Research Networking Programmes

Short Visit Grant ☒ or Exchange Visit Grant ☐

(please tick the relevant box)

Scientific Report

Scientific report (one single document in WORD or PDF file) should be submitted online within one month of the event. It should not exceed eight A4 pages.

***Proposal Title**:* Local adaptation in marginal populations in high mountain plants: an integrated perspective

Application Reference N°: 5893

1) Purpose of the visit

The evaluation of local adaptation in organisms under different selection pressures is one of the objectives pursued under the frame of this project. In this sense, the high-mountain plant Silene ciliata Pourret, distributed across mountain systems in Balkan Peninsula, the Apenines, the French Massif Central and the North of the Iberian Peninsula is a good model organism to work with. The Central System in Spain is the southernmost distribution limit of this species, where it can be found from 1900 m to 2500 m elevation. In the context of this project the utilization of genomic techniques is planned, in order to know the genes involved in the local adaptation processes. This knowledge about the response to environmental changes by these organisms will allow and support the decision making when developing conservation strategies.
Prof. Widmer and his research team have great knowledge concerning the use of these methods in Silene species. The main purpose of this short stay is to optimize the processes related to the extraction of mRNA in field conditions and the preparation of resulting data in the early stages of the process of transcriptome analysis. A secondary objective is getting acquainted with the genotyping by sequencing technique, which may help in the characterization studies to be undertaken with S. ciliata populations, as an alternative to the SSR markers that we are using at present.

2) Description of the work carried out during the visit

* RNAm extractions from S. ciliata plant material previously sent to ETH have been carried out. General considerations previous to the lab work point out the following issues:

- It is better to extract mRNA from young tissues, such as buds or young leaves. Although the use of leaves could be advisable because it is easier to collect the tissues, the presence of chlorophylls may result in worse quality of the extract. Nevertheless, the questions we are asking in the context of this project can be solved with this material and the quality of the extracts would be good enough.
- Large quantities of plant material may spoil the extraction process. According to the protocol used, no more than 100 mg of tissue should be used.
- It is advisable to use gloves when collecting the plant material in order to avoid pollution of samples and the disruption of the RNA molecules with RNAses in our hands. It is essential to flash freeze the tissue in liquid nitrogen in the moment of colletion.
- Samples must be in LN until its usage or at least in -80º C freezers.
- Filter tips are recommended for this kind of lab work.

* Two RNAm extractions were carried out using commercial kits following slightly modified protocols.

* Quantification and quality assessment of the extracts were done using a fluorometer and Bioanalyzer 2100 respectively. The quality of the RNA was measured according to the RIN method (RNA Integrity Number) proposed by Agilent Technologies and following the RNA 6000 nano kit protocol, recommended for the quantities of RNA we were working with (Total RNA: 5-500 ng/ul, mRNA: 25-250 ng/ul). Quantification of RNA was made with a fluorometer, again following manufacturer instructions (Qubit fluorometer).

* Next objective was to get some knowledge on the Next Generation Sequencing (NGS) techniques. This has been done holding some meetings with senior and junior researchers in the host group in ETH (Prof. A. Widmer, Dr. M. Fischer or Dr. M. Paris) to discuss some of their newest research activities in this area and explain to them the main issues we are interested in regarding the Silene ciliata experiment that we are planning to undertake in Spain. Also discussions with young researchers (A. Florez) were held. The reading and discussion of trending papers on these topics was also fundamental for the understanding of processes and results.

* I have also attended the department meetings organized in ETH for discussing their new scientific articles (Science meetings held on Thursday mornings) and to the group meetings, led by A. Widmer, held after this Science meeting.

3) Description of the main results obtained

RNA extractions were successful as checked with the quality and quantity assessment.

* Quality was evaluated according to the RIN number (scale 1-10), and values were 7.2 and 7.8 for the samples measured with the Bioanalyzer 2100.
* RNA quantities obtained from the extraction process were also good, reaching concentrations of 73.4 µg/µl and 141 µg/µl.

* Discussions with ETH researchers were also really productive. The benefits of using pooled samples for the planned analyses were discussed. Although the use of pooled samples implies the loss of information at the individual level, it can provide information at the population level for expression, allele frequencies and also on effective sizes of populations which will meet the requirements set in the project. We, at the URJC team, have decided then to use this pooled samples approach instead of individual samples as initially foreseen. The benefits of using third generation techniques (Single Molecule Real Time Sequencing) and platforms other than Illumina were also discussed. Conclusions on this issue are that it is more advisable to use Illumina (HiSeq or MiSeq) than other techniques due to two main reasons, 1) questions posed in this project can be solved with these techniques and 2) the wide use of Illumina platforms makes available a lot of information and references to support our results in the future. Finally the use of the Genotyping by Sequencing (GBS) techniques has been discarded for the time being because the information that can be provided with them, will be available with the construction of cDNA libraries from pooled samples of mRNA.

4) Future collaboration with host institution (if applicable)

This short stay has been very useful to establish contacts of experts in genomic approaches with Silene species. Both at the personal and team levels, future collaboration with the genomic studies on Silene ciliata is expected at the time of sequencing the material and in the processing of the resulting information in Spring 2014

5) Projected publications / articles resulting or to result from the grant *(ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant)*

No ongoing publications exist at the time being, but they are likely to arise if this collaboration continues when the research is finalized. ESF will be acknowledged in all future publications that might result from this collaboration.

6) Other comments (if any)

A change in dates of the visit, due to calendar impediments with the technician helping in the lab work, was authorized by ESF. Final dates were from 21st October to 3rd of November instead of the initially proposed dates (30th of September to 13th of October)