

STSM REPORT

Short term scientific mission, Reference code: COST-STSM-863-04605

Applicant: Sandro Frati – CReSO, Consorzio di Ricerca, Divulgazione e Sperimentazione per l'Ortofrutticoltura Piemontese, Cuneo, Italy

Host: Prof. Edward Żurawicz, Instytut Sadownictwa i Kwiaciarnictwa (ISK), Skierniewice, Poland

Period: from april 15th to april 30th, 2009

Objective of STSM

The objective of this STSM was to improve the knowledge about the berry production and characterization in Poland, with the aim to transfer this knowledge to the Piedmontese (Northwestern Italy) area. The activity carried out during this STSM was articulated in visits to the different facilities of the Host Institute, thanks to the supervision of Prof. Żurawicz.

Department of fruit breeding, facility of biotechnology

The department of fruit breeding has a big and modern facility for the application to the biotechnological tools to the needs of fruit breeding. The Responsible is Dr Małgorzata Korbin. The practical activity has been carried out through the explanation of different experiments and the participation in many of them:

1. Gene expression of *Verticillium dahliae* resistance in plants of strawberry. *V.dahliae* is one of most important pathogens for this crop in Poland and in other Countries characterized by generally cold weather. The aim of this experiment was to determinate the expression of resistance to this pathogen in artificially inoculated plants. The plants used for this study belong both to susceptible and tolerant varieties. After the inoculation and the appearance of symptoms, DNA was extracted through CTAB method. On this genomic DNA a fingerprint was obtained using different molecular tools, such as RAPD or ISSR, with the aim to find a specific band for the resistance to *V.dahliae* by comparing the tolerant and susceptible plants. A parallel experiment was made by analyzing the RNA to determinate the gene expression for resistance.
2. Identification of varieties of strawberry through AFLP. The genomic DNA of plants belonging to two varieties was extracted. Then, the AFLP technique is composed by two main steps: the digestion of DNA with some (one or more) restriction enzymes and ligation of specific adaptors, the selective amplification with primer designed on these adaptors. With the electrophoretic separation of PCR product on agarose gel it was afterward possible to observe different profiles, which can be compared with the known varieties. Another application of AFLP technique is the screen of resistance to herbicide Basta™ of corn progenies.
3. Visit to the DNA sequencer and to the bioanalyzer. The Host Institute owns a sequencer, which is useful in order to recognize the sequence of a specific part of DNA. This application can be applied for identification of genes. Another useful instrument is the bioanalyzer, which allows to visualize the result of a PCR just by putting few microliters of

samples into the machine instead of preparing the traditional gel. The bioanalyzer gives in real time a detailed picture of a “virtual” gel.

4. GMO. The laboratory for GMO is separated from other facilities, according to the normative. The ISK carries out a research activity to “discover” the presence of certain molecular modification both on foodstuff and plants.
5. Preparation of acrylamide gel. This kind of gel is used in vertical electrophoretic chambers and allows a very good separation of bands. The gel is putted between two plate glass, which are carefully sealed. At the end of the run the gel is dippen into a acetic acid solution in order to fix the DNA into the gel, and then in a staining solution (AgNO₃). After a wash with distilled water, the gel is ready to be read. This technique is very useful to find polymorphisms, for example in new progeny of plants.
6. Molecular tools for identification of specific characters in progenies. The practical applications of these techniques are many and helpful for early evaluation of progeny for a specific character. For instance, a specific analysis have been carried out to investigate on heritability of the character for sweetness of fruits in progeny of apple. Given the polymorphisms of two parental for this character it was possible to compare the polymorphisms of progeny at early stage of development. It was, finally, possible to determinate if a certain progeny was more similar to one or to another parent. This experiment has also been included in this STSM because could be easily applied to berries.
7. Real-Time PCR. During this STSM it was possible to set an experiment of Real-Time PCR in order to get acquainted with it. The DNA template was from apples but, as for the previous point, it was included because the technique is applied to every other DNA. The goal of this experiment was to study the gene expression of genes responsible for the sugar content in fruits. The fruits were picked in May (immature) and at harvest time, the cultivar were Retina and Topaz. With this reaction it was possible observe the higher expression of this gene at harvest time.

Department of fruit breeding, facility of traditional breeding

The facility of traditional breeding is directly led by Prof Żurawicz. The research activity is headed to the selection of new varieties of fruit crops (top fruits and berries). Among berry, blackcurrant has a big importance in Poland, where it is processed in for the production of many foodstuff. The breeding program has the aim to improve many characters, such as production and nutritive auality. The ISK is now studying around 100 crosses.

During the blossoming period of every species the pollen of one parental is taken, while the flowers of the other parental are emasculated. It is just necessary to apply the collected pollen on the other flower to get the desired combination. The branch with the experimental crosses are then covered in order to avoid any contamination. At harvest time the ripen fruits will be collected and the mature seeds will be taken and sewed at the proper time. With this method the ISK is able to get around 5000-10000 progenies of apple and 4000 of gooseberry every year.

Also in this part of the Department of fruit breeding, a general overview of experiments and trials was included into STSM program:

1. Autofertility of blackcurrant: many varieties which belong to this important specie are not autofertile, causing troubles to the growers, who are sometimes forced to plant more

than one variety in their orchards. ISK is carrying out a very simple experiment to investigate on the autofertility of blackcurrant. Few branches of a plant were covered with a tissue sleeve in order to prevent the contamination from external pollen. After the ripening of fruit it was necessary to determinate the production of fruit inside and outside the sleeve.

