

Scientific Report on a Short-Term Scientific Mission

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Host: Beatrice Denoyes-Rothan, INRA – UREF

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Purpose: The Mission had following objectives:

1. Knowledge of some molecular techniques specifically designed to SSR analyses and polyacrylamide gel electrophoresis with silver nitrate staining.
2. Selections of SSR primer pairs which would be informative for a mapping population creating by 'Ottoman' x 'Camarosa'.
3. Selections of SSR primer pairs which would be informative for evaluation of relatedness of 'Ottoman' to other *F. ×ananassa* and progenitor species, *F. chiloensis* and *F. virginiana*.

Program:

I have worked at the INRA, UREF, Villenave d'Ornon Cedex, France under the supervision of Dr. Beatrice Denoyes-Rothan. During my mission, I have had several opportunities to apply molecular techniques from DNA quantification to gel development. At the end of the mission, I have accomplished all the objectives given above. I have also experienced several issues such as gel scoring from different studies and the utilization of molecular data generated from SSR such as genetic diversity and mapping studies for octoploid strawberry species. Dr. Denoyes-Rothan also prepared several lectures for me on the fundamentals of mapping in polyploidy fruit species.

During my mission, I have had chances to interact with many researchers from INRA who are conducting research activities of the several aspects of fruits. I have discussed several possibilities of the cooperation between INRA and institutes in Turkey with research activities in small fruits.

Description:

I started the mission having some general information of the molecular laboratories and some safety interactions. Then, we initiated our work. We studied two sets of the strawberry populations. The first set consisted of 10 genotypes, two of them were *F. ×ananassa*, four of them were *F. chiloensis* and four of them were *F. virginiana*. This first set was used to evaluate the effectiveness of the SSR primers for diversity studies and identify some primers that can be used to reveal the genetic background of ‘Ottoman’ an old Turkish cultivar. The second set consisted of six hybrids from a mapping population generated by ‘Ottoman’ x ‘Camarosa’. This set was used to identify SSR primers which can be used in mapping study. Further information regarding genotypes backgrounds was given in [Table 1](#).

First, we quantified the DNAs of the genotypes by measuring the concentrations and running them on agarose gel. Later, we tested few protocols for PCR and optimized the conditions for the genotypes tested for SSR primers. We tested a total of 24 SSR primer combinations. The amplification products from these reactions were tested on agarose gel and they all amplified the expected repeats in our populations. Later, the PCCR products were separated on polyacrylamide gels and then developed by silver nitrate staining. Further information regarding the PCR, agarose gel and polyacrylamide gel conditions can be found in [Rousseau-Gueutin et al., 2008](#).

The bands were scored as 0 (no bands) and 1 (presence of the band) resulting in a binary data file. The data set was used to perform Principle Coordinate (PCoA) and cluster analyses using NTSYS program ([Rohlf, 1997](#)). First, a similarity matrix was generated using Jaccard coefficients. This matrix was then, used for PCoA. For cluster analysis, the UPGMA (Unweighted Pair Group Method using Arithmetic Average) method was used to construct dendrograms. The bootstrap values for the clusters were calculated by 1000 replicates using PAUP program ([Swofford, 1998](#)). The representativeness of dendrograms was evaluated by estimating cophenetic correlation for the dendrogram and comparing it with the similarity matrix, using Mantel’s matrix correspondence test ([Mantel, 1967](#)). The result of this test is a cophenetic correlation coefficient, r , indicating how well dendrogram represents similarity data.

Results:

All of the 24 primer pairs generated bands on agarose and polyacrylamide gels. The banding patterns of the 24 SSR pairs were evaluated on the mapping populations. All primers produced some banding patterns which are present in only one parent and segregating among

the hybrids. Because of the small population size, it is not possible to conclude if all of these bands could be used in mapping; however, the SSR tested indicated a great potential for mapping for the population tested. Fig. 1 shows an example of the patterns.

A subset of the SSR primer combinations, 19 SSRs, was evaluated for population I and the results are presented in Table 2. Nineteen SSR primer combinations generated 110 bands 100 of which were polymorphic. The average % polymorphism was 91% for the population tested. The multivariate analyses indicated that the genotypes tested could be divided into groups (Figure 2). Cluster analysis, for example, indicates that ‘Ottoman’, Darrow 72 and CA 1541 separated from the other genotypes. The nodes separating Eagle 14 and LH 50-4 was supported by bootstrap values. For the rest of the genotypes, *F. chiloensis* and ‘Camarosa’ was separated from the *F. virginiana* genotypes. A high *r* value of 0.89 indicated that the dendrogram generated from cluster analysis is a good representation of the similarity matrix.

