

COST Action:FP1202

Name: Eleni Tzingidou

STSM Title: “Genetics of *Arbutus unedo* marginal population and management under climate change”

Location: NATIONAL BOTANIC GARDENS, Dublin, Ireland

Host: Dr Colin Kelleher

Home institution: Aristotle university Thessaloniki, Greece

Home institution supervisor: Philippos Aravanopoulos

Purpose of the STSM.

Very little is known about the population genetics or phylogeography of *Arbutus unedo* to date and so this project aimed to investigate this, particularly for the Greek samples. The cpDNA is available online and this was used to try to assess regions for variability. For specific results we designed specific primers for *Arbutus* areas for particular areas of the cpDNA. The project aimed to screen these regions for variation using a selection of samples from a wide geographical base including Greek samples. *Arbutus unedo* L. or as the common name “the strawberry tree” belongs to the family Ericaceae. *Arbutus* consists of approximately 11 species. *Arbutus unedo* is a small tree that can reach a height up to 10 meters. The main distribution of the species is in the Mediterranean region and along the Atlantic coast of Spain and Portugal, with outlier populations in the south-west and the north-west of Ireland at the northern limit of the species. The most southern populations in Europe are in Greece in the area of Crete. It also grows in north Africa.. The trees grow independently as isolated individuals or in small populations. Many products are produced using *Arbutus* today. In Portugal an alcoholic beverage called medronho is made from the fruit and in Greece a strong alcoholic drink Tsipouro is also produced. The fruit is red in color when ripe and is used to produce jams. The fruit are also particularly desirable for birds. The flowers are white in the shape of bells and are pollinated by bees. The honey from *Arbutus* feeding bees has a characteristic bitter taste but because of the substance *Arbutin* honey, regulates cholesterol and purifies the blood and has low glucose level.

Description of the work carried out during the STSM.

A set of *Arbutus unedo* samples were available in the DNA bank and the tissue bank in the National Botanic Gardens. These samples were used as comparisons against Greek samples. Samples were available from Ireland, France, Spain, Portugal, Italy and Greece. Two populations from Greece were sampled; one from Vasilika by Philippos Aravanopoulos (Aristotle university of Thessaloniki faculty of Forestry and Natural Environment) and another from Chalkidiki by Roula Nannou. From each individual fresh leaves were collected. While sampling the aim was to have about 40m apart the individuals to avoid collecting multiple plants from the same parent. Samples from Chortiatis were carried in ice and after that stored in the freezer (-80 C), Samples from Chalkidiki were sent by courier fresh and transferred to silica gel on arrival in Ireland. The quality of leaves which were stored in silica gel were better than those which were stored on the ice.

In the beginning we tried different methods for the DNA extraction with the Nucleo spin plant II kit (Macherey-Nagel) using buffer p11 and with the Qiagen DNeasy plant mini kit. The results were not satisfactory so we used the Nucleo spin plant kit II (Macherey-Nagel) using buffer p12 with minor modifications of the manual. Optimization of the extraction were performed by adding 80µl Dithiothreitol (DTT)(10ng/µl) and 10 µl of proteinase K (800 units/ml) allowing for the release of the DNA from its proteins and further degradation of the proteins respectively. The incubating time was extended to 60 min. The quantity and the quality of DNA obtained was assessed spectrophotometrically at 260 nm and 280 nm using Nanodrop

2000 (Thermo Scientific) (image 1), furthermore with electrophoresis on 1.5% agarose gel with Sybr Safe (Invitrogen) and 40 min of migration at 100V. The extractions were stored at -20C.(Fig1)

