

Scientific Report on a Short-Term Scientific Mission

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Host: Walter Faedi, C.R.A. – Istituto Sperimentale per la Frutticoltura

Place: Forli, Italy

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Purpose: The Mission had two objectives:

1. Knowledge of some molecular techniques specifically designed to analyze DNA and genetic variability of plant's genome.
2. Discrimination of genotypes members of *Fragaria* genus's species (*F. ×ananassa*, *F. virginiana*, *F. chiloensis*) through SSRs markers.

Programme:

I have worked in the Istituto Sperimentale per la Frutticoltura. During my mission I have conducted molecular work on strawberries using SSR. The details of my study will be explained. At the same time, I had visits to the strawberry breeding program led by Dr. Walther Faedi. In one trip, we went to Verona area where there was a unique system for fall and spring strawberry productions. I have attended a grower day and visited several growers along with the research station located near Verona region. In another trip, we have seen several strawberry growers along with the research station and a strawberry nursery in Cesana area.

During my mission I had chances to interact with many researchers from Istituto Sperimentale per la Frutticoltura. I have seen the instruments for several analyses. Also, Dr. Faedi and I had discussions many times where he explained his methods and activities regarding strawberry breeding.

Description:

During my mission, Maura Bonoli helped me for the molecular work. The objective of my study was to learn how to make SSRs and visualize the resulting bands on polyacrylamide gel electrophoresed on vertical gel. As planting material we have used 20

strawberry genotypes whose descriptions are given Table 1. In short, Ottoman, Arnavutkoy, Kara Cilek and Tuylu are Turkish old cultivars whose origins are not known. These cultivars are morphologically similar to pure *F. chiloensis* clones. Therefore, we aimed to test their molecular similarity to the *F. ×ananassa*, *F. chiloensis* and *F. virginiana*. The cultivated strawberries, *F. ×ananassa*, are presented by both American and European cultivars while the progenitor species *F. chiloensis* and *F. virginiana* were represented by supercore genotypes developed by Dr. Hancock *et al.* (2001 and 2002).

I isolated DNA in Turkey and carried them to the Italy. We first quantified the DNA. After several tries, we optimize the amount of DNA concentration to be used in each PCR tube. Then, we have used different PCR product on the polyacrilamide gel for optimum imaging. Then using these 20 genotypes we have used SSR primers from Cipriani *et al.* (2006). After the PCR reactions, we have first checked the presence of PCR product using agarose gel. After confirming the PCR product, we have separated them on the polyacrilamide gel.

Results:

The results of the SSR analysis must be confirmed by at least two replications. However, due to the limited amount of time, we carried the analysis only once. All the results presented in this report come from single run. Therefore, the validity of these results should be confirmed by at least one more run.

Thirteen primers from Cipriani *et al.* (2006) were selected and studied. The PCR products of these primers were tested on the agarose gel. Although there were some similarities, the banding patterns were not identical to the Cipriani *et al.* (2006). The discrepancies were present for primer pairs 004, 005, 007, 008, 010, 017, 011, 019. For example, for primer pairs 007 Cipriani *et al.* (2006) received amplification for *F. ×ananassa*; but not for *F. chiloensis* and *F. virginiana*. In our work, however, we have amplification product for all genotypes but Sugar Lia. The result indicate that the SSR primers studied may be specific to the genotypes but not to the species (Table 2).

The PCR products from nine primers pairs (001, 002, 003, 004, 005, 007, 008, 010, 011) were scored on polyacrilamide gel. For the populations we have identified 13, 3, 13, 7, 4, 11, 8, 5 and 2 bands respectively. The average polymorphic bands per primer pairs for our population were 7.3. These results suggest that SSR are powerful molecular markers to evaluate strawberry populations with different genetic background.

Using the data from these nine SSR primer pairs we constructed a cluster by UPGMA method on PAUP 4.0. The resulting cluster is presented in Figure 1. In the cluster, The *F. virginiana* genotypes LH 50-4 and JP 95-1 looked different from other genotypes. The Sweet Charlie and Camarosa, the American *F. ×ananassa* cultivars, separated from rest of the genotypes. After these, there were two big clusters: in one group there were four Turkish genotypes (Ottoman, Arnavutkoy, Kara Cilek and Tuylu) and rest of the genotypes: all *F. chiloensis* and other all *F. ×ananassa* cultivars. Surprisingly, the two *F. virginiana* genotypes RH 43 and RH 30 were within the later cluster. Although the Turkish genotypes cluster together and separated from all other *F. ×ananassa* and *F. chiloensis* genotypes our results are not adequate to support either of the ultimate hypotheses that if they are more closely related to *F. ×ananassa* or *F. chiloensis* molecularly. Therefore, the results from more primers pairs should be included to complete our study. Once again care must be taken to evaluate our data as they are not replicated as well.

