

Ph.D. thesis assessment report

Deciphering the diversity and functionality of fungal endophytes associated with *Salicornia europaea* L.

Bliss Ursula Furtado

Nicolaus Copernicus University in Torun, Faculty of Biological and Veterinary Sciences, Department of Microbiology

Main supervisor: Prof. Katarzyna Hryniewicz, Nicolaus Copernicus University, Poland

Co-supervisors: Prof. Torben Asp, Aarhus University, Denmark, Dr. Niels Roulund, DLF Seeds A/S, Denmark

Salicornia europaea is a plant species capable of withstanding extreme soil salinity. This makes it an interesting target for the study of mechanisms of plant adaptation to salinity, a study area with practical applications for agriculture. Among the possible mechanisms for plant adaptation to stress, those contributed by the plant microbiome should be considered, however, the function of the microbiome on plant adaptation to salinity is largely unknown. This thesis is a study of *S. europaea* and its microbiome in relation to salinity adaptation. Two different populations of *S. europaea* were studied, one at a naturally saline area where plants are expected to have a long history inhabiting the site, the other at a site where the source of salinity are industrial residues, and a more recent history of inhabitation is expected. In terms of conductivity and other parameters, salinity is greater at the natural than at the industrial site, and in fall over spring at both sites. Plant shoots and roots from both populations were compared across seasons in order to detect effects on plant gene expression and microbiome structure. The thesis consists of a general introduction plus three different chapters, each one corresponding to a different study already published in a scientific journal.

The introduction contains some general information about halophytic plants and endophytic microorganisms. In this part I missed some information about known anatomical and/or physiological modifications and mechanisms related to salinity adaptation in *Salicornia europaea*. Although in chapter 1 it is mentioned that there are "specific morphological features that enable plants to adapt to salinity.." (p. 44), those are not mentioned in the text. Also, the section about the problem of salinity in agricultural soils is very general and could have been more detailed. For example, some information about the relationship between irrigation and salinity, or the extent of salinity damage in agricultural soils in terms of area could have been included.

The first chapter reports an RNA sequencing study of shoots and roots of *S. europaea* plants from the two locations, at two different seasons. A transcriptome consisting of 38384 genes was assembled and these genes were classified according to gene ontology categories. Differentially expressed genes (DEG) were detected mostly when samples from different seasons were compared, not across sites or plant organs. A detailed analysis of 30 DEG found in all comparisons was done, and the differential expression of some of these genes was confirmed by quantitative PCR. The Additional

file 3 with the list of the 30 selected DEG could have been included in the thesis. Two of these genes are RNA dependent RNA polymerases, to my knowledge these are not common plant components, and this could have been discussed. In this chapter I missed a numerical summary of the analysis of differential expression, showing the number of DEG genes detected above a given threshold for each possible comparison (site1 vs. site 2; shoot vs. root, spring vs. fall), this is not easy to appreciate in some of the gene expression plots (Figure 4). Also, the heading of additional file 4 is confusing, according to it there are 38k genes differentially expressed.

The second chapter reports the results of a study where a culture-independent approach was used to identify bacterial and fungal endophytes of roots and stems of *S. europea* at the two sites above mentioned, in two seasons. Overall, thirty one bacterial and six fungal taxa were identified. In terms of structure, bacterial assemblages differed between sites and organs, but not between seasons. It is proposed that the history of salinization, not the salinity value itself, is the main driver of site differentiation. A single bacterial taxon, *Marinimicrobium*, was identified as a possible member of the core microbiome, due to its abundance in samples. Fungal communities were dominated by a *Paradendryphiella* sp., and it is not clear to me why a bacterial taxon is proposed as a member of the core microbiome, and this fungal taxon is not. Perhaps, more information about the criteria used to define the core microbiome should have been included. The fungal microbiome identified is dominated by pleosporalean taxa, and this could have been linked in the discussion to the dark septate endophytes, mentioned in the introduction. A cocorespondence analysis indicated relationships between the fungal and bacterial communities, in such way that the composition of one would influence the composition of the other. It is proposed that the bacterial metabolism may prepare adequate niches for particular fungal species. In addition, a bioinformatics study linking the bacterial assemblages detected to metabolic pathways associated to the microbiome was done. Several pathways related to carbohydrate metabolism and other functions were reported to occur.

In Chapter three a direct isolation approach was used to survey the fungal microbiome of *S. europea*. After strain identification, a series of biochemical and physiological characteristics such as production of siderophores, polyamines, indole acetic acid, several enzymes, and salinity tolerance were determined for a group of 39 strains. In addition, plant inoculations with six fungal strains were made into two varieties of *Lolium perenne*, one of them was symbiotic with *Epichloë festucae* (E+), and the other variety non symbiotic (E-). It is interesting that for the fungal isolation, bacterial isolation techniques such as grinding samples to paste, or the use of serial dilutions were used. As a result of the survey, forty different fungal taxa were identified. This diversity is much greater than the one observed in the culture-independent survey of chapter 2, and this could have been discussed in the last section of the thesis. The biochemical and physiological characteristics of the fungi are resumed in a table, and perhaps a multivariant analysis of these data, could have been made to search for connections between the variables measured, and organ, site of origin, or taxonomic similarity of strains. In the inoculation tests the six strains positively affected several shoot growth components of the E+ variety of *Lolium perenne*, but this effect was restricted to only two strains in the E- variety. This result shows that some *Salicornia* endophytes can be beneficial for an agronomic

species, and in the future it would be interesting to know the response of inoculated plants to salinity stress. In the discussion about why the effect of endophytes differ between both varieties it is important to know if these are truly different plant varieties, or if they consist of the same plant variety, but differing only in their *Epichloë* status.

This thesis is an original work which produces new and useful knowledge about a very interesting system in a field of science that is currently growing and attracting much attention, that of plants and their microbiomes. The results were obtained using various techniques, and range from a plant transcriptome to descriptions of microbiome composition, structure and function, including possible agricultural applications. The thesis is well written, and its conclusions are well supported by the evidence obtained. The main body has been published in three well considered journals: BMC Plant Biology, Applied and Environmental Microbiology, and Plant and Soil, and Ms. Furtado is the first author of these three publications. Considering the above, in my opinion this dissertation is appropriate for the next steps of the procedure leading to the Ph.D. degree, and could be considered as distinctive, being eligible for an award.

Sincerely,



Iñigo Zabalgogea, Ph.D.
Research Scientist
Salamanca, November 12, 2019