

**General information on Short Term Scientific Mission (STSM)**

Host institution: Institute of Biosciences and BioResources, National Research Council - Division of Florence, Sesto Fiorentino-Firenze (IT); Dr. Giovanni Giuseppe Vendramin.

Period: 05/06/2014 to 14/06/2014

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STSM Applicant: Nicolas-George Eliades, Ph.D.

**Subject: Improving genetic, demographic and biological knowledge in marginal and peripheral forest trees populations: maintaining genetic resources.****Purpose of the Short Term Scientific Mission**

The main purpose of this STSM was to improve and update the knowledge of a young researcher on various forest genetics aspects, with a focus on evolutionary factors which are the basis for the adaptation of forest tree populations into different geographical sites (marginal and peripheral populations). Further, the STSM aimed to increase the skills of the young researcher on molecular markers (more specifically on new-generation sequences) and on various tools for analysing genetic variability.

**Description of the work and main results**

During the STSM I had the opportunity to gain familiarity with new molecular techniques currently used for investigating demography and adaptation processes of wild plant populations. This knowledge is a powerful tool for developing sustainable management strategies for genetic resources of marginal/peripheral populations which are particularly important for adapting forests to global changes. The marginal/peripheral populations are particularly threatened by climatic changes because the changes act in combination with other disturbances originating from human activities.

More specially, in the first two days of my STSM I had the chance to become familiar with the host institution's infrastructure on molecular genetics (laboratory and used methods). Further, through discussions with Dr Vendramin and his group we exchanged information regarding our common research interests with more focus on plant species' adaptive abilities. Admittedly, the levels of genetic variability and diversity significantly influence the adaptive abilities of the forest populations. Thus, the knowledge of the genetic variability distribution within and among the marginal/peripheral populations is important for the preservation of gene resources for *in situ* and for *ex situ* conservation actions.

At the practical level, I was given the opportunity to learn about the general approach on designing and developing nuclear microsatellites (SSRs) markers. The SSR marker was developed during 1990s

and acted as a tool for various studies (i.e. demography studies, breeding). Often the SSRs derived from non-coding/anonymous genomic regions, such as genomic survey sequences and bacterial artificial chromosomes. However, in the last years, due to the availability of large-scale gene/EST (expressed sequence tag) sequence information for various plant species, SSR markers can easily be developed in silico. The availability of genome sequences and transcriptome from different plant species in gene banks allow us to design and develop relatively easily a high amount of nuclear SSRs. The genus of *Cedrus* ssp. and more specifically the species of *Cedrus brevifolia* was used as a study case for studying the steps for SSRs discovery and primer design. Initially, transcriptome from *C. atlantica* was used for searching the SSRs motive, using the software *SSR locator.v1*. Running of *SSR locator.v1* filter and evaluating the observed SSRs motives within the input data (large-scale gene / expressed sequence tag / transcriptome). After the evaluation of the software outcomes, the next step was the design of primer pairs for each of the final decided SSRs. The primer design was carried out by the *Primer3: WWW primer tool* web software. This practical training on the theory of SSRs primer design was a useful skill since the transcriptome data is expected to increase in the gene banks in the future, allowing the researchers to easily develop and test a high amount of SSRs for target species.

The next-generation DNA sequencing (NGS) technologies have led to the development of rapid genome-wide single nucleotide polymorphism (SNP) detection applications in various plant species. The improvements in sequencing and the general decrease of cost per gigabase of sequence are nowadays allowing NGS to be applied not only to the evaluation of small subsets of parental inbred lines, but also to other studies (i.e. mapping and characterization of traits of interest in much larger populations, investigating gene diversity). Such an approach, where sequences are used simultaneously to detect and score SNPs, therefore bypassing the entire marker assay development stage, is known as genotyping-by-sequencing (GBS). All this theoretical frame of NGS I studied in a practical way in this STSM, since the host institution has the relative equipment (i.e. Ion torrent, Ion Chef System, Ion OneTouch2, Ion OneTouch ES). At the same time I had the opportunity to see and practically work with raw data of GBS in conifer species (available by the host institution), thus increasing my skill and knowledge on this molecular technique. More specifically, working with the software *SNPviewer* enables genotyping data to be viewed as a cluster plot by GBS.

Furthermore, I learned (in theory and in practice) about a stochastic analysis of the evaluation of demographic history and neutral parameterization on the performance of  $F_{ST}$  outlier tests. Lively discussions on the  $F_{ST}$  outlier tests theory and how this method is applied in population genetics allowed me to learn new statistical techniques. The practical test based on the two major theories on  $F_{ST}$  outlier tests was done using the softwares Arlequin, Lositan and Bayesca.



*Future collaborations*

This STSM was a good option for me as an early stage researcher, to increase my skills and knowledge of the modern genetic technics that will be used to investigate the gene diversity and population structure on plant species. In addition, this STSM has enhanced collaboration between two different institutions which were not related in any way in the past. Both sides have agreed to maintain collaboration, and, hence, will go further in the near future by submitting proposals in EU programmes (i.e. Horizon 2020, Marie Skłodowska-Curie Actions, LIFE+). Generally, this collaboration can be reinforced with the study of the adaptation and response to climate change of forest species with important environmental and commercial value. I believe that such STSM is important for strengthening not only the scientific but also the personal relationships among different researchers.