Future Collaboration:

In the future, I would like to visit Dr. Faedi's research program one more time when the program makes their selection probably in early spring. Also, we may continue our molecular work either in Istituto Sperimentale per la Frutticoltura or Mustafa Kemal University. The work can be extended to the some member of *F. vesca* genotypes collected from different parts of Turkey.

Comments:

I am extremely glad that I have visited Dr. Faedi's strawberry program. I have not only learned new molecular techniques and generated valuable results but also had a change to interact with a very active and successful breeding program. I believe the STSMs are excellent opportunities for similar purposes.

Acknowledgements

I would like to thank COST program for giving me for this opportunity. I thank Dr. Faedi for hosting me in his instituting and his program. I also thank for his explanations and sharing his experience with me. The thanks are extended to the Ms. Bonoli who patiently thought me the molecular work. Finally, I would like to thank all the researchers of Istituto Sperimentale per la Frutticoltura for their great hospitality.

References

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Table 1. The genotypes and their description studied by SSR primers developed by (Cipriani *et al.*, 2006).

No	Genotype	Description
1	Ottoman	<i>F. ×ananassa</i> ; a local cultivar in question
2	Arnavutköy	<i>F. ×ananassa</i> ; a local Turkish cultivar
3	Kara Cilek	<i>F. ×ananassa</i> ; a local Turkish cultivar
4	Tuylu	<i>F. ×ananassa</i> ; a local Turkish cultivar
5	Camarosa	<i>F. ×ananassa</i> ; an American cultivar (California)
6	Sweet Charlie	<i>F. ×ananassa</i> ; an American cultivar (Florida)
7	Queen Elisa	<i>F. ×ananassa</i> ; a European cultivar (Italian)
8	Dora	<i>F. ×ananassa</i> ; a European cultivar (Italian)
9	Sugar Lia	<i>F. ×ananassa</i> ; a European cultivar (Italian)
10	Record	<i>F. ×ananassa</i> ; a European cultivar (Italian)
11	2 BRA 1A	<i>F. chiloensis</i> ssp. <i>chiloensis</i> f. <i>chiloensis</i>
12	CA 1541	<i>F. chiloensis</i> ssp. <i>chiloensis</i> f. <i>chiloensis</i>
13	2 PAL 2C	<i>F. chiloensis</i> ssp. <i>chiloensis</i> f. <i>patagonica</i>
14	2 TAB 4B	<i>F. chiloensis</i> ssp. <i>chiloensis</i> f. <i>patagonica</i>
15	Auke Lake	<i>F. chiloensis</i> ssp. <i>pacifica</i>
16	Scotts Creek	<i>F. chiloensis</i> ssp. <i>pacifica</i>
17	LH 50-4	<i>F. virginiana</i> ssp. <i>glauca</i>
18	RH 43	<i>F. virginiana</i> ssp. <i>glauca</i>
19	JP 95-1	<i>F. virginiana</i> ssp. <i>virginiana</i>
20	RH 30	<i>F. virginiana</i> ssp. <i>virginiana</i>

Table 2. The amplification patterns of several SSR markers from both Cipriani *et al.* 2006, and our study for 20 genotypes from *F. ×ananassa*, *F. chiloensis* and *F. virginiana*.

No.	Genotype	Primer												
		001	002	003	004	005	006	007	008	010	017	011	014	019
Results from Cipriani et al., 2006														
1	<i>F. ×ananassa</i>	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	?	N
2	<i>F. chiloensis</i>	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	?	N
3	<i>F. chiloensis</i>	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	?	Y
4	<i>F. virginiana</i>	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	?	N
Results from our study														
1	Ottoman	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
2	Arnavutköy	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
3	Kara Cilek	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
4	Tuylu	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
5	Camarosa	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
6	Sweet Charlie	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
7	Queen Elisa	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N
8	Dora	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
9	Sugar Lia	Y	Y	Y	N	N	Y	N	Y	Y	Y	Y	N	N
10	Record	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
11	2 BRA 1A	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
12	CA 1541	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	N	N
13	2 PAL 2C	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
14	2 TAB 4B	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	N	N
15	Auke Lake	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N	N	N
16	Scotts Creek	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
17	LH 50-4	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
18	RH 43	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
19	JP 95-1	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N
20	RH 30	Y	Y	Y	N	?	Y	Y	Y	Y	Y	Y	N	N

Figure 1. UPGMA dendrogram of 20 strawberry genotypes form from *F. ×ananassa*, *F. chiloensis* and *F. virginiana* evaluated by 13 SSR primers form Cipriani *et al.* 2006.

