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

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# Chapter 6

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General discussion

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Interactions between plants and soil microbes may be positive, neutral or negative. This outcome will depend on plant and soil microbial community characteristics and composition, as well as on the compatibility of the molecular signalling by both partners. The rhizosphere is the first interface of interaction between soil microbes and plant roots. Some microorganisms present in this compartment are able to attach to the root rhizoplane and even to colonize the root endosphere compartment for association or to interact with the plant. Specifically for sorghum, little is known about how different sorghum genotypes recruit and benefit from soil microbial communities. The increasing demand for food and bioenergy crops, allied to the increasing necessity of crops that are resistant to drought conditions, make sorghum a promising plant species to be studied regarding the benefits of the root microbiome. The overall aim of my thesis was to investigate the dynamics of the sorghum root microbiome and to explore the beneficial effects of the root microbiome on sorghum growth and drought stress tolerance. In this chapter I discuss the main findings of my studies and I will provide future perspectives for the application of the results.

### **Factors that drive the dynamics of the microbial community in the rhizosphere**

Many factors affect the rhizosphere microbial community composition including soil factors, plant growth stage and plant species or genotype (Nihorimbere *et al.*, 2011). Although many studies have addressed the influence of these factors on the rhizosphere microbial community composition (Garbeva *et al.*, 2004, Berg & Smalla, 2009, Aira *et al.*, 2010, Marques *et al.*, 2014), most of them did not evaluate these factors simultaneously. In this thesis, I present the relative contribution of soil type, plant genotype and plant growth stage in shaping the sorghum rhizosphere microbial community (**Chapters 2 and 3**). I found soil to be the main factor in driving the assembly of the sorghum rhizosphere microbial community followed by plant growth stage and plant genotype.

While many studies point to soil as the major determinant of root microbial community composition (Singh *et al.*, 2007, Xu *et al.*, 2009), others showed that the influence of plant species or plant genotype on the rhizosphere microbial community composition is larger than the impact of soil characteristics (Miethling *et al.*, 2000, Aira *et al.*, 2010). Such contradictory results may be explained either by the use of different plant genotypes and soil types, or by different sampling strategies applied (Wieland *et al.*, 2001). To disentangle which factor, soil or plant genotype, is the main driver of the structure of the rhizosphere microbial community of sorghum, I compared both factors during plant growth. Whereas at the early stages of plant growth, soil showed a preponderant role in the structuring of sorghum rhizosphere bacterial community, at later stages of plant growth, the sorghum genotype

showed to be an important driver of rhizobacterial recruitment. My explanation is that, at the early stages of growth, plant still does not yet have a well-developed root system, which can widely interfacing soil to interact with the soil microbial community. During root growth, roots increase their contact surface with soil and so with the soil microbial community as well as that the quality and quantity of rhizodeposits will increase and so plant roots will likely have an increasing role in shaping the rhizosphere bacterial community. In this sense, plant genotype effect on the rhizosphere microbial community would not be a consequence of asynchronous growth, but instead, the result of the relative influence of roots and root-produced rhizodeposits at a given time (Micallef et al., 2009). Plant genotypes with different growth rates and differences in root development may release different types and amounts of root exudates, which compounds will attract different microbes from the soil-borne microbial pool and assemble a specific microbial community in the rhizosphere (Dunfield & Germida, 2003, Inceoglu et al., 2010). Chaparro *et al.* (2013) suggested that at early stages of plant growth roots exude more simple compounds used by a more diverse community of microorganisms, and at later stages plant roots release more complex compounds selecting more specific microbes. The understanding of the shaping forces of the rhizosphere microbial community structure of different plant genotypes and at different growth stages may help the improvement of strategies and management practices for controlling soil-borne pathogens (Wu *et al.*, 2016).

Many studies already showed the influence of plant genotype on the microbial community composition of the rhizosphere of several crops (Aira *et al.*, 2010, Sugiyama *et al.*, 2012, Marques *et al.*, 2014). However, only few studies evaluate the genotype effect consistency in different soils (Peiffer *et al.*, 2013). Here I provided evidence that the genotype effect on the sorghum rhizosphere bacterial assembly and on bacterial and fungal co-variance was soil dependent, and most strongly occurred in the less fertile Clue Field soil. Corroborating with this evidence, Nicolitch *et al.* (2016) found that beech trees had a higher enrichment of bacterial taxa in the rhizosphere in a nutrient poor soil compared with nutrient rich soil. The authors suggested that such enrichment may be related to the intensification of rhizodeposition in order to recruit microorganisms that could effectively play a role in nutrient mobilization (Nicolitch *et al.*, 2016).

