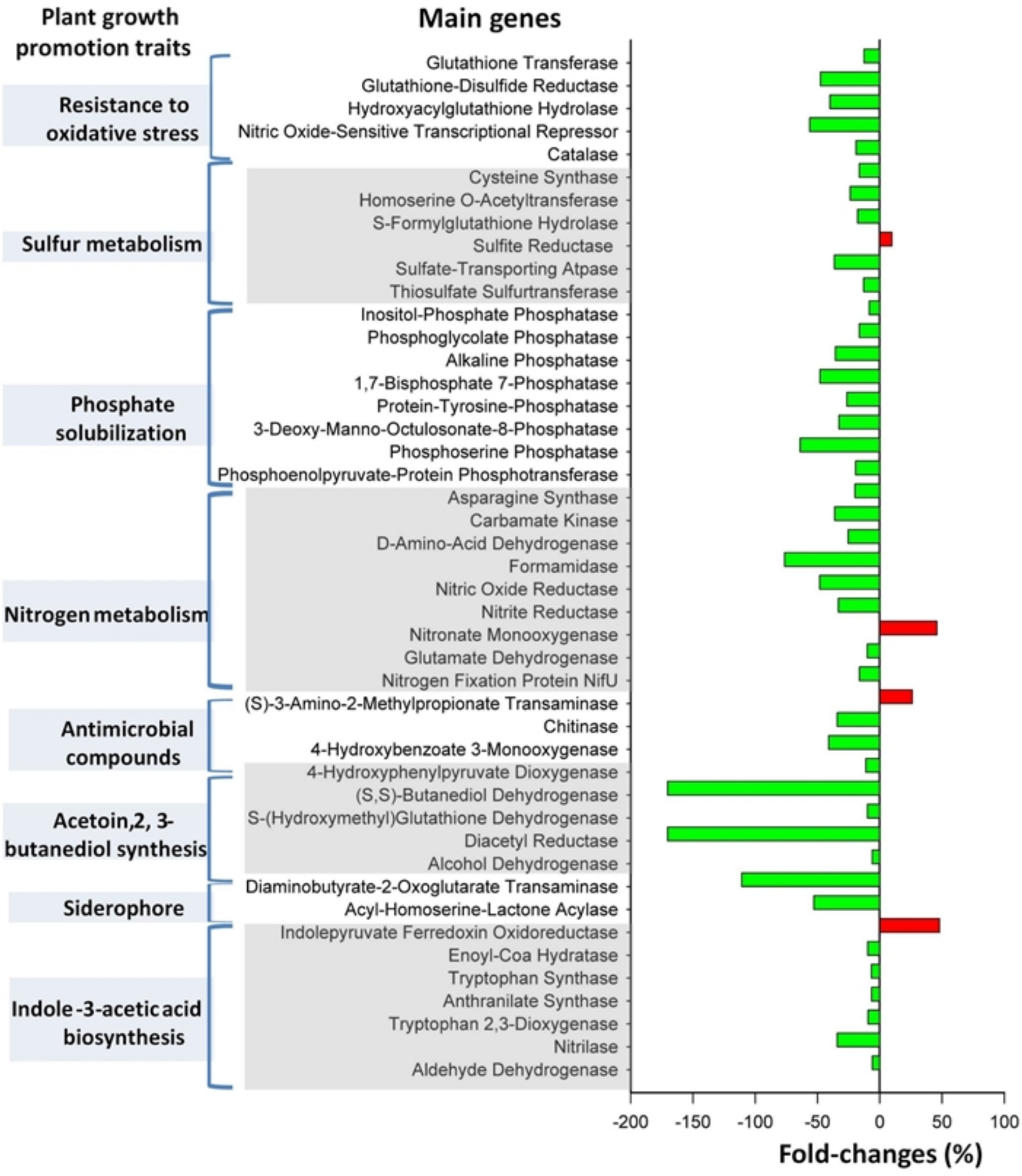
a

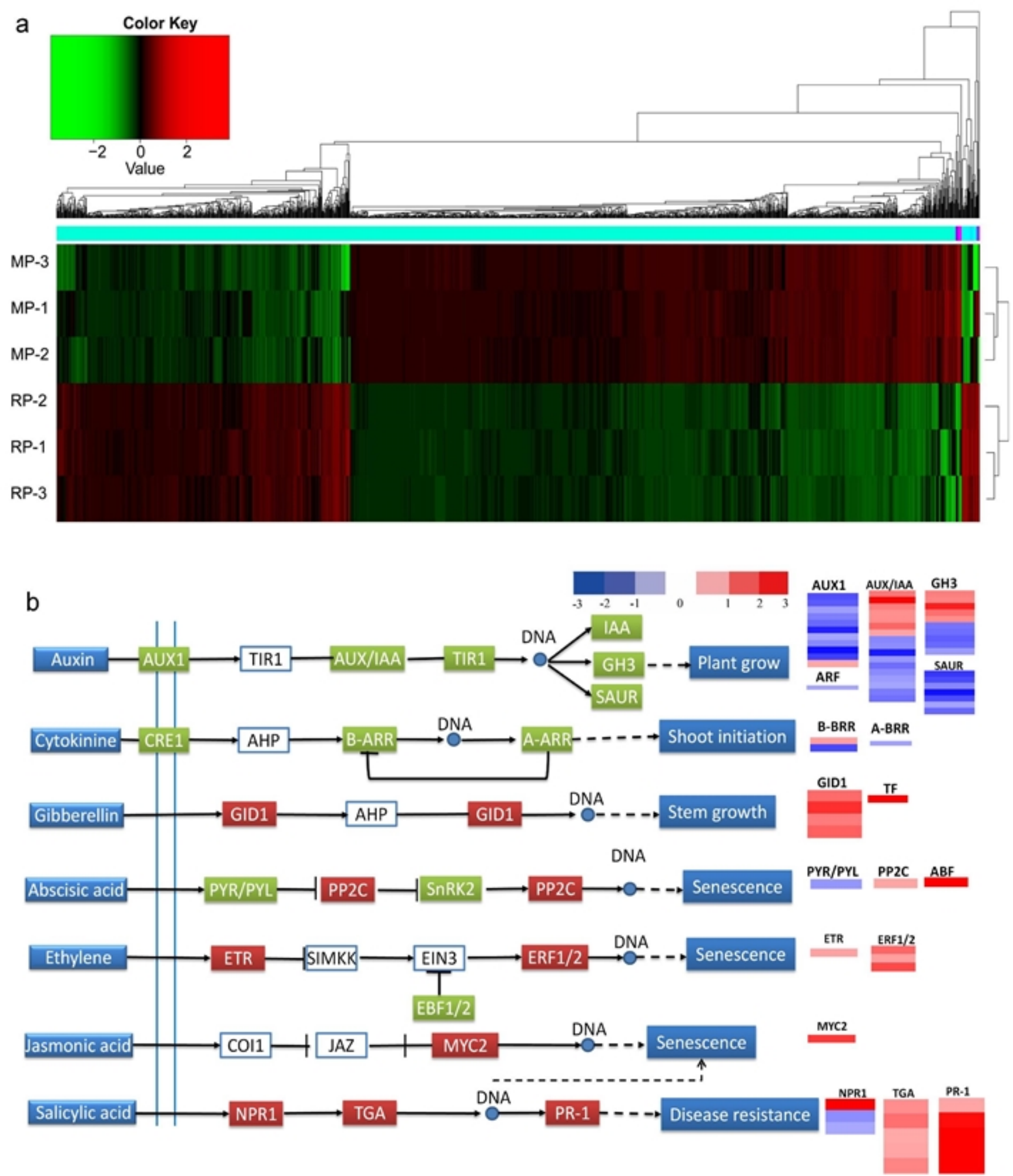


Sterile cultivation with bacterial suspension

b




Plant growth promotion traits
Main genes
Resistance to oxidative stress
Glutathione Transferase
Glutathione-Disulfide Reductase
Hydroxyacylglutathione Hydrolase
Nitric Oxide-Sensitive Transcriptional Repressor
Catalase
Sulfur metabolism
Cysteine Synthase
Homoserine O-Acetyltransferase
S-Formylglutathione Hydrolase
Sulfite Reductase
Sulfate-Transporting Atpase
Thiosulfate Sulfurtransferase
Phosphate solubilization
Inositol-Phosphate Phosphatase
Phosphoglycolate Phosphatase
Alkaline Phosphatase
1,7-Bisphosphate 7-Phosphatase
Protein-Tyrosine-Phosphatase
3-Deoxy-Manno-Octulosonate-8-Phosphatase
Phosphoserine Phosphatase
Phosphoenolpyruvate-Protein Phosphotransferase
Nitrogen metabolism
Asparagine Synthase
Carbamate Kinase
D-Amino-Acid Dehydrogenase
Formamidase
Nitric Oxide Reductase
Nitrite Reductase
Nitronate Monooxygenase
Glutamate Dehydrogenase
Nitrogen Fixation Protein NifU
Antimicrobial compounds
(S)-3-Amino-2-Methylpropionate Transaminase
