The DARS team (Adaptive Development of Rice and Sorghum) studies development mechanisms in rice and sorghum in relation to adaptation to climate change.

Research objectives

In rice, the team studies the formation and differentiation of the root cortex to improve water use efficiency through the formation of root aerenchyma, air cavities **(Figure 1)**. Water use efficiency in rice and cereals is related to the ability to extract and transfer water at the root level, which in turn is correlated with anatomical parameters such as the presence of aerenchyma and the ratio of cortex to vascular tissue surface area. In the field, these parameters are influenced by the presence of rhyzobacteria that are able, in symbiosis with cereals, to modulate the differentiation of the cortex and block or stimulate the formation of aerenchymes **(Figure 1)**.

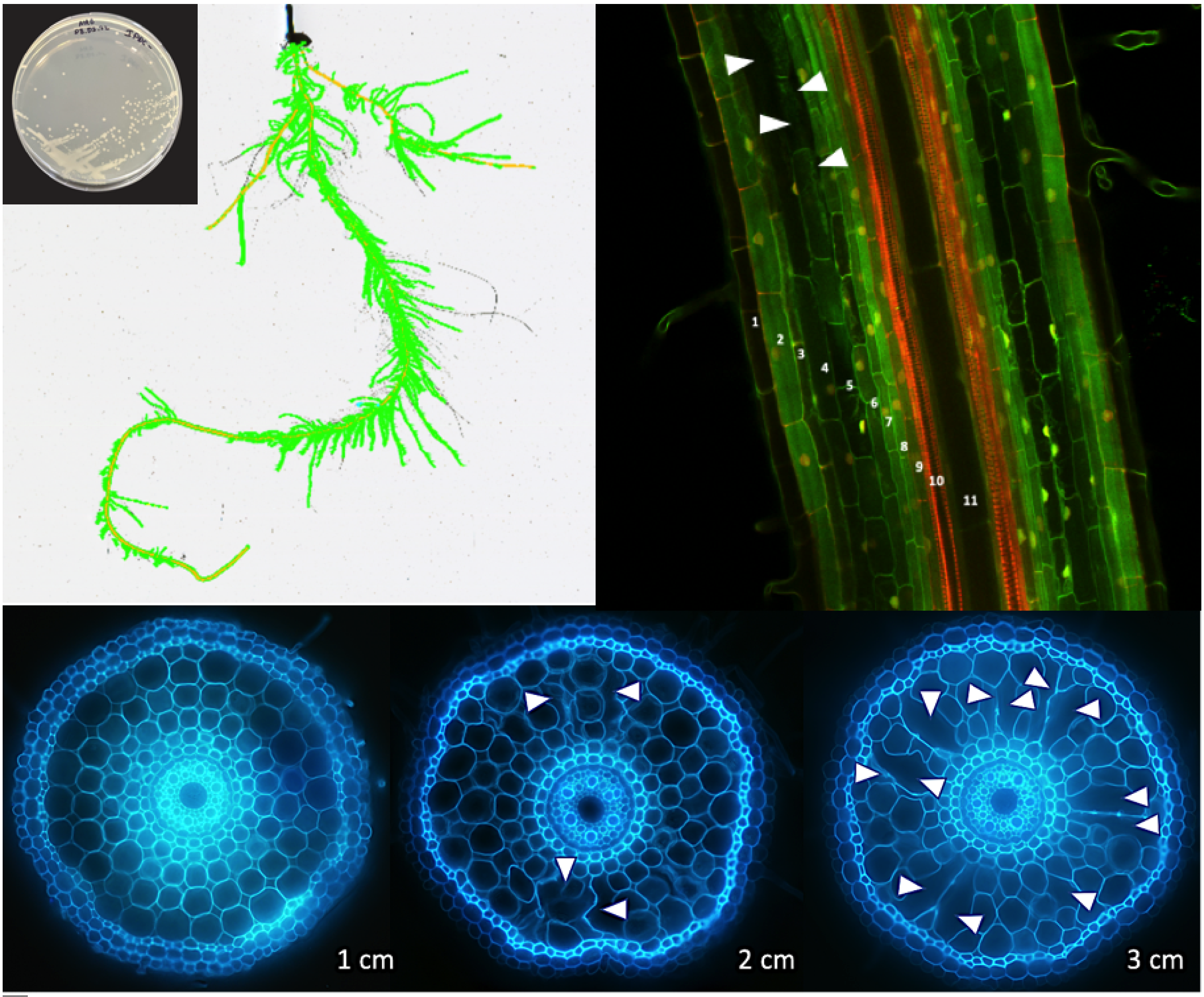

Fig. 1: From left to right and top to bottom. Architecture analysis of a rice root system using SMARTROOT after inoculation with azospiriullum brasilense sp245 ipdc- (insert). The coronary roots stand out in yellow and the lateral roots in green (photos Joanye Canguio). Median multiphoton view of a rice root p35 :YFP counterstained with propidium iodide at the aerenchyma formation zone and treated with ClearSee (photo Christophe Périn). Aerenchyma formation (white arrowheads) in the rice seminal root at 1, 2 and 3 cm from the root tip (photos Sergi Navarro). White arrows: formation of aerenchyma by cell death and detachment of the cortex walls.

