Sink or swim: submergence tolerance and survival strategies in Rorippa and Arabidopsis

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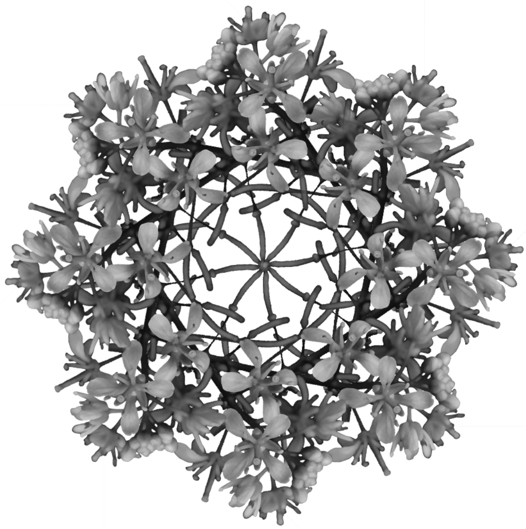

CHAPTER 6

Variation is often underappreciated.

Conclusion

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CHAPTER 6

Lamarck in his Zoological Philosophy (1809) mentions the dramatic impact of submergence on plants as follows:

> "So long as *Ranunculus aquatilis* is submerged in the water, all its leaves are finely divided into minute segments; but when the stem of this plant reaches the surface of the water, the leaves which develop in the air are large, round and simply lobed. If several feet of the same plant succeed in growing in a soil that is merely damp without any immersion, their stems are then short, and none of their leaves are broken up into minute divisions, so that we get *Ranunculus hederaceus*, which botanists regard as a separate species."

This strong selective force affects the life cycle of many plant species and has led to the evolution of a wide range of adaptations and a gradient of plant species in flood-prone areas (Vervuren, 2003; Van Eck *et al.*, 2004). Submergence also has a high impact on crops since it decreases the productivity approximately by 20% worldwide. Thus, in order to sustain both agricultural crops and natural plant populations, it is important to understand why and how some species are better adapted to low oxygen conditions. The aim of this thesis is to unravel the underlying mechanisms of various adaptations evolved in plants to cope with severe effects of submergence. For this purpose we studied the variation both within and between species that show different submergence tolerances. We investigated submergence tolerance mechanisms in tolerant *Rorippa* and *Rumex* species inhabiting flood-prone areas and in the intolerant model plant Arabidopsis.

Using inter-species variation

Escape, quiescence and additional strategies in Rorippa

In Chapter 2, we investigated the nature of extreme submergence tolerance in two *Rorippa* species that show little mortality under complete submergence even after months. *Rorippa amphibia*, inhabiting sites that are flooded for longer periods, shows an escape strategy with an elongated stem and elongated leaves. In order to supply the energy and building blocks for this growth, reserve carbohydrates are depleted faster. And if the elongating stem cannot reach the surface before the carbohydrates reach emergency levels, plants may not survive. However, once the stem reaches the water surface and the gas exchange is re-established, the shoot flourishes above the water level and forms adventitious roots on the stems. The elongated slender stem often detaches from the belowground tissues and settles on the embankment again where conditions are more favorable. Contrastingly, *Rorippa sylvestris*, inhabiting sites flooded for shorter periods, adopts a quiescence strategy and ceases its growth until the waters subside. This strategy leads to a slower depletion of the precious carbohydrates and as a result to a longer survival under complete submergence. *Rorippa sylvestris* also clonally grows from the carbohydrate rich belowground tissues after floods reside. So clonal growth constitutes a big part of the submergence survival mechanisms of both *Rorippa* species although they show different modes of action. The effectiveness of these strategies might explain why flood-prone areas are often populated with clonal plants (Sosnova *et al.*, 2010).

In rice these strategies are also present but without the additional detach and disperse strategy of *R. amphibia* or clonal expansion from roots of *R. sylvestris* (Xu & Mackill, 1996; Hattori, 2007). So when studying submergence strategies in different species, it is important to note the ecological context as well as the repertoire available to the plants , as both are responsible for the evolution of different mechanisms of submergence tolerance. These two *Rorippa* species constitute an important model system for studying submergence tolerance, firstly because their adaptations to cope with flooding are extremely effective, and secondly, because *Rorippa* is closely related to the model plant Arabidopsis, which facilitates genetics and genomics research in *Rorippa*. Thus, the use of molecular tools available for Arabidopsis can greatly accelerate further submergence research in these species.