I found that the African sorghum cultivar SRN-39 cropped on the less fertile Clue field soil recruited a higher abundance of *Burkholderia*, *Cupriavidus* (*Burkholderiaceae*), *Acidovorax* and *Albidiferax* (*Comamonadaceae*) (**Chapter 2**) than in the more fertile soil, and hosted the strongest interaction between fungal and bacterial communities in rhizosphere (**Chapter 3**) of all cultivars considered here. Furthermore, cultivar SRN-39 showed better growth responsiveness when inoculated with the bacterial strains IAC/BECa 135 (*Burkholderia tropica*) and IAC/BECa 152

(*Herbaspirillum frisingense*) than the other cultivars (**Chapter 5**). Although the mechanisms of the sorghum genotype effect on microbial community selection and structure remain unclear, I speculate that differences in rhizodeposition could be the primary reason for the observed effect.

Rhizodeposition varies qualitatively and quantitatively over different plant genotypes (Aulakh et al., 2001). As an example, I found that cultivar SRN-39 produced the strigolactone molecule Orobanchol at levels 300 to 1100 times higher than the other six studied cultivars. Strigolactones are considered an additional class of hormones that besides being involved in the plant morphological regulation (Koltai, 2014), are responsible to stimulate the seed germination of parasitic plants such as *Striga hermonthica* and *Orobanche* spp. (Bouwmeester et al., 2007), as well as hyphal-branching and symbiosis of the arbuscular mycorrhizal fungi - AMF (Akiyama et al., 2010), and are used by bacteria to communicate with each other in order to sense population density (Proust et al., 2011).

Different bacterial species have been shown to reduce the infection of parasitic plants on different host plants (Miché et al., 2000, Mabrouk et al., 2007, Hassan et al., 2009). Specifically regarding sorghum cultivar SRN-39, Hassan et al. (2009) showed that the inoculation of *Pseudomonas putida* and *Azospirillum amazonas* reduced infestation of *Striga* compared with non-inoculated plants. From a few studies available regarding bacteria as biocontrol of *Striga*, most of them focus on the influence of bacterial isolates rather than total soil bacterial community. Sorghum crops are grown in different soils and interact with a diversity of microorganisms whose composition may have different effect on *Striga*. Because SRN-39 is a *Striga*-resistant cultivar that recruited significantly higher abundance of specific groups of bacteria and has a strigolactone profile that is different from the other 5 studied cultivars, I recommend that bacterial community selected by SRN-39 should be subject of future investigations to test their effect on *Striga* suppression. An important uncertainty that arises in my study and that should be subject of investigation in future studies is whether the tolerance of SRN-39 to *Striga* is directly and exclusively based on the plant genetic background acquired during breeding, or if there is a participation of a specific microbial community recruited in the rhizosphere that contributes to *Striga* tolerance. Furthermore, despite that high levels of Orobanchol may play a role in rhizobacterial community assembly, also exudates other than strigolactones could play a role. Thus, additional analyses including root exudate of different sorghum genotypes are required.

## **Sorghum rhizoplane bacterial community and drought stress tolerance**

As powerful as the effect of soil on the microbial community of the rhizosphere, is the effect of climate on the microbial community in soil (Lladó *et al.*, 2018). Considering the importance of precipitation as a climatic event that could drive changes in microbial communities, in **chapter 4** we tested 5 different soils in search for well-adapted microorganisms that could alleviate drought stress of sorghum plants. For this purpose, the approach presented in this chapter allowed us to check for the contribution of root-associated microorganisms to water stress alleviation thereby minimizing the effect of chemical and physical soil characteristics.

Worldwide, the lack of water on agricultural systems causes huge crop productivity losses with serious economic and social consequences both on the local and global scale. Despite the possibility of artificial irrigation to overcome drought generally small farmers cannot afford this technology to guarantee their crop yields due to the high costs of irrigation (Bakhsh *et al.*, 2015). Low moisture conditions imposes physiological stress on plants such as suppression of photosynthesis, decrease in a chlorophyll content, and stomatal closure to reduce water loss (Rizhsky *et al.*, 2004, Rahdari *et al.*, 2012). Such physiological stresses limit plant growth and make plants more vulnerable to certain diseases (Diourte *et al.*, 1995, Farooq *et al.*, 2012, Tesso *et al.*, 2012). Specifically in sorghum, plants under drought stress are more susceptible to stalk and charcoal rot disease caused by *Macrophomina phaseolina* (Diourte *et al.*, 1995, Tesso *et al.*, 2005).