In sorghum, the team is studying the mechanisms involved in the storage of grain proteins and their digestibility to improve nutritional quality. Indeed, the low digestibility of grain proteins is an obstacle to the use of sorghum, a cereal species adapted to arid conditions, in human and animal nutrition. The impact of climate change, and in particular recurrent thermal stresses on the nutritional quality of grains, is also the subject of our studies **(Figure 2)**.

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ed sorghum field. Top right, sorghum panicle diversity. Bottom, kinetics of development and maturation of sorghum grains (photos C. Périn, Nancy Terrier).

Background and issues

Rice and sorghum: cereals of global importance for human nutrition

Rice (Oryza sativa L.) is a plant of considerable sociological, economic and cultural importance. It is cultivated on more than 150 million hectares, with 600 million tons produced annually, making it the leading cereal for human consumption. A 50% increase in production must be achieved in the next 20 years on non-extendable arable land and in an unstable climate context. Rice is the model monocot and cereal and has remarkable genetic resources with 22 wild species and more than 100,000 samples of cultivated accessions; favorable biological characteristics, it is a self-pollinating plant with a short cycle; the existence of macro- and micro-colinearity of its genome with that of other cereals; the existence of numerous molecular resources, and a significant scientific community. Rice is grown in a wide range of cropping systems, water regimes (rainfed, flooded, irrigated and floating) and environments, and the genetic resources of this plant represent a considerable and original reservoir of alleles for rice adaptation to different constraints.

Sorghum (Sorghum bicolor (L.) Moench) is a cereal belonging to the Poaceae. It is currently the 5th most important cereal in the world in terms of grain production, behind wheat, rice, maize and barley, with 61.5 million tons produced annually. In the European Union, France and Italy are the two main producers of sorghum, but more than half of the sorghum consumed in the EU is imported. The grain of this cereal can be used for animal feed, which is the main use in developed countries, and for human consumption (mainly in Africa and Asia). Its agronomic characteristics are major assets for its cultivation in a context of climate change and reduced chemical inputs. Indeed, this C4 photosynthesis plant is capable of a better carbon assimilation at high temperature than C3 plants, which allowed it to develop under tropical climates, growing at an optimal temperature of 30°C. In addition, it has a high water use efficiency, giving it a high tolerance to drought, and nutrients, in particular mineral nitrogen (notably thanks to a deep root system), short growth cycles (3-4 months), and hardiness towards pests and diseases. In particular, sorghum uses water more efficiently than corn under water-limiting conditions. Not only does sorghum consume little nitrogen fertilizer, but 40% of the nitrogen mobilized by the crop is returned to the soil in organic form. Its use in crop rotation cycles is therefore extremely positive for the overall nitrogen cycle on a plot. Sorghum thus represents an alternative to corn in areas where water availability is critical. With these characteristics, sorghum, on which 500 million people already depended daily in 2011, will most certainly become an essential crop for global food security by 2050, given the need to reduce inputs and in a context of climate change.

Adaptation to contrasting soil water conditions in rice: role of root aerenchyma in tolerance to submergence and growth under arid conditions.

Water use efficiency in rice and cereals is related to the ability to extract and transfer water at the root level. This water extraction and transfer capacity is correlated with anatomical parameters such as the presence of aerenchyma, air cavities, and the ratio between the surface of the cortex and vascular tissues. These anatomical parameters are related to the formation and differentiation of the cortex. In the field, these parameters are influenced by the presence of rhyzobacteria that are able, in symbiosis with cereals, to modulate cortex differentiation and block or stimulate aerenchyma formation. Compared to other cereals, rice naturally produces aerenchymes whatever the growing conditions (constitutive aerenchymes) whereas other cereals only produce aerenchymes under abiotic stress conditions, and of course under submergence, and constitutes an ideal model to identify the genes and characterize its formation mechanisms.

Aerenchyma are air channels that form by cell death of a tissue present in roots and stems, the cortex. Aerenchyma, under submerged conditions (waterlogging), allow the roots to receive the oxygen necessary for their proper functioning. Indeed, when the roots are submerged, oxygen is present at much lower concentrations than in the air and the diffusion of oxygen in water is very slow. Under these conditions, the roots will very quickly asphyxiate. In arid conditions, they also play an important role because the presence of many dead cells reduces the amount of energy to be used to extract water and nutrients from the soil. This available energy can be reallocated to the aerial parts or to grain filling. This role explains the presence of so-called inducible aerenchyma in plants that grow in arid conditions such as sorghum or millet, compared to rice. On the other hand, under favorable conditions, aerenchyma have a negative impact on plants because the presence of aerenchyma reduces the radial transfer of water and nutrients. Indeed, the passage of water and nutrients is essentially done by the living cells, the aerenchymes not allowing this passage. There is therefore a trade-off between the presence of aerenchyma and soil moisture conditions.

