



FA1203: Sustainable management of *Ambrosia artemisiifolia* in Europe (SMARTER)

Short Term Scientific Mission Report

Test of potential EST- & SSR markers on different populations of common ragweed

STSM details

COST STSM Reference Number: COST-STSM-FA1203-161014-051278

Timing of STSM: planned betw. 16-10-2014 to 16-11-2014

Applicant details

Dr. SCALONE Romain

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Host details

Dr. LE CORRE Valérie

INRA Dijon, UMR Agroecology, Dijon, France

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Summary of the STSM

This STSM permits to identify 58 EST-SSR markers (Expressed Sequence Tag – Simple Sequence Repeats) potentially functional within three different populations of common ragweed originating from France, Germany and USA and to start the genotyping with new 10 SSR markers of a total of 384 individuals coming from 20 European and North American populations. We will use these new EST-SSR and SSR markers as tools (i) to compare the robustness between the two types, (ii) to determine the genetic diversities within different *Ambrosia* populations and (iii) to realize QTL mapping during late 2015.

Purpose of the STSM

Within SMARTER, a new Task Force called “Genetics on Ambrosia” composed by experts in molecular population genetics from 5 different European countries has been recently created in March 2014 in Dijon (France).

The main conclusion formulated by them during the Dijon meeting and presented in a poster at the conference EWRS “Weeds and Invasive Plants” taking place on the SupAgro Campus of Montpellier (France) in May 2014 was that the number of genetic markers specific to *Ambrosia* is too limited to realize proper population dynamics studies.

The purpose of the STSM was to identify and increase this number.

For that, the objectives of the applicant were to:

- Test 37 EST-SSR markers on dozens of individuals coming from one German and one American population.

These 37 EST-SSR markers have previously been selected by the post-graduate MEYER Lucie during her Master thesis from a first group of 97 EST-SSR tested on French populations only.

- Test a second group of new 77 EST-SSRs, never tested before, on dozens of individuals coming from French, German and North American populations.
- Start to analyse the genotyping results of 384 Ambrosia individuals tested with new 10 SSR markers.

The 10 SSR markers have previously been selected by tests during the Master thesis of MEYER Lucie on French populations only.

Description of the work carried out during the STSM

Week 1

- Meeting with the INRA administrators, the technicians and the workers of the working group “Agroecology”.
- DNA extractions of German and American samples from fresh leaves material.
- Preparation of buffer solution and agarose gel.
- Test 3 EST-SSR (of the group of 37) together with the post-graduate MEYER Lucie for the same German and American individuals.
- Compare the PCR results of the 3 EST-SSR markers done by SCALONE Romain and MEYER Lucie, as control.
- Test the rest of the 37 EST-SSR markers on German and American individuals.
- Meeting with MEYER Lucie and LE CORRE Valérie to discuss about the results of the test of the first group of 37 EST-SSR markers on German and American individuals by comparing with the previous results obtained by MEYER Lucie on French populations.

Week 2

- Reception of the primers of the second group of 77 EST-SSR markers (never tested before).
- Preparation of the primers (dilution), buffer solution and agarose gel.
- Selection of the French samples, which will be included in the test with the German and American samples.
- Test of the new 77 EST-SSR markers on French, German and American individuals.

Week 3

- Preparation of buffer solution and agarose gel.
- Test of the second part of the 77 EST-SSR markers on French, German and American individuals.

Week 4

- Preparation of buffer solution and agarose gel.
- Test of the last set of EST-SSR markers on French, German and American individuals.

- Meeting with MEYER Lucie and LE CORRE Valérie to discuss about the results of the test of the second group of 77 EST-SSR markers on French, German and American individuals.
- Reception of the results of the genotyping of the 384 *Ambrosia* individuals with the new 10 SSRs from the INRA Genomic Platform of Clermont-Ferrand.
- Training to use the genotyping softwares (Genemapper and PeakScanner) to analyse the SSR-genotyping.
- Meeting with MEYER Lucie and LE CORRE Valérie to discuss about the analysis of the genotyping results.

Description of the main results obtained

Within the first set of 37 EST-SSR markers, already tested on French populations, 25 EST-SSR markers are amplified in German and American individuals. Three are working only on French and German individuals, but not on American ones, while nine are working only on French individuals.

Within the second set of 77 EST-SSR markers, never tested before, 33 EST-SSR markers are amplified from individuals coming from all the three different countries (France, Germany and USA). One is working only on German and American individuals but not on French ones, while 40 are not working at all. Three EST-SSR markers have to be re-checked to confirm potential positive conclusions.

In total, two third (67.6 %) of the EST-SSR markers already validated on French populations are positive on German and American individuals too, while more than 40% of the 77 EST-SSR markers, never tested before (42.9%), have been selected after positive results on all the three types of individuals (French, German & American).

A total of 58 new EST-SSR markers (on a total of 173 tested; 33.5%) can be used now for genotyping.

Future collaboration with the host institution

The send of German and American samples will be realized soon in order to include them in the new genotyping sampling testing the 58 EST-SSR markers newly identified.

A video-meeting is already planned to take place at the middle of January 2015 in order to compare the genotyping results of the 384 individuals tested with 10 SSR markers and analyzed separately by SCALONE Romain and MEYER Lucie. Then, discussions about the results of the genotyping will start at the same time than the writing process.

Foreseen publications/articles resulting from the STSM

The genotyping of the 384 individuals with 10 SSR markers as well as the identification and the genotyping of French, German and American individuals with the new 58 EST-SSR markers will be the main part of a technical publication aiming to compare the robustness of the two new sets of microsatellites (SSR) markers depending to their different origins (nuclear neutral versus EST-based).

The results of the genotyping of the 384 individuals with the new 10 SSR markers will be part of a publication aiming to compare the genetic diversity levels of several European and North American populations, as the light of their phenological traits, observed in a parallel common garden experiment realized in Sweden and in Croatia during summer 2012.

Confirmation by the host institution of the successful execution of the STSM

Follow up. Start by den 6 december 2014. Due by den 6 december 2014.

From: Valérie LE CORRE <valerie.le-corre@dijon.inra.fr> Sent: fr 2014-12-05 09:12
 To: maurizio.vurro@ispa.cnr.it; SMARTER@unifr.ch
 Cc: Romain Scalone
 Subject: COST-STSM-FA1203-21009

Dear Dr Maurizio Vurro,

I am writing to confirm that, thanks to COST-STSM-FA1203-21009, Dr Romain Scalone has been visiting our laboratory, "UMR Agroecology" at INRA Dijon, France for one month in November 2014. He mainly worked with Lucie Meyer, a post-graduate student from our lab who recently developed a new set of microsatellite markers for *Ambrosia artemisiifolia*.

Most of his work consisted in laboratory work aiming at testing a large set of PCR primers targeting EST-SSRs, that is, microsatellites located within expressed coding regions within the *A. artemisiifolia* genome. His work has been largely successful, since we now have in hands a new set of several tens of markers ready for further validation via genotyping. In addition, a large sample of the populations that Romain studied in his common garden experiments in Uppsala was genotyped with the markers previously developed by Lucie Meyer. Analyses of data are underway.

The work accomplished during the stay of Romain Scalone in Dijon is only the beginning of a collaboration that is to be continued during the very next months, with the aim of publishing new sets of microsatellites markers of different origins (nuclear neutral vs EST-based) and compare their robustness and usefulness for analyzing genetic diversity of *A. artemisiifolia* populations.

Best regards,

Valérie Le Corre

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