

COST Action FP1106: Short term scientific mission  
“Plant hormones (IAA and ABA) and polyamines as the biomonitors of drought stress and heat shock in oak provenance tests”

Reference number: COST-STSM-FP1106-11208

22<sup>nd</sup> April -31<sup>st</sup> May, 2013.

"CNR- IBIMET Institute of biometeorology"

Bologna, Italy

## REPORT

### ❖ Purpose of the STSM

Short term scientific mission at the "CNR- IBIMET Institute of biometeorology" was conducted in the period between 22<sup>nd</sup> April and 31<sup>st</sup> May 2012., at CNR-IBIMET Institute of biometeorology at laboratory for biochemistry and gas chromatography which presents a center of excellence regarding research related to plant hormones analysis as well as volatile organic compounds emission and its mitigation of pollution in environment.

Through application of the most modern scientific methods and techniques such as gas and liquid chromatography coupled with mass spectrometry we wanted to quantify contents of growth regulators such as indole-3 acetic acid (IAA) and abscisic acid (ABA) as well as polyamine contents in leaves of oak provenances and to examine role of these plant hormones as indicators of stress conditions particularly regarding increased temperature and different levels of drought.

The aim of my visit was transfer of knowledge and know-how skills regarding:

- 1) Isolation and purification methodology based on solid phase extraction (SPE)
- 2) Acquiring the knowledge about gas and liquid chromatography coupled with mass spectrometry (GC/MS and LC/MS) and its application in plant hormone and polyamine determination and quantification
- 3) Learning about methodology for derivatization of polar compounds and synthesis of reagents for derivatization of IAA and ABA and polyamines into their esters
- 4) Learning about chromatograms analysis and peak integration as well as interpretation of gained chromatograms
- 5) Learn how to maintenance, tune and optimize GC/MS equipment
- 6) Procession of the brought samples
- 7) Data analysis and establishing the scientific concept for future publication of gained results
- 8) Harmonization of laboratories methodologies in order to accomplish a platform for further interlaboratory exchange of samples

Main goal of this STSM was to estimate levels of plant hormones IAA, ABA and polyamines in leaves of two different oak species (*Quercus robur* and *Q. cerris*) and to examine their role as stress indicators under drought and heat shock stress.

### ❖ Description of the work carried out during the STSM

Samples of leaves of different oak species (*Quercus robur* and *Q. cerris*) that were grown under the different levels of drought in the soil which were maintained and measured according to the field water capacity in pots in greenhouse were sampled in Serbia, then lyophilized and brought to CNR-IBIMET Institute for further analysis of plant hormone and polyamine contents. Drought treatments were established with different watering regimens of 30% and 60% field water capacity as well as

controls. First week we prepared all needed solutions and solvents for extraction of IAA and ABA coupled with stable isotope labeling techniques of samples, and we optimized existing methodology (Barkawi et al., 2007) for different matrices (root and leaves) and we adjusted methodology for oak samples and I did detailed literature survey to realize what expected results could be. We process extracted samples with two levels of purification based on solid phase extraction (SPE) on amino columns and then on C18 columns using mixture of different solvents for elution of interfering compounds (Ludwig-Muller et al., 2008). After evaporation of solvents, samples were undergone derivatization with diazomethane which we synthesized weekly in adequate apparatus, to transfer these polar analytes into methylesters, to improve resolution and analytical efficiency, increase detectability and reduce tailing of polar compounds on GC/MS (Cohen J., 1984). We were able to process six samples per day so in summary, for entire STSM we processed 60 samples of oak leaves using this methodology. I was also introduced with principles of GC/MS technique and was shown practically how to maintenance, tune and optimize the instrument as well as how to interpret gained chromatograms and how to do integration of peak areas as well calculation of determined and identified analytes, ABA and IAA. All samples were determined in triplicates using selected ion monitoring (SIM) mode of GC/MS and ions m/z 130, 136, 190 and 194 were tracked for identification and quantification of IAA and ABA.

Barkawi L.S., Yuen Yee T., Tillman J. A., Pederson B., Calio J., Al-Amier H., Emerick M., Normanly J., A high-throughput method for the quantitative analysis of indole-3-acetic acid and other auxins from plant tissue, *Analytical biochemistry* 372 (2008) 177-188

Ludwig –Muller J., Georgiev M., Bley T. Metabolite and hormonal status of hairy roots cultures of devil's claw (*Harpagophytum procumbens*) in flasks and in a bubble reactor, *Process biochemistry* 43 (2008) 15-23

Cohen J. Convenient apparatus for the generation of small amounts of diazomethane, *Journal of chromatography*, 303 (1984) 193-196.

#### ❖ Description of the main results obtained

Main goal we succeeded to achieve with this short term scientific mission was to establish, adjust and set protocols for plant hormone and polyamine isolation and determination in *Quercus species* as well as to examine is there changes in these parameters under the drought stress. Another goal was to estimate differences in between two species *Q. robur* and *Q. cerris* in their response to drought induced stress in order to get information which one is more tolerant to drought stress which will find application in forest management strategies. Also, we made a good collaboration and discussion between researcher from Serbia and host Italian researchers. Furthermore, there was great exchange of knowledge and know how skills regarding determination of metabolites and also of knowledge related to how abiotic stress such as extreme events such as increased temperature and drought influence on metabolic pathways of polyamine and plant hormones and what plant responses of oaks to this stress is represented by. This gained knowledge is based on theory and literature survey as well as on practical level based on laboratory examination.

All gained data will be profoundly explained in joint scientific publication which will present one of the most important outcome derived from this scientific mission.

❖ **Description about how the results contribute to the Action aims**

Results gained from this STSM present example of multidisciplinary and comprehensive research approach which can contribute to broaden our understanding about tree's response to abiotic stresses and extreme environmental conditions and to show interaction between climate changes, plant species and soil conditions. This kind of subtle parameters could be included at comprehensive forest research sites (Supersites III) or be implemented in some other future long term monitoring systems which could give us information about plant's responses and their adaptability to climate changes, particularly drought and heat shock and increased temperatures. Quantification of ABA as a signaling molecule, presents crucial link between disturbances in water regimen caused by climate changes and increased air temperatures and this kind of scientific investigation could present sound platform for further investigations about extreme climate events-related risks. This STSM offered solid ground for further collaboration between Institutes and interlaboratory exchange and presented great tool for knowledge and skills transfer regarding application of newly acquired technologies and modern instrumentations such as GC/MS.

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

The confirmation by the host institution is in a separate file.

❖ **Authorization to post the report at the Action website**

**I hereby authorize posting this report at the action FP1106 website**

Novi Sad, 28<sup>th</sup> June, 2013.

Sincerely yours,

M.Sc. Marko Kebert