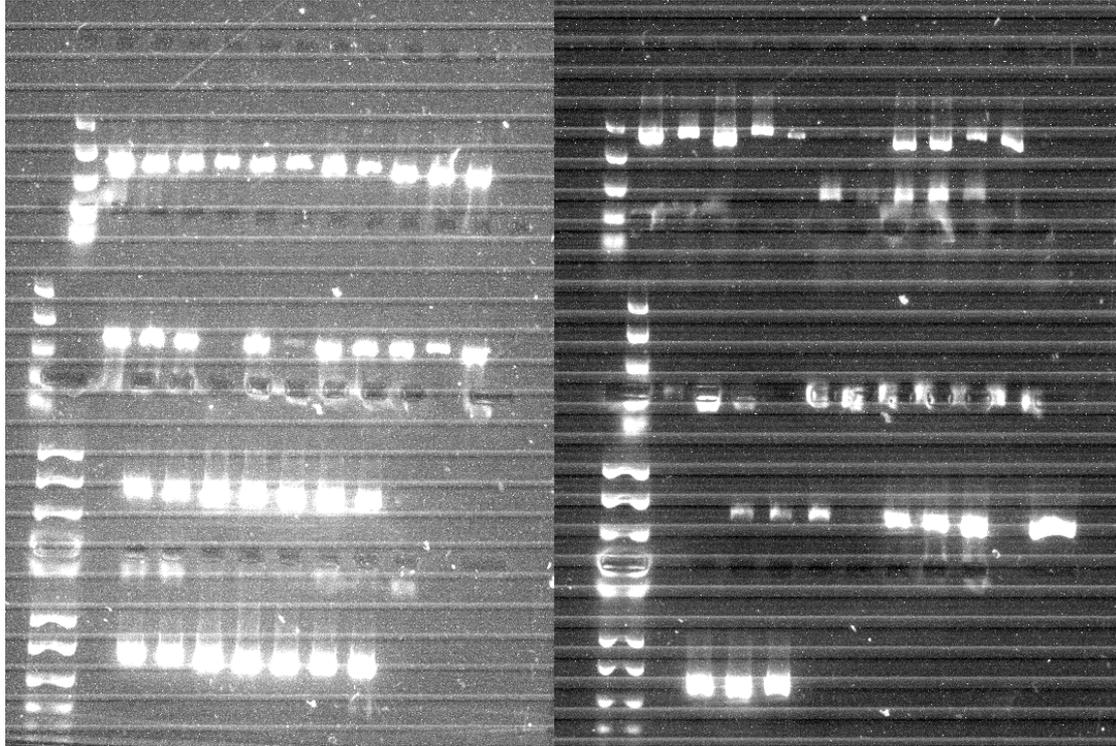


Fig.1 Image of PCR products electrophoresis on 1.5% agarose gel with Sybr Safe (Invitrogen)

Genes and primers screening

Gene regions were selected based on Martinez et al. 2013. Primers were developed from the *Arbutus unedo* cpDNA using Primer 3. After choosing the appropriate primers (petA_14F, cemaA_30F, ndhF_989F, ITS_{Arbutus}_F, TrnS(GCU), TrnHf) we continued with the PCR reaction, each PCR reaction contained 8µl water, 10µl MyTaq Mix 2x, 0.5µl each forward and reverse primers at 10µl and 1µl of extracted DNA in a final 20µl volume. The initial denaturation step (2 min, 94C) was followed by 29 cycles of 1 min at 94C, 1 min at 58 C and 1 min at 72C finally 72c for 10 min and hold it to 10 min. The PCR reaction products were separated by electrophoresis in agarose gel (1.5%), and detected using Sybr Safe (Invitrogen) under blue light after 20 min of migration at 100V (image 2). The products were sent for sequencing to Macrogen and afterwards were aligned and cleaned using Sequencher (Gene Codes Corporation).

Description of the main results obtained.