Overall, principle coordinate analysis results were in agreement with those of cluster analysis. In Figure 3, *F. chiloensis* and *F. virginiana* genotypes were separated although 2 TAB 4B was among the *F. virginiana* genotypes. Although ‘Ottoman’ seemed to be more closely related with *F. chiloensis* genotypes; ‘Camarosa’ appeared to be closer to *F. virginiana* groups. Of course more genotype and data are needed to make statistically valid conclusion; however, the analyses of these small sets demonstrated the power and appropriateness of the SSR used in this study.

Future Collaboration:

In the future, I would like to visit INRA as well. I will also attempt to introduce Turkish scientists who are working on small fruit species and have changes to collaborate on scientist from Europe. Finally, I would like to initiate joint projects with researcher from INRA in the future on molecular characterization of plant genetic resources.

Acknowledgements

First, I would like to thank COST program for giving me for this opportunity. I thank Denoyes-Rothan for hosting me. The gratitude is extended to the Dr. Aurelie Petit and Dr. Aniko Horvath for their help in the laboratory during the mission.

References:

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Table 1. The genotypes and their description studied by SSR primers during the study.

Genotype	Description
Set 1	
Ottoman	<i>F. ×ananassa</i> ; a local Turkish cultivar
Camarosa	<i>F. ×ananassa</i> ; a cultivar from California
Darrow 72	<i>F. chiloensis</i> ssp. <i>chiloensis</i> f. <i>chiloensis</i>
2 TAB 4B	<i>F. chiloensis</i> ssp. <i>chiloensis</i> f. <i>patagonica</i>
CA 1541	<i>F. chiloensis</i> ssp. <i>chiloensis</i> f. <i>chiloensis</i>
Pigeon Point	<i>F. chiloensis</i> ssp. <i>pacifica</i>
NC 96-35	<i>F. virginiana</i> ssp. <i>virginiana</i>
Eagle 14	<i>F. virginiana</i> ssp. <i>virginiana</i>
JP 95-1	<i>F. virginiana</i> ssp. <i>virginiana</i>
LH 50-4	<i>F. virginiana</i> ssp. <i>glauca</i>
Set 2	
Ottoman	<i>F. ×ananassa</i> ; a local Turkish cultivar
Camarosa	<i>F. ×ananassa</i> ; a cultivar from California
F1	Hybrid of Ottoman x Camarosa
F2	Hybrid of Ottoman x Camarosa
F3	Hybrid of Ottoman x Camarosa
F4	Hybrid of Ottoman x Camarosa
F5	Hybrid of Ottoman x Camarosa
F6	Hybrid of Ottoman x Camarosa

Table 2. SSR primers, the sizes of their amplification products, total and polymorphic band numbers and their % polymorphism for 10 strawberry genotypes from *F. ×ananassa*, *F. chiloensis* and *F. virginiana*.

No.	Primer pair	Sizes (bp)	Band number		% Polymorphism
			Total	Polymorphic	
1	Primer 1	120-148	7	7	100
2	Primer 2	160-184	4	3	75
3	Primer 3	188-208	8	6	75
4	Primer 4	190-200	2	1	50
5	Primer 5	253-265	3	3	100
6	Primer 6	280-294	2	1	50
7	Primer 7	230-238	3	2	67
8	Primer 8	258-264	2	1	50
9	Primer 9	194-196	2	1	50
10	Primer 10	222-228	2	2	100
11	Primer 11	170-210	9	7	78
12	Primer 12	165-188	7	7	100
13	Primer 13	220-250	13	13	100
14	Primer 14	162-210	7	7	100
15	Primer 15	160-240	13	13	100
16	Primer 16	188-200	6	6	100
17	Primer 17	170-218	7	7	100
18	Primer 18	280-310	6	6	100
19	Primer 19	190-220	7	7	100
Total / Mean			110	100	91

Figure 1. An example of gel picture generated by SSR for Population I (*Fragaria chiloensis* and *F. virginiana* accessions) and mapping population. For mapping population possible informative bands are marked with red dots w possible monomorphic bands were marked with blue squares.

