Microbial communities associated to common bean seed

A mechanism of local adaptation of plants?

STEPHANIE M. KLAEDTKE[1,2], MATTHIEU BARRET[3,4,5], VÉRONIQUE CHABLE[2], MARIE-AGNÈS JACQUES[3,4,5]

[1] INRA de Rennes, France, www.rennes.inra.fr/sad-bcrp, stephanie.klaedtke@rennes.inra.fr
[2] SEED, Université de Liège campus Arlon, Avenue de Longwy 185, 67000 Arlon, Belgium
[3] INRA, UMR1345 Institut de Recherches en Horticulture et Semences, Beaucouzé, France
[4] Agrocampus Ouest, UMR1345 Institut de Recherches en Horticulture et Semences, Beaucouzé, France
[5] Université d'Angers, UMR1345 Institut de Recherches en Horticulture et Semences, SFR4207 QUASAV, Beaucouzé, France

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Summary: The effects of crop genotype and cultivation site on microbial communities associated to seed of *Phaseolus vulgaris* were assessed on 33 seed lots. These seed lots were obtained by multiplying 5 initial seed lots of different cultivars in two contrasting environments for 2 years in 3 replicates. An additional commercial control lot was introduced the second year. The diversity of fungal and bacterial communities was analyzed by high-throughput sequencing of the bacterial 16S rRNA gene and the fungal ITS1 region, respectively. Results showed that the structure of the fungal and the bacterial communities is significantly affected by the cultivation site.

Background

Small scale organic artisan seed producers generate and market seed of traditional cultivars of common bean (*Phaseolus vulgaris*) in Western Europe. Several seed-borne pathogens represent phytosanitary challenges for common bean seed production. In particular, the agents responsible for the common bacterial blight of bean such as *Xanthomonas axonopodis* var. *phaseoli* and *X. fuscans* subsp. *fuscans* are listed in European Council Directive 2000/29/EC, Annex II A II, as harmful organisms. We emit the hypothesis that different conceptions of plant health are confronted in debates concerning the problem of managing these agents as quarantine pests. While some stakeholders define plant health as "absence of disease" and as objective entity, others argue for a salutogenic position “focusing on more complex interactions between plants and pathogens, such as induced resistance phenomena” in order to “move towards health” (Döring *et al.*, 2011). The application of beneficial microorganisms to seeds for bio-control of plant pathogens has become an important research topic (Berg, 2009). However, the role of indigenous seed microbial communities in plant adaptation to local environments has not been studied. Whereas seed-borne pathogens are frequently quantified on commercial seed lots, the presence and potential functions of other microorganisms are left aside. These microorganisms may be of particular importance when seed lots are multiplied on the same farm year after year. In a research project in cooperation with the *Croqueurs de Carottes*, an association of French and Belgian artisan seed producers, we analyzed the diversity of microbial communities on common bean seed from two cultivation sites to investigate the possible influence of microorganisms on the adaptation of plants to contrasting environments.

Material and Methods

The influence of the seed production site on the structure of the seed microbiota was assessed on 33 seed samples obtained from two cultivation sites. These seed samples were obtained by multiplying 5 initial seed lots in two organic farms in Brittany and Luxembourg in three replicates in 2012 and 2013. Four of these seed lots consisted of farm seeds of traditional cultivars; one was seed of the commercial variety 'Calima' obtained from a breeding company and used as a commercial control. The initial seed lots were thus exposed to contrasting biotic and abiotic environments for two consecutive years. In addition, commercial seed of 'Calima' was again sown in 2013. One cultivar ('Rognon de Coq') did not yield sufficient seed for sampling in Luxembourg. Total genomic DNA extraction was performed on 200-seeds subsamples collected from each seed lot. The composition of the bacterial and fungal communities was analyzed by high-throughput sequencing (Illumina MiSeq v. 2.0 platform, 250 bp paired-end reads) of the bacterial 16S rRNA gene and the fungal ITS1 region, respectively. Low quality reads were removed using the standard operational procedure of Mothur (Schloss *et al*., 2009; Kozich *et al*., 2013). High-quality sequences were then grouped in operational taxonomic units (OTUs) at a genetic distance of 0.03. Only OTUs with a minimum threshold of 1 ‰ relative abundance were conserved for further analyses and defined as abundant OTUs (aOTUs). Variation of bacterial and fungal diversity across samples was assessed by calculating unweighted Unifrac and Yue & Clayton distances, respectively. Non-metric dimensional scaling (NMDS) ordinations were used to observe sample clustering. Analysis of molecular variance (AMOVA) test was performed to test if the observed clustering was statistically significant. The effect of sampling was verified by taking 3 sub-samples each from 2 large seed lots.

Results and Discussion

A total of 169 aOTUs were detected in the fungal communities, with 123 aOTUs present in Brittany and 106 in Luxembourg. *Sordariomycetes* (relative abundance of 34,5%), *Tremellomycetes* (22,3%), *Dothideomycetes* (17,8%), *Wallemiomycetes* (7,8%) and *Eurotiomycetes* (3,6%) were the most abundant fungal classes observed in Brittany, while *Dothideomycetes* (25,6%), *Sordariomycetes* (22,8%), *Leotiomycetes* (18,7%), *Eurotiomycetes* (11,6%) and *Agaricomycetes* (9,5%) were most abundant in Luxembourg. A total of 217 aOTUs were detected in the bacterial communities, with 176 aOTUs present in Brittany and 136 in Luxembourg. *Gammaproteobacteria* were by far the most abundant bacterial phylum in both cultivation sites, with

a relative abundance of 89,6% in Brittany and 78,6% in Luxembourg. *Alphaproteobacteria* (5,9% and 4,4%, respectively), *Betaproteobacteria* and (4,6% and 1,5%) *Bacteroidetes* (3,2% and 3,0%) were strongly represented in both sites. On contrary, *Firmicutes* were enriched in Brittany (4,6% and 0,5%, respectively).

