Objectives and research hypothesis

Floods have become more frequent, more severe in recent years in many parts of the world, a trend that is linked to ongoing climate change (FAO, 2015). Beyond their obvious impact on the affected populations, **flood periods are responsible for significant crop losses, with most cereals being sensitive or even very sensitive to flooding with 60% of the total world crop loss for the 2003-2013 period (FAO, 2015)**. Water is a medium where gases diffuse very slowly. Submersion leads very quickly to the blocking of two essential biological processes, photosynthesis and respiration, which in turn blocks growth and ultimately production therefore affecting yields (Sasidharan and Voesenek, 2015). Survival to submersion is highly variable in the plant kingdom and includes various survival mechanisms involving anatomical, architectural and metabolic adaptations. Even within the same species as rice, there is a wide diversity of responses to submergence. Some rice cultivars are tolerant to a relatively short period of flooding through a “quiescence” strategy consisting in inhibiting all growth and metabolism until the water slide decreases (Xu et al., 2006). Floating rice has a totally different adaptive response called “avoidance” consisting in considerably lengthening the internodes in response to submergence, which allows the always emerged part of the plant to supply the submerged parts with oxygen (Hattori et al., 2009). The SUB1A and SK1/SK2 genes respectively under the quiescence and the escape strategies were already identified (Hattori et al., 2009; Xu et al., 2006). **Besides these mechanisms found in some rice specific cultivars, a broader mechanism of flooding tolerance is also present in rice involving anatomical adaptations typical of aquatic plants named aerenchyma (Nishiuchi et al., 2012).**

Aerenchyma (Figure 1), are air-filled cavities that connect the aerial parts and roots, allowing them to maintain their breathing and growth under submerged conditions (Nishiuchi et al., 2012; Rebouillat et al., 2009; Yamauchi et al., 2018).

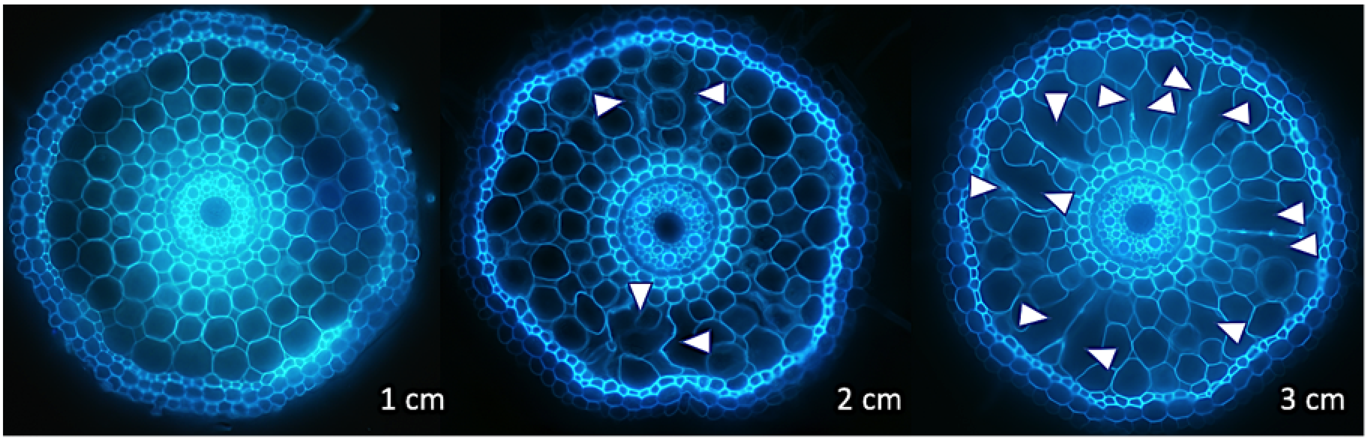

Fig. 1: Aerenchyma formation (white arrowheads) in the rice seminal root at 1, 2 and 3 cm from the root tip (photos Sergi Navarro). White arrows: formation of aerenchyma by cell death and detachment of the cortex walls.

The formation of aerenchyma leads to a decrease in mechanical strength during root soil penetration. In most submergence-tolerant species that form aerenchyma, there are also compensation mechanisms to maintain mechanical strength by formation of a ring of cells layers outside cortex (Nishiuchi et al., 2012; Striker et al., 2007). In rice, outer tissues such as highly lignified sclerenchyma tissue, and to a lesser extent exodermis, form a belt that compensates for this decrease in mechanical strength and is an important part of flooding tolerance in a lot of submersion-tolerant varieties (Rebouillat et al., 2009). Interestingly, there are aerenchyma-forming species in all groups of flowering plants, monocotyledons, dicotyledons and basal angiosperms (Seago et al., 2005). **Lysogenic aerenchyma are formed by cell death and the fusion of cortex cell walls, comprising several cell layers in rice (Rebouillat et al., 2009; Seago et al., 2005).**

Formation of aerenchyma can be subdivided in four phases in rice (Kawai et al., 1998; Mounier et al 2020a). In a first phase inside root meristem, called cortex formation, the endodermis-cortex stem cell divides several times to form the outer cortex and a layer of endodermis (Pauluzzi et al., 2012). The elongation phase follows the initial phase when the median cortical cells elongate radially. Besides cell elongation, the acidification of these cells is also one of the first early elements distinguishing these cortex cells from their neighbors (Kawai et al., 1998; Mounier et al 2020a). In the third so-called cell death initiation phase, the cells of the median cortex layers begin to undergo programmed cell death where first aerenchyma become to form (Kawai et al., 1998; Mounier et al 2020a). The last phase, the cell death expansion phase, starts when cell death extends radially to other cortical cells (Figure 2).