2. Covering of raspberry and strawberry: in Polish countryside it is common, and necessary, to cover the primocane raspberries and the strawberries with some material in order to protect the crop from the winter frost. The ISK is testing and trying different plastic and tissue materials with this purpose.

3. Damages of frost on different strawberry cultivars: the plants, which belong to the more diffused varieties in Poland, have been cultivated for a week at 7 and 15°C. These plants have then been exposed to cold temperatures (-5, -10, -15 e -20) for different periods of time, with the aim to determinate which of them were more susceptible to cold temperatures.

4. Quantification of vitamin C: this is a very important parameter, suitable when the proper determination of quality is required. It is possible to quantify the vitamin C through the use of Reflectoquant, Merck®. During the STSM this analysis have been done on frozen blackcurrant. The sample was homogenized and diluted, and then a particular paper strip is dipped into it; by putting the strip into the instrument the reading of the concentration is immediately possible.

During the staying to the facility of traditional breeding, a visit to the experimental station of Dąbrowice has been planned. In Dąbrowice, few kilometers far from Skierniewice, the ISK is testing in open fields the most important varieties and selection of their species. A practical trial, also included into this STSM, was about the controlled impollination of sour cherry (cv Kordia x other selections). It was planned to include this experience because during the STSM, the last two weeks of April, it was not possible to do it on berries. This technique could be applied on every other species anyway.

Besides the trials which are directly carried out into the ISK's facilities, the Institute also organize trials of new plant materials in private farms.

Fig. 1 – Pictures of facility and activities carried out at traditional breeding division



Department of food safety

This department carries out chromatographic analysis for both residues of pesticides and mycotoxins in foodstuff. The Responsible is Dr. Artur Miszczak. The activity included in this STSM has only been finalized to pesticide analysis, since it is a more important goal for the reality of CReSO.

The facility has been validated for around 170 residues, the analysis are carried out for the Polish Ministry of Agriculture, as monitoring, and for private companies. The chromatographic techniques are both liquid (HPLC-MS, HPLC-MSMS) and gas chromatography (GC-MS). For most of samples, the extraction is carried out through QuEChERS protocol, which can be slightly modified for some matrix. This facility is the State reference laboratory for the two closer Polish regions. In every room of the building temperature and humidity are constantly kept the same in order to do not change the outcome of analysis.

The STSM program for this facility included the extraction of sample and the following analysis through HPLC-MS.

Extraction

1. The matrix, previously frozen, is minced in dry ice;
2. Add 10 ml of acetonitrile, vortex for one minute;
3. Add 4 ml of MgSO₄ and then 1 g of NaCl, vortex for one minute;
4. Centrifuge for one minute at 5000 rpm;
5. Filtration with filters on syringe (diameter 0.2 micrometers);
6. Analysis

This extractive technique is very suitable for high quantity of samples, in view of the fact that it is very quick and accurate.

During the STSM, the development of HPLC method for thiacloprid has been made. Thiacloprid is important in Italy, since it is diffused for treatment of berries in Italy. The retention time was unknown.

The instrument was equipped with a triple quadrupole MS. The steps were the following:

1. Visualization of the whole spectrum in order to search the interesting ions;
2. When the interesting ion is found, the fragmentor is searched;
3. Check of the higher quantity of ion fragments;

The STSM into the Department of Food Safety included, lastly, the calculation of the linearity of Chlormequat spiked samples, in order to get acquainted with this type of analysis.

Department of food processing and storage

This department is composed by two separated facilities, one for food processing and another for food storage. The persons who planned the activity for this Department were Dr. Dorota Konopacka and Dr. Jarosław Markowski for food processing, and Dr. Krzysztof Rutkowski and Dr. Anna Wawrzyńczak for food storage.

The analysis of consistence are carried out through an automatic machine, named Instron. This instrument is able to apply always the same strength to the samples in order to avoid the influence of the operator.

The Department also carries out tests for juiciness of fruits. These tests are useful, for instance, to evaluate the suitability of a new storage condition. The berry is weighted and then pressed together with two sheets of Whatman™ paper, which will adsorb the almost totality of juice. After press, the juice is weighted again, and the percentage of juice is determined. Every lot of fruit (berries or top fruit) is subjected to three analysis: hardness, juiciness and sensorial.

The facility of food processing carries out many trials on new ways of processing, in order to save the nutritional value of fruit. During this visit the observation of dried blackcurrant was possible. This facility own an HPLC in order to determinate the concentration of vitamins and nutrient in fruits.

The facility for food storage is a modern building which owns around ten big climatic chambers. The quality of fruits is there evaluated through NIR reading. With an only, non-destructive analysis, it is possible to read the sugar content, the titratable acidity, the color and the hardness.

Conclusions

This two weeks visit at Instytut Sadownictwa i Kwiaciarnictwa of Skierniewice got many important hints to plan similar activities in Piedmont region, where the production of berries is also significant. The experience at ISK of particular interest and allowed to improve my knowledge on the main different cultivation techniques adopted for berry cultivation in Poland.

Furthermore, the visit to the different Departments and the meetings with the researchers was extremely useful for gaining interesting informations on their activities and strategies used for the different breeding programs but also for promoting the exchange of different genetic material (cultivars and selections) and further collaborations.

The success of this STSM was mainly due to the kind and accurate help of Prof. Żurawicz, who took care of every detail of my staying in Skierniewice and at the Institute. I would also like to thank every person who showed me their work and their facilities, the crew of every lab was very open and I really could improve my knowledge. I am extremely grateful to Instytut Sadownictwa i Kwiaciarnictwa and to COST Euroberry project for this opportunity.