In the case of the analyses of the fungal ITS region, sub-sampling of 200 seeds was considered sufficient due to a high proportion of OTUs common to the 3 subsamples of both tested seed lots (54% and 67%, respectively). However, limitations to the subsampling were detected for the analyses of bacterial 16S RNA genes, as 1 sample out of 3 had very few OTUs for both seed lots. We conclude that larger sub-samples would be required to avoid sampling effects for the analyses of bacterial communities.

The effect of cultivation site on both fungal and bacterial communities was found to be significant at p=0,001 (Figure 1). Seed microbial communities were thus shaped by the cultivation site within two reproduction cycles. Further research steps are required to investigate whether these differences in microbial communities between sites may involve an adaption to local environmental conditions. This could be done through an analysis of the functions of abundant OTUs found in the seed samples.

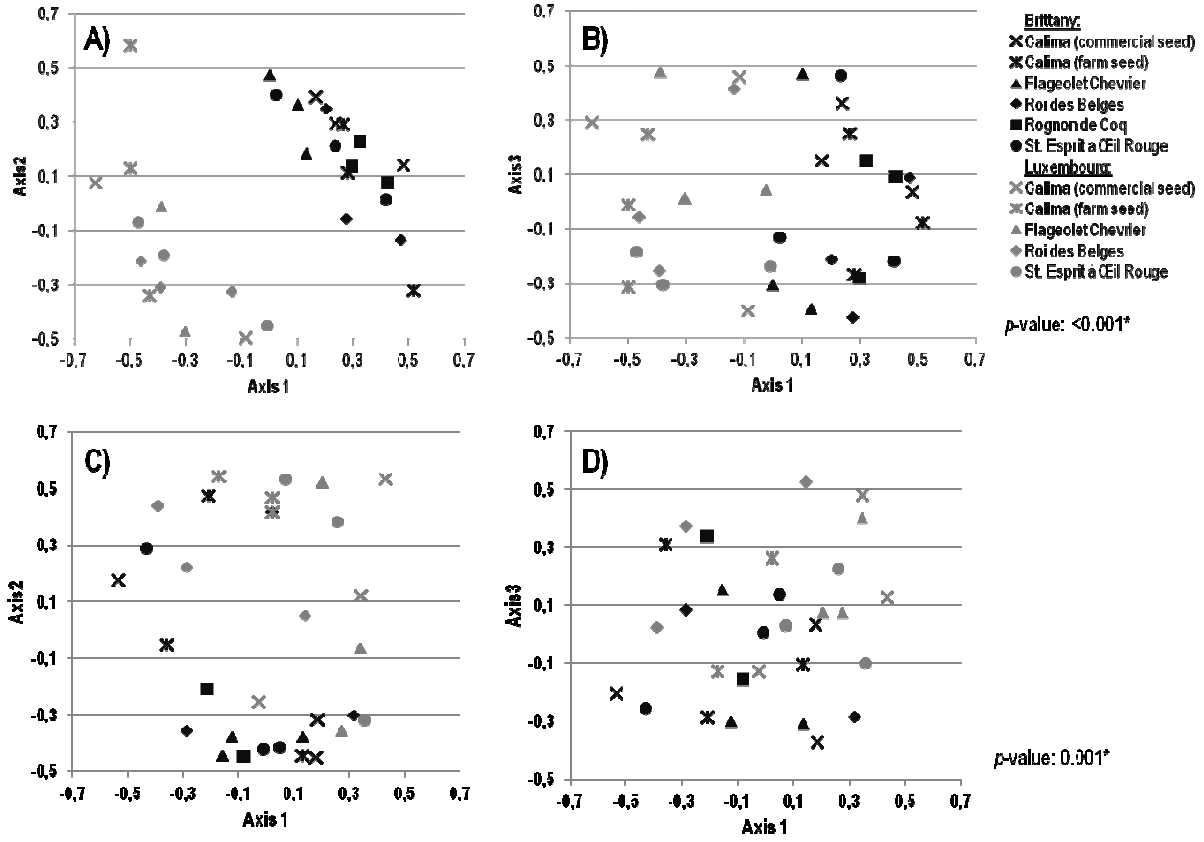

Figure 1. Diversity of microbial communities associated to common bean seeds of different varieties and cultivation sites. β-diversity of fungal communities calculated by unweighted Yue & Clayton distance and visualized in 3 dimensions by NMDS (lowest stress: 0.16, R^2: 0.82), A) and B). β-diversity of bacterial communities calculated by unweighted Unifrac distance and visualized in 3 dimensions by NMDS (lowest stress: 0.15, R^2: 0.88), C) and D). *P*-values (AMOVA) indicate the significance level of the effect of cultivation site on microbial communities.

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