PoolSeq analysis of the selection of the *Rhizobium* genotypes by the legume host plant.

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ABSTRACT
Rhizobium leguminosarum establishes highly specific nitrogen-fixing symbioses. We have applied a Pool-Seq approach to study plant host selection of genotypes. Our results confirm, at the genomic level, previous observations regarding plant selection of specific genotypes

INTRODUCTION
Rhizobium leguminosarum bv. *viciae* establishes highly specific nitrogen-fixing symbioses with different legume genera (*Pisum*, *Lens*, *Lathyrus* and *Vicia*). The molecular bases of specificity, establishment and functioning of the symbioses have been described from the bacterial (Murray *et al.*, 2011) and plant (Yokota and Hayashi, 2011) points of view.
Thanks to the advent next generation sequencing techniques and bioinformatics tools, the genome of a large number of rhizobial mycrosimbiont strains have been sequenced.
Nevertheless, there are aspects of the *Rhizobium*-legume interaction that are yet to be explained, and they could be relevant for efficient inoculant design.
Classic studies using trap plants provided evidence that, given a choice, specific hosts select specific genotypes of rhizobia which are, apparently, particularly adapted to that host (Mutch and Young, 2004; Louvrier *et al.*, 1996).
In the Pool-Seq approach (Kofler *et al.*, 2011), population genomics insights are gained through high-throughput DNA sequencing of pools of bacterial isolates.
We have applied a Pool-Seq approach to study plant host selection of genotypes from the available genomic diversity in a well-characterized soil.

MATERIAL AND METHODS
A well-characterized agricultural soil (INRA Bretennieres) was used as source of rhizobia. Plants of Pisum sativum, Lens culinaris, Vicia sativa and V. faba were employed as traps. We pooled 100 nodules from each host, and the pooled DNAs were sequenced (BGI-Hong Kong; Illumina Hi-seq 2000, 180 bp PE libraries, 100 bp reads, 12 Mreads). Reads were quality filtered with FastQC and Trimmomatic. Filtered reads were mapped with Bowtie2 using Rhizobium leguminosarum bv. viciae 3841 as reference genome. Single Nucleotide Polymorphisms (SNPs) were called with VarScan (minimum coverage 20 reads, minimum frequency 0.1). Results were visualized with SeqMonk and IGV.

RESULTS AND DISCUSSION
An important fraction of the filtered reads were not recruited by the reference genome (Table 1), suggesting that plant soil isolates contain a large number of genes that are not present in the reference genome.
Single Nucleotide Polymorphism (SNP) analysis was carried out with Pool-Seqs, and a plant-specific SNP distribution was observed within the nodDABC cluster.

Population dissimilarities were obtained from the complete SNP genome analysis. Pairwise Euclidean distances were calculated from SNP frequencies with SPSS software (Table 2).

Table 1. Reads mapped and unmapped against *R. leguminosarum* bv. *viciae* 3841.

	PEA	LENTIL	FAVA	VETCH
Total reads	11,697,508	11,893,439	11,905,328	11,862,580
% Mapped reads	84.17	77.79	77.24	81.88
% Unmapped reads	16.24	23.27	23.29	18.22

Table 2. Population dissimilarities.

	Pea	Lentil	Fava	Vetch	Rlv 3841
Pea	0				
Lentil	76	0			
Fava	90	56	0		
Vetch	54	58	65	0	
Rlv 3841	157	187	195	170	0

Our results confirm, at the genomic level, previous observations regarding plant selection of specific genotypes. We expect that further, ongoing comparative studies on differential Pool-Seq sequences will identify specific gene components of the plant-selected genotypes.

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