A promising alternative to overcome the deleterious effect of drought on different crops is the interaction of plants with beneficial soil microorganisms. Microorganisms that are adapted or tolerant to drought conditions can help plants to overcome drought and enhance plant growth, nutrition and plant resistance to water stress by, for instance, by influencing the expression of AtRAB18 and AtLT178 stress responsive genes (Sukweenadhi *et al.*, 2015) and conferring induced systemic tolerance (IST) to drought stress (Yang *et al.*, 2009). Furthermore, plants under water stress conditions produce the hormone ethylene to regulate the plant homeostasis and to reduce root and shoot growth. However, in the presence of bacterial ACC deaminase less ethylene is formed, avoiding the reduction on plant growth (Glick, 2012). Although, soil moisture may impose physiological stress on microbial communities limiting or inhibiting their development (Bouskill *et al.*, 2013, Armada *et al.*, 2014), bacteria do employ strategies to overcome water deficiency that include accumulation of compatible solutes, production of exopolysaccharide and production of spores as dormant life form (Barnard *et al.*, 2013).

Drought and the rewetting of dry soils are considered disturbances for soil microorganisms. The degree to which microbes respond to disturbances is related to the sensitivity or tolerance of the



organism as well as the disturbance intensity and severity. In our experiment described in **chapter 4**, I applied two pulses of water disturbance at the pre-flowering plant growth stage as this is the most critical point of sorghum development when plants face such stress (Emendack *et al.*, 2018). The pre-flowering stage is a crucial stage in the assembly of the rhizosphere microbial community under limited water conditions because drought can delay the microbial root colonization at early development stage of sorghum growth (Xu *et al.*, 2018).

In order to assess which species of the rhizomicrobiome are able to alleviate water stress on plants, studies may directly consider the effect of microorganisms on plant growth, production and nutrition at water limited conditions (Xu *et al.*, 2018) as well as indirectly, by assessing bacterial mediated hormonal modulators such as the exogenous Indole Acetic Acid (IAA) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity that contribute to a physiological response and stress tolerance (Govindasamy *et al.*, 2017, Armada *et al.*, 2018). Here I directly determined the effects of the bacterial community on sorghum growth and root architecture modification at water deficiency and under normal water supply conditions. Although I found that different soils and water moisture content played a role in sorghum growth and root architecture modification, no relationship between plant growth at water limited conditions and bacterial community composition was found.

Nevertheless, at water deficiency, the rhizoplane of drought susceptible (DS) sorghum lineage previously planted in Cerrado and the rhizoplane of drought tolerant (DT) sorghum lineage previously planted in Sorghum field soil showed the highest abundances of the bacterial families *Caulobacteraceae* and *Rhizobiaceae*, respectively. The Cerrado soil comes from a savannah-like vegetation characterized by extreme temperatures, fire and water stress (Borghetti *et al.*, 2005). Sorghum field soil came from a semi-arid region with half of the annual average precipitation as compared to the locations where the other soils were sampled. In general, microbial communities that have experienced drying and rewetting cycles may be more resistant to changes under these conditions than those that have not (Evans & Wallenstein, 2012, Bouskill *et al.*, 2013). Hence, the degree to which the soil microbial community can resist environment fluctuations may be influenced by climate history. Although *Caulobacteraceae* and *Rhizobiaceae* families which are known to include species tolerant to drought and thermal conditions (Bouskill *et al.*, 2013, Nunes *et al.*, 2018) did not show effect on the plant growth, the possibility cannot be excluded that a high density of bacterial cells of specific species isolated from these families could alleviate plant stress. Thus, I suggest that future research should identify and isolate strains belonging to the water stress tolerant

*Caulobacteraceae* and *Rhizobiaceae* families and test them as possible bioinoculant to promote plant growth under abiotic stress conditions.

