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## **The rhizomicrobiome of Sorghum ; impact on plant growth and stress tolerance**

Schlemper, T.R.

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**Author:** Schlemper, T.R.

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# Summary

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Soil microbes may have positive and negative interactions with plants. Positive interactions include symbiotic associations between plants and microbes promoting plant growth and nutrient uptake, whereas negative interactions include pathogenesis and competition for nutrients. Thus the composition of the rhizomicrobiome which is related to several factors such as plant species, plant genotype and soil type is of utmost significance for plant growth and health.

Sorghum is currently the 5<sup>th</sup> most cultivated cereal worldwide and is an economically important crop used for animal feed and human food, in particular for subsistence farmers in Sub-Saharan Africa. The research described in this PhD thesis explores the main factors that determine the sorghum rhizomicrobiome assembly, and how its composition modulates plant growth and stress tolerance. The approaches employed in the present study were *in vivo* bioassays, bacterial inoculation and next-generation sequencing to assess the taxonomic composition of the sorghum rhizomicrobiome.

To investigate the relative impact of the factors soil type, plant genotype and plant growth stage on the sorghum rhizobacterial community composition seven different sorghum genotypes were grown in the greenhouse in two different soil types (Clue Field – CF and Vredepeel – VD) and evaluated at four different plant growth stages (days 10, 20, 35 and 50). The results showed that the composition of the bacterial community was most strongly influenced by soil type followed by plant growth stage and plant genotype (Chapter 2). Furthermore, at early stages of sorghum growth, the rhizosphere bacterial community composition was mainly driven by soil type, whereas at later stages plant genotype became a more important factor (Chapter 2). Moreover, one of the genotypes, SRN-39, that was grown in abandoned CF soil, had significantly higher relative abundances of *Acidobacteria* GP1, *Burkholderia*, *Cupriavidus* (*Burkholderiaceae*), *Acidovorax* and *Albidiferax* (*Comamonadaceae*) than the other six cultivars. This result indicates that the genotype effect on the sorghum rhizosphere bacterial assembly was soil dependent.

In order to assess the dynamics of bacterial and fungal communities in the sorghum rhizosphere two sorghum genotypes (SRN-39 and BRS 330) were grown in the greenhouse in two different soil types (CF and VD) and evaluated at three different plant growth stages (days 10, 35 and 50). The results showed that sorghum genotype SRN-39 promoted a stronger co-variance between bacterial and fungal communities when grown in abandoned Clue Field soil. Furthermore, the decrease in relative abundance of the fungus genus *Gibberella* over plant growth stages was followed by a decrease of the bacterial families *Oxalobacteraceae* and *Sphingobacteriaceae*. We suggest that there may be a link between these organisms, as both bacterial families are known to be antagonistic

to fungal activity. Notwithstanding, this hypothesis needs to be experimentally assessed in future studies (Chapter 3).

Aiming to investigate if sorghum plants pre-cultivated in soils with different bacterial community composition responded differently to water deficiency conditions, two sorghum lineages (drought susceptible and drought tolerant) were grown in five different soils for 21 days and transplanted with their rhizoplane microbial community to a standardized substrate at different moisture conditions. The results showed that at water deficient conditions high abundances of the *Caulobacteraceae* family in the rhizoplane of the drought susceptible lineage and of the *Rhizobiaceae* family in the rhizoplane of the drought tolerant lineage occurred in particular at the plants that were pre-grown in two soils with a history of low rainfall regimes, Cerrado and Sorghum field, respectively. These results suggest that pre-cultivation of sorghum in soils with a history of low rainfall regimes selected for representatives of the *Alphaproteobacteria* which may provide a selective advantage at water deficient conditions (Chapter 4).

In order to investigate potential plant growth –activity of so-called PGPB (Plant Growth Promoting Bacteria) on sorghum, five endophytic bacterial strains originally selected as PGPB of sugarcane were inoculated in four sorghum cultivars. Inoculation of the cultivars SRN-39 and BRS330 with *Burkholderia tropica* strain IAC/BECa 135 or *Herbaspirillum frisingense* strain IAC/BECa 152 resulted in significant increases in plant biomass. In particular cultivar SRN-39 showed better growth responsiveness resulting in significantly larger plant biomass when inoculated with the bacterial strains IAC/BECa 135 (*Burkholderia tropica*) and IAC/BECa 152 (*Herbaspirillum frisingense*) than the other cultivars (Chapter 5). This study shows that these strains are promising PGPB strains for use as bioinoculant in sorghum cultivation.

In conclusion, the research presented in this thesis showed for the first time the simultaneous impact of factors plant genotype, growth stage and soil type on the sorghum rhizosphere bacterial community composition. Furthermore, sorghum genotype SRN-39 showed interesting interactions with its rhizomicrobiome which may be employed further to design sustainable sorghum cultivation. Overall, the results described in this thesis highlight the importance of studies on the rhizomicrobiome of sorghum which could contribute to find potential microbial candidates to be used in sustainable agricultural management practices to improve sorghum health and productivity.