Regulation of the strategies and oxygen sensors

In Chapter 3, we made use of this relatedness to clone *Rorippa* group VII ethylene response factor (ERF) genes as possible candidates for controlling these strategies, given that *SUB1* and

SNORKEL genes of rice are also members of this subfamily (Xu, 2006; Hattori *et al.*, 2009). We showed that all group VII ERFs form two syntenic blocks, implying that in angiosperms group VII ERFs are derived from two ancestral genes. We also used two *Rumex* species; *Rumex palustris* and *Rumex acetosa* that show escape and quiescence strategies, respectively, investigating if there was a parallel evolution in the regulation patterns of these genes in *Rumex* and *Rorippa*. We did not observe a clear signal for convergent evolution in regulation patterns, but we did identify members from synteny block I to be good candidates for being oxygen sensors, and possibly increasing submergence tolerance. We also did not observe a clear-cut correlation between preservation of carbohydrate reserves and submergence tolerance of species. Our results indicate that the initial acclimation processes is important and leads to depletion of carbohydrates. However species lacking the internal mechanisms to cope with submergence stress show faster mortality, even if the carbohydrates are conserved, as observed in *Rumex acetosa* that is flooded less frequently in its natural habitat (Nabben *et al.*, 1999).

Using within-species variation

Although a species not occurring in flood-prone areas, Arabidopsis does show variation in submergence tolerance (Vashisht *et al.*, 2011). By using this variation in two accessions Col (*gl1*) and Kas-1, we identified a submergence tolerance QTL, *Come Quick Drowning 1* (*CQD1*), by performing a quantitative trait loci (QTL) analysis. It is important that this locus does not include any growth related QTLs. It is likely that gene(s) in this QTL are responsible for an acclimation process, since Arabidopsis does not display any escape or quiescence strategies. We also performed an RNA-seq experiment to capture the earlier responses of these two accessions to identify differentially regulated genes between the accessions. Interestingly, we found that many genes from the ERF gene family were differentially regulated under submergence in the two accessions. The recent identification of group VII ERFs as oxygen sensors that initiate low oxygen induced transcription suggests that these genes are necessary for acclimations to low oxygen stress (Gibbs *et al.*, 2011; Licausi *et al.*, 2011). Knock-out mutants of these genes show decreased submergence tolerance and over-expresser lines, correspondingly, have a higher survival.

A network of trade-offs

Depths and durations of floods are a major determinant of survival. Survival mechanisms set trade-offs between the carbohydrate contents (depleted or conserved depending on the strategies adopted) and acclimation processes to prevent severe tissue damage. If elongating species cannot reach the surface before all carbohydrates are depleted the plant will surely die. Likewise if plants do not acclimate but the floods last very long, even if the carbohydrate conservation is sufficient, the tissue damage will lead to a higher mortality. Conversely, if floods are short and transient, a quiescent plant showing no acclimation and thus conserving more carbohydrates in the initial phase of submergence might flourish faster than a plant that used carbohydrates for acclimations that soon were obsolete. Thus, there is a tight balance between all these parameters and as a result submergence tolerance is the result of a complex network affected by all these trade-offs set by different flooding regimes.

Future Perspectives

In order to understand the nature of these adaptations present in *Rorippa*, *Rumex* and also to some extent Arabidopsis, it is crucial to identify the genes responsible for higher tolerance. Group VII ERFs are good candidates for dissecting the mechanisms of the escape and quiescence strategies in both *Rorippa* and *Rumex*. Functional analysis of these genes might aid identifying the regulation mechanisms. In *Rumex*, the hormonal regulatory patterns are well studied in particular. Similar research might also accelerate the submergence research in *Rorippa* to resolve the key components regulating these strategies and their extreme tolerance. Likewise in Arabidopsis, *CQD1* locus sets a good candidate to understand the intraspecific variation in different ecotypes of an intolerant plant. Further fine-mapping and candidate gene analyses in this region may reveal important actors in submergence tolerance. Studying a wide range of tolerant species inhabiting flood-prone areas will help us to unravel the stories of nature and fully appreciate the variation that is essential for the survival of any organism in a rapidly changing environment.