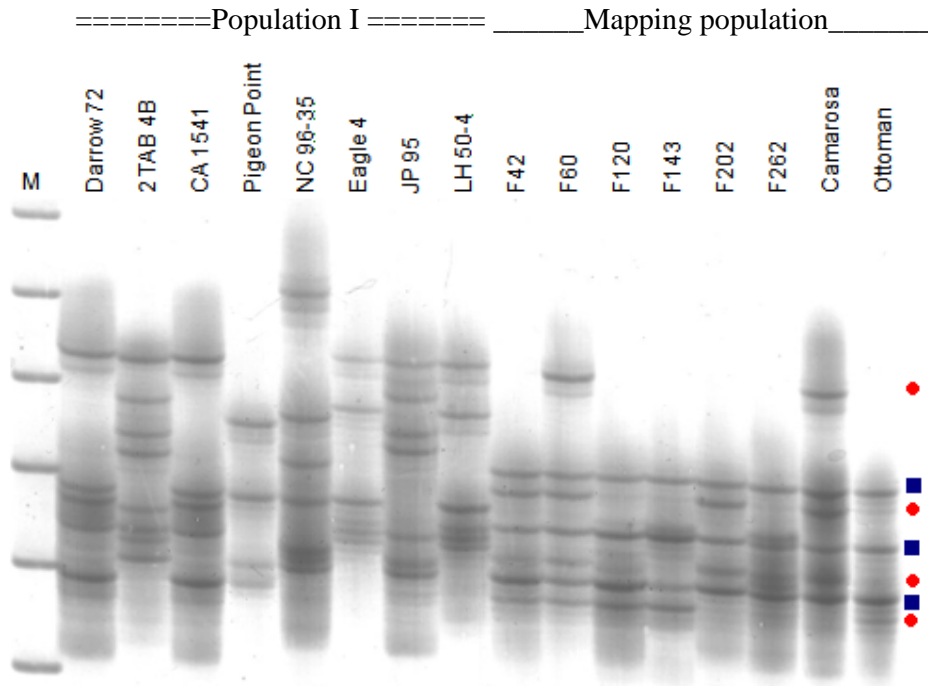


Figure 2. UPGMA dendrogram of 10 strawberry genotypes from *F. ×ananassa*, *F. chiloensis* and *F. virginiana* evaluated by 110 bands generated from 19 SSR primers pairs. *Fragaria chiloensis* and *F. virginiana* genotypes were marked with red dots and blue squares, respectively while x was used for *F. ×ananassa*.

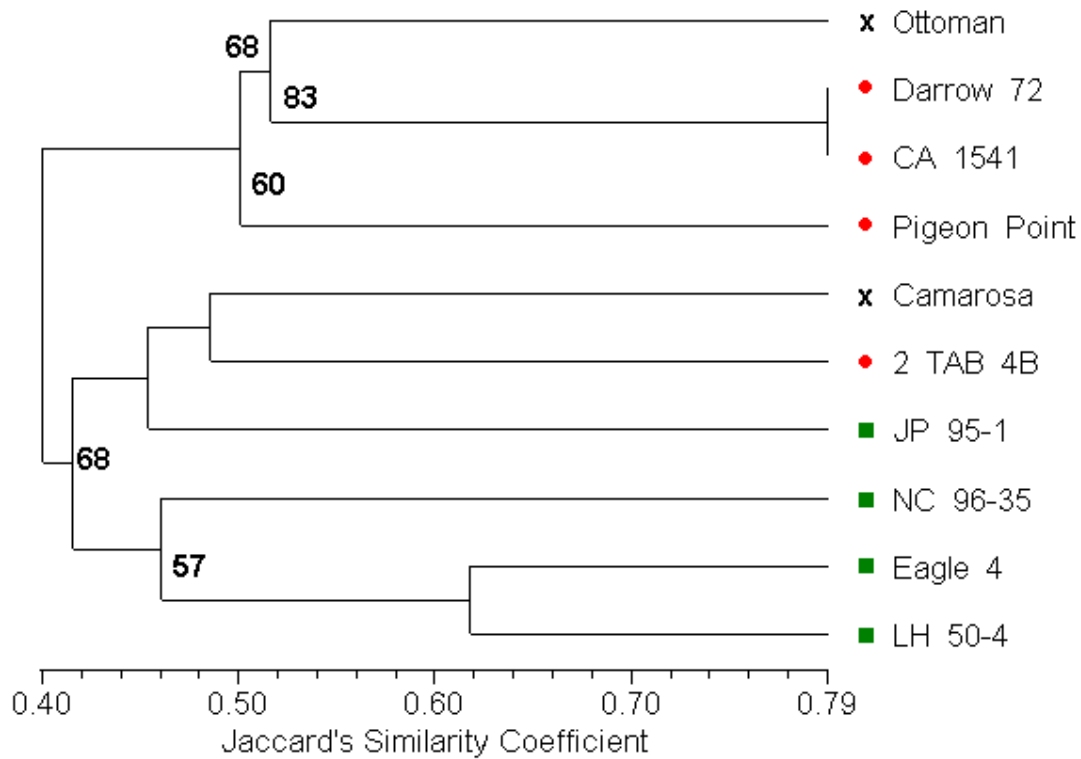


Figure 3. Three-plot of principle coordinate analysis of 10 strawberry genotypes from *F. ×ananassa*, *F. chiloensis* and *F. virginiana* evaluated by 110 bands generated from 19 SSR primers pairs. *Fragaria chiloensis* and *F. virginiana* genotypes were marked with red dots and blue squares, respectively while × was used for *F. ×ananassa*.

