SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator.

Action number: CA18201
STSM title: Population genetic tools for studying of rare species
STSM start and end date: 12/09/2021 to 18/09/2021
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PURPOSE OF THE STSM:

This mission was planned to contribute to the conservation of *Cedrus libani* in Turkey. As an initial study, my STSM aimed to examine whether there is genetic differentiation of two Cedrus species represented with four *C. libani* individuals and two *Cedrus atlantica* ones through known nSSR molecular markers. Microsatellites are the most widely used and adaptable genetic markers in population genetics, with several uses. Microsatellites are naturally codominant, highly polymorphic, easily typed, and Mendelian inherited, which makes them ideal for researching population structure and pedigree analysis, as well as discovering variations between closely related species. The Mediterranean cedars have a lot in common in terms of look. Molecular studies have revealed continuous variations between Cedrus species. Using a new collection of nuclear microsatellite markers, researchers found that Lebanese *C. libani* is more closely related to *C. brevifolia* than Turkish *C. libani* (Karam et al. 2019), arguing for *C. brevifolia* to be classified as a variety or subspecies of *C. libani*. The expected success of the microsatellite markers we used in our laboratory study in distinguishing individuals suggests that it would be beneficial to use nSSR markers in *C. libani*, which is considered to have 35 origins in Turkey.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

As a forest engineer, I had an excellent theoretical sense of how intensive and demanding it was to work in a population genetics laboratory. First, while Prof. Gemeinholzer showed the whole department and introduced all of the technical team one by one, she also gave detailed information about the function of each laboratory and technical room. Later in the day, Prof. Gemeinholzer gave some theoretical knowledge for the work to be done. In this notification, after introducing the concept of molecular markers, she gave extensive information about the purposes for which these markers are used, in which locations they are located on the DNA and their types. After that, she gave information about the **microsatellite** markers we used explicitly in this study and their importance in population genetic studies.

On Prof. Gemeinholzer's recommendation, I visited the city of Göttingen to examine herbarium specimens of the Cedrus species. Here I had the opportunity to study many well-dried and preserved Cedrus species. Even I saw the representatives of a few species from the mid-1800s remained intact. The individuals I have selected here have been left to be sent to Kassel for further use. After returning to Kassel during the day, I reviewed the articles and briefing notes on microsatellite markers.

Under the supervision of the laboratory Tech. Ms.Bersch, DNA isolation of four individuals from four *C. libani* and two individuals from *C. atlantica* was performed according to the DNeasy Plant Mini Kit protocol. The functions of all chemical substances included in the protocol and used to obtain the pure DNA molecule were explained in detail. (Buffer AP1, Buffer AW1, etc.). After getting the pure DNAs, the amounts of these DNAs

were measured in the spectral photometer. To be used in the next step, the DNA amounts should be a minimum of 10ng/µl for each sample.

We performed the Polymerase Chain Reaction (PCR) process of 6 samples that we isolated the previous day. I learned that the key ingredients of a PCR reaction were Taq polymerase, primers, template DNA, and nucleotides (DNA building blocks). These ingredients were assembled in a tube, along with cofactors needed by the enzyme, and were put through repeated cycles of heating and cooling that allow DNA to be synthesized in a PCR machine. After a 2-3 hour period, we ran the PCR products we removed from the machine in agarose gel in a low electric current for 25-30 minutes. In this process, which I did for the first time, we could not see any band formation in the gel we looked at under UV light, but I learned what it was like from the photographs of the samples made before.

Prof. Gemeinholzer provided information on how to use GenAlex 6.5, a population genetic analysis program. Referring to the gel electrophoresis images, she gave a considerable briefing on how to detect inter-and intra-population variation with comparative samples.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

After the isolation, the DNA concentrations, 260/280 and 260/260 purity ratios of the six samples we obtained are as follows:

Sample Code	DNA Concentration (≥ 10ng/µl)	260/280 (~1.8)	260/230 (~1.8-2.2)
1	98.298	1.943	2.539 ⬆
2	7.508 ⬇	1.588 ⬇	9.353 ⬆
3	83.152	1.415 ⬇	0.746 ⬇
4	74.219	1.639 ⬇	1.484 ⬇
5	25.893	2.629 ⬆	0.518 ⬇
6	59.812	1.661 ⬇	2.177

As seen from the table, all but one (sample 2) DNA concentrations are greater than 10ng/µl, which is the minimum amount that can be used in the PCR process. High concentration samples can be diluted and used.

As for 260/280 purity rates, it is generally accepted that DNA of relative purity will yield an A_{260}/A_{280} ratio of ≥ 1.8 on a scale with a maximum of 2.0. A ratio below 1.8 including samples 2,3,4 and 6 indicates protein contamination in the DNA samples and a need for further purification. For any DNA sample with a 260/280 ratio (sample 5), more than 1.8 indicates the presence of RNA as contamination. It is always suggested to give RNAse treatment at the time of DNA extraction to get a pure DNA sample.

As to 260/230 purity rates, this ratio is used to indicate the presence of unwanted organic compounds such as Trizol, phenol, Guanidine HCL, and guanidine thiocyanate. Generally, acceptable 260/230 ratios are in the range of 1.8 – 2.2. Values higher than this (samples 1 and 2) may indicate contamination with the aforementioned compounds. A low A 260/A230 ratio (samples 3,4 and 5) may be the result of carbohydrate carryover (often a problem with plants), residual phenol from nucleic acid extraction, residual guanidine (usually used in column-based kits), glycogen used for precipitation.

After the PCR process was finished, we placed the PCR products of six samples on agarose gel and conducted electrophoresis at a low current for 25-30 minutes. When we looked at the PCR products that we put in an agarose gel under UV light, we could not see any bands. The probable cause of this situation may be an experimental error such as pipetting, contamination in the process that I did on my own from start to finish.

Through my discussions with Prof. Gemeinholzer, I learned a lot about the importance and use of microsatellite markers. According to this:

A genetic marker can be used to pinpoint the position of a specific section of genetic information on a chromosome. Microsatellite markers are one form of genetic marker used to assess genetic diversity. Short

tandem repeats (STRs) or simple sequence repeats are other names for microsatellites (SSRs). They're simple DNA segments with a repeating nucleotide sequence. These microsatellites are found all over the genome. Microsatellite markers are inherited from both parents, making them useful for population genetic studies. They are useful for population genetic studies because many are considered highly polymorphic.

At last, the fact that this polymorphism is highly effective in determining the genetic diversity and variation within and between populations has been demonstrated very clearly with an applied detailed example made in GenAlex software.

FUTURE COLLABORATIONS (if applicable)

I plan to be able to use microsatellite markers, whose activity I have seen clearly, on *Cedrus libani* origins in Turkey shortly. As a result of my last interview with Prof. Gemeinholzer, she stated that she wanted to do such a study. The Turkish Scientific and Technological Council of Turkey (TUBİTAK) provides such an opportunity to researchers in the working group of a Cost Action program. I will have received my Ph. D. degree by the next June. Until that time, I am going to work on a project proposal and subsequently applying for it.