The results from the sequence were aligned with Bioedit and checked with BLAST. Only in the trnHF and ndhF regions we found variation between the different regions. The samples from Greece Athens and Ireland seems to separate from the others

without any particular geographical separation in the areas of Europe (.The markers identified in this short project will be valuable to a larger scale study of *Arbutus unedo*. In Mediterranean climates mainly summers are very hot and dry as well as robust winds leading to devastating fires which often are repeated at short intervals. As result *Arbutus unedo* like other plants of the particular spreading zone to be fire resistant, the seeds are very light so they can be dispersed over a wide range, also the fruit can be transported away through kilometers of birds that have with results even after a fire there is a saved genetic material away from fire area so the new seeds will grow away from the burning area. *Arbutus* seedlings can grow in an environment with direct sunlight so they can grow up independently in areas after fire .Furthermore *Arbutus* is protective for the ground since it protects against corrosion also is high resistant in extreme temperature changes. As a result *Arbutus unedo* could become a proposed species for tree planting both in the Mediterranean and in other parts of continental Europe in case of climate change especially after fire.

Future collaboration with the host institution

In future investigations more cpDNA regions can be assessed for variation between different regions of Europe with more samples from different areas. Also it will be very useful since there is a very small variation between the different regions to compare also the phenotype and the adaptability of the different regions to choose the appropriate regions to collect plants with desirable futures for vegetative production or seed production in a commercial program to use them for reforestation after fires.

Foreseen publications/articles resulting from the STSM

The work developed during the STSM will be published as soon as the processing of results will finish

Acknowledgments

First I would like to thank Dr Philippos Aravanopoulos for given me the opportunity to work with the National Botanic Gardens in Ireland Also Dr Colin Kelleher for our wonderful cooperation and for helping me to analyze the results. Finally I would like to express my sincere thanks to the COST Action for this professional opportunity which provide me.

References

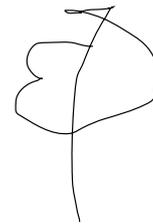
Filomena Gomes, Rita Costa, Maria M. Ribeiro, Elisa Figueiredo, Jorge M. Canhoto (2013) Analysis of genetic relationship among *Arbutus unedo* L. genotyping RAPD and SSR markers.

Martínez-Alberola F, del Campo EM, Lázaro-Gimeno D, Mezquita-Claramonte S, Molins A, et al. (2013) Balanced Gene Losses, Duplications and Intensive Rearrangements Led to an Unusual Regularly Sized Genome in *Arbutus unedo* Chloroplasts. PLoS ONE 8(11): e79685. doi:10.1371/journal.pone.0079685

[CK2] the Arbutoidae (Ericaceae): Implications for the Madrean-Tethyan Hypothesis. Systematic Botany. 2001 Jan 1;26(1):131–43.

Signature: 
Valentina Garavaglia (Oct 14, 2014)

Email: valentina.garavaglia@fao.org



COST ACRTION FP2012 REPORT

EchoSign Document History

October 14, 2014

COST Action FP2012
Name: Eleni Tzingidou
STSM Title: "Genetics of *Arabidopsis thaliana* marginal population and
management under climate change"
Location: NATIONAL BOTANIC GARDENS, Dublin, Ireland
Host: Dr Colin Kelleher
Host institution: Aristotle university Thessaloniki, Greece
Host institution supervisor: Philippos Aravanopoulos

Created:	October 14, 2014
By:	Eleni Tzingidou (eleni.sa99@yahoo.com)
Status:	SIGNED
Transaction ID:	XF8JX865B39233J

“COST ACRTION FP2012 REPORT” History

-  Document signed in Reader by Eleni Tzingidou (eleni.sa99@yahoo.com)
Signature Date: October 14, 2014 - 12:53 PM GMT+3 - Time Source: device
-  Document uploaded by Eleni Tzingidou (eleni.sa99@yahoo.com) from Reader
October 14, 2014 - 12:59 PM GMT+3 - IP address: 46.12.14.102
-  Document emailed to Valentina Garavaglia (valentina.garavaglia@fao.org) for signature
October 14, 2014 - 12:59 PM GMT+3
-  Document viewed by Valentina Garavaglia (valentina.garavaglia@fao.org)
October 14, 2014 - 1:01 PM GMT+3 - IP address: 168.202.38.206
-  Document e-signed by Valentina Garavaglia (valentina.garavaglia@fao.org)
Signature Date: October 14, 2014 - 1:11 PM GMT+3 - Time Source: server - IP address: 168.202.38.206