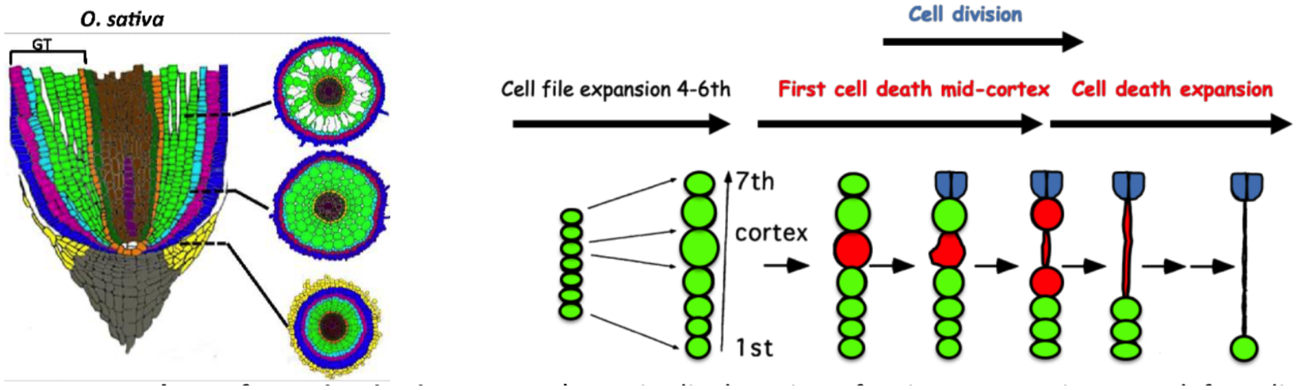

Fig. 2: A) Longitudinal section of a rice root meristem on left, radial sections on right. Cortex is color coded in green. From bottom to top, radial sections of the meristematic stage of cortex formation, elongation phase with radially enlarged mid-cortex cells, mature aerenchyma formation stage. Aerenchyma are visible as white cavity in the last stage. B) Detailed stages of aerenchyma formation. In red, first cell to undergo cell death that propagate radially. Note the most distal cell do not undergo cell death as well as the cortex cell layer adjacent to the exodermis (in bleu), divided tangentially

When aerenchyma formation is achieved, some bridges of living cortical cells as well as the outermost layers of cortex remain alive. **Rice has constitutive aerenchyma that are formed regardless of the growing conditions (rainfed or submerged) but the aerenchyma are induced in a more distal position inside root meristems in submerged conditions (Yamauchi et al., 2018)**. In other cereals, the appearance of these aerenchyma is only induced by submersion, for instance in maize (Yamauchi et al., 2016; Mounier et al 2020a) suggesting that some of these tolerance mechanisms are retained between these cultivated cereals and that transferring high-level of tolerance from rice to other cereals should be feasible. Moreover, it is now well established that root cortex aerenchyma reduce the metabolic cost for soil exploration and thus a promising way to develop varieties with water, nitrogen and phosphate use efficiency in cereals (Lynch JP., 2015).

Programmed cell death is triggered and is under the control of ethylene, in rice but also in maize and other cereals. If the biosynthesis or perception of ethylene is blocked, there is no formation of aerenchyma confirming its key role (Justin and Armstrong, 1991). In addition, it has been shown that ROS (Reactive Oxygen Species) downstream of ethylene biosynthesis also have an important role in triggering cell death. Cells that undergo cell death have an increase in the expression of ethylene biosynthesis genes such as ACO (ACC oxidase) and ACS (ACC synthase) and ROS production genes such as RBOHH (respiratory burst oxidase homolog H) (Yamauchi et al., 2017). Conversely, there is an increase in the transcription of ROS detoxification genes like Metallothionein in cells and tissues that do not experience cell death (Yamauchi et al., 2017).

However, the control of the initiation phase is independent of ethylene, suggesting that its formation is controlled by development and/or hormonally in rice by independent

mechanisms and signals (Justin and Armstrong, 1991). This phase is a priming phase where cortex cells become competent to respond to ethylene and where first events of aerenchyma formation are visible (Kawai et al., 1998; Mounier et al 2020a). This is a critical phase to identify upstream regulator of aerenchyma differentiation. Few recent results suggest a complex interplay in formation and priming of aerenchyma in rice. Overexpression of a peptide, OsEPF1, has led to the early appearance of aerenchyma in rice, suggesting that peptide/receptor signaling pathways are also involved in the establishment of aerenchyma and may be in PCD (Mohammed et al., 2019). Moreover, the formation of aerenchyma also appears to be under the control of auxin through the AUX/IAA and ARF auxin dependent pathways (Yamauchi et al., 2019).

But to date, no key genes, cortical cell death regulators or priming genes for the initiation phase of cell death have yet been identified. The main objective of this project is to identify the gene network involved in aerenchyma formation. Moreover, due to the close relationship of cereals and the existence of some partial common conserved mechanism of inducible aerenchyma formation between rice and other cereals it should be possible to transfer rice flooding tolerance mechanisms to other cereals.

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