One of the hypotheses we are working on is to optimize rice growth under rainfed conditions (intermediate) by reducing or even eliminating root aerenchyma (CRAZYRICE project, Phenomen team collaboration, C. Rebolledo). We also seek to modulate the presence of aerenchyma in rice or sorghum varieties in order to improve their yield under a wide range of crop conditions. In collaboration with the LIST (Dr Ghoniem) and the University of Lille (Pr Elati), we seek to isolate the gene networks involved in the establishment of aerenchyma by a reverse genetic approach (ANR GREENER project, leader C. Périn).

We are currently characterizing strains of PGPR bacteria, Azospirullum brasilense and Pseudomonas fluorecens for their ability to induce or suppress root aerenchymes in rice (Rhyzocortex UM/AGAP project, leader F. Varoquaux). These bacteria are already used to improve the yield of cereals, including rice, in several countries, such as South America. We have also identified bacterial strains (e.g. Azospirullum brasilense sp245 ipdc-) mutated for plant hormone biosynthesis or catabolism genes and are studying their impact on aerenchyma formation and other root anatomical parameters.

Protein quality in sorghum

Despite the agronomic advantages of sorghum, the low digestibility of sorghum grain proteins by gastrointestinal proteases is a major obstacle to the wider use of sorghum for animal (monogastric) and human food. This digestibility is at least 25% lower than that of other cereals (wheat, corn, rice). This can be attributed to the interaction of proteins with other grain compounds such as starch or tannins, but also to the structure of the sorghum grain's reserve proteins, the kafirins, which represent 70% of the grain's proteins. Sorghum grains can contain from 7.6 to 13% of proteins. Kafirins are assembled in an organelle called protein body (PC). The low digestibility of kafirins would be linked to the three-dimensional network of these PCs, structured by numerous disulfide bridges between cysteines. The rate of proteins linked by disulfide bridges increases during grain development but the proteins are still partially reduced in the mature grain; cooking favors the appearance of additional disulfide bridges by oxidation, which would explain the observed loss of digestibility.

Our working hypotheses are i) that a low diversity for protein content and protein digestibility is available within temperate breeding programs (Europe) and that it can be enriched through the use of a larger genetic base, ii) that a better understanding of the molecular mechanisms responsible for protein digestibility and iii) that access to medium and high throughput phenotyping methods for digestibility and protein content will allow the development of more efficient breeding strategies. Work to test these hypotheses is being developed (Sokafi, BAP, INRAE; NitroSorg, CASDAR, leader N. Terrier). The impact of recurrent hot stresses on the quality of sorghum grains, and in particular on protein content and digestibility, is also being evaluated (PARSEMA project, INRAE, project leader C. Granier).

Translational biology: two complementary cereal models

Sorghum is a C4 plant grown mainly in arid conditions, whereas rice is a semi-aquatic C3 plant grown mainly in irrigated conditions. These two species are therefore at two ends of the spectrum of cereal growing conditions.

They are two complementary species and their comparative study will make it possible to test the genericity or specificity of the genes and developmental mechanisms studied. For example, sorghum, which forms aerenchyma under arid conditions, can be used to determine whether genes involved in aerenchyma formation in rice have a conserved function and to compare the mechanisms of their establishment.

Conclusion

We aim to identify and characterize the genes involved in these two developmental and adaptive processes in these two species using a molecular genetic approach combining mutant analysis (CRISPR/CAS9), imaging (Laser Capture Microdissection, multiphoton imaging), transcriptomics, high-throughput phenotyping, network analysis and association genetics.

Partnership

This work is carried out in collaboration with the Evolutionary Genomics and Population Management (GE^2pop) and Genetics and Varietal Innovation (GIV), Phenomen teams of the unit, using the latest cellular imaging approaches developed on the PHIV platform. The University of Lille (Mohamed Elati

University Professor at INSERM U908 Cellular Plasticity and Cancer, LIST Mohammad Ghoniem), ANR GREENER project, as well as the CERES Diade team (Pascal Gantet, University of Montpellier Professor, Masteroot ANR project in progress) are our main partners in Montpellier The work on sorghum grain quality is built in collaboration with the IATE (Montpellier), BIA (Nantes), BOA and PEAT (Tours) units, the Eurosorgho and RAGT2n seed companies and the ITAVI technical institute.