Chitinase
4-Hydroxybenzoate 3-Monooxygenase
Acetoin,2, 3-butanediol synthesis
4-Hydroxyphenylpyruvate Dioxygenase
(S,S)-Butanediol Dehydrogenase
S-(Hydroxymethyl)Glutathione Dehydrogenase
Diacetyl Reductase
Alcohol Dehydrogenase
Siderophore
Diaminobutyrate-2-Oxoglutarate Transaminase
Acyl-Homoserine-Lactone Acylase
Indole-3-acetic acid biosynthesis
Indolepyruvate Ferredoxin Oxidoreductase
Enoyl-Coa Hydratase
Tryptophan Synthase
Anthranilate Synthase
Tryptophan 2,3-Dioxygenase
Nitrilase
Aldehyde Dehydrogenase
-200
-150
-100
-50
0
50
100
Fold-changes (%)


a
Color Key
−2
0
2
Value
MP-3
MP-1
MP-2
RP-2
RP-1
RP-3
b
-3 -2 -1 0 1 2 3
AUX1
AUX/IAA
GH3
ARF
SAUR
Auxin
AUX1
TIR1
AUX/IAA
TIR1
DNA
IAA
GH3
SAUR
Plant grow
Cytokinine
CRE1
AHP
B-ARR
DNA
A-ARR
Shoot initiation
B-BRR
A-BRR
Gibberellin
GID1
AHP
GID1
DNA
Stem growth
GID1
TF
Abscisic acid
PYR/PYL
PP2C
SnRK2
PP2C
DNA
Senescence
PYR/PYL
PP2C
ABF
Ethylene
ETR
SIMKK
EIN3
EBF1/2
ERF1/2
DNA
Senescence
ETR
ERF1/2
Jasmonic acid
COI1
JAZ
MYC2
DNA
Senescence
MYC2
Salicylic acid
NPR1
TGA
DNA
PR-1
Disease resistance
NPR1
TGA
PR-1