### **Plant growth promoting bacteria inoculated on sorghum**

To be considered as PGPB, bacterial strains need to fulfill at least two of the three criteria of effective colonization, plant growth stimulation and biocontrol (Weller *et al.*, 2002, Vessey, 2003). To investigate if bacterial strains characterized as PGPB in sugarcane would affect sorghum growth, I inoculated the rhizosphere of four sorghum cultivars (SRN-39, Shanqui-Red (SQR), BRS330, and BRS509) with five sugarcane endophytic bacterial isolates *K. radicincitans* (IAC/BECa 99), *E. asburiae* (IAC/BECa 128), *P. fluorescens* (IAC/BECa 141), *B. tropica*, (IAC/BECa 135) and *H. frisingense* (IAC/BECa 152) (**chapter 5**). The results indicated that bacterial strains characterized as PGPB in sugarcane were also able to promote growth of sorghum. Sorghum and sugarcane are genetically closely related; intergeneric hybrids between these two species have already been reported (Bowers *et al.*, 2003). Whereas the evolutionary divergence between sorghum and maize have been estimated to have occurred approximately 15–20 million years ago, sorghum diverged from sugarcane approximately 5 million years ago (Paterson *et al.*, 2004). Given the genetic proximity of sorghum and sugarcane and taking into account that these two species are often reported to be part of the same crop rotation system (Tew *et al.*, 2008, May *et al.*, 2013), it is really promising that PGPB isolates of the one crop could have a positive effect on the other which is useful in a crop rotation or intercropping system. However, the effect of bacterial strains on sorghum growth was different dependent on sorghum genotype. Sorghum cultivar SRN-39 had the best performance with a significant increase on root dry biomass when inoculated with isolates *B. tropica* and *H. frisingense* (*Burkholderiales* Order), and a significant higher shoot biomass when inoculated with *E. asburiae* and *H. frisingense*, compared with the control. Interestingly, cultivar SRN-39 also showed higher relative abundance of specific rhizosphere bacterial families belonging to *Burkholderiales* Order (**Chapter 2**), and a stronger rhizosphere bacterial and fungal co-variation with significant contribution of representatives of *Burkholderiales* Order (**Chapter 3**) much more than the other cultivars. It seems that this cultivar has a greater potential in interacting with representatives of *Burkholderiales* Order, and so, its growth seems to be more impacted by them than the other cultivars. As already mentioned, this cultivar produces the strigolactone molecule Orobanchol at levels 300 to 1100 times higher than the other six studied cultivars, and we can not rule out the possibility that this group of hormones contributes to the plant genotype specificity in the interaction with the aforementioned soil microbes and PGPB isolates. However, to confirm this hypothesis, future studies are required in order to directly test the

influence of the strigolactone molecules and other exudates mediating this interaction. Variation in root exudation among agricultural crop genotypes has the potential for effective plant manipulation in order to create specific positive effects on the beneficial members of the rhizosphere microbiome (Bakker *et al.*, 2012).

## Concluding remarks

The results presented in this thesis indicated that soil and plant genotype play a crucial role in structuring the sorghum rhizosphere microbial community. As soils with low fertility showed to induce plant genotype specificity in interaction with bacterial and fungi communities, investigations on the impact of low soil fertility on the sorghum root microbial community could provide a first step in identifying microbial candidates to improve sorghum nutrient-use efficiency in low-input agricultural cropping systems in resource poor regions. This was clearly illustrated by the observation that in the less fertile Clue Field soil, the sorghum cultivar SRN-39 had significantly higher relative abundances of representatives of *Burkholderiaceae* and *Comamonadaceae* families with a stronger co-variance between bacterial and fungal community than the other cultivars. Besides the genotype effect of sorghum cultivar SRN-39 on the recruitment of the bacterial community in the rhizosphere and on the co-variation of bacterial and fungal communities, the inoculation of *Burkholderia tropica* and *Herbaspirillum frisingense* in sorghum cultivar SRN-39 also showed a greater growth effect than at the other cultivars. Future studies are required to obtain a more complete understanding of the mechanisms involved in rhizodeposition processes in sorghum and differences therein in different genotypes, in particular related to strigolactones production and their role in the interaction of soil microorganisms with sorghum. Similarly we strongly recommend that future studies should identify and isolate strains testing them as possible bioinoculant to alleviate water stress on sorghum. The higher abundance of *Caulobacteraceae* and *Rhizobiaceae* at water deficiency conditions illustrate the strain specificity at water stress conditions.

How specific functions of the microbial community of the rhizosphere of sorghum are affected in different soils, at different plant growth stages, plant genotype and drought stress is an intriguing subject which I highly recommend to be addressed in future studies. Taking into account the promising findings of our study, we suggest that microbiome engineering should be considered as an integral part of sorghum crop management practices in order to safeguard sorghum production at stress conditions.