Report on research visit • British Scholarship Trust

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Home Institution: Institute for Adriatic Crops and Karst Reclamation, Split Croatia

Host Institution: University of Reading, School of Agriculture, Policy and Development United Kingdom

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Glimpse of inspiration

Agriculture of 21st century – a time when agriculture is facing its gratest challenges in terms of reconciling increase in food demand, human population and environmental awareness with farming land constantly decreasing and with seemingly intractable climate change on top of all. This scholarship provided me an opportunity to experience the vibrant atmosphere at one of the world's core centres directly coping with such issues. The University of Reading gathers scientists and students from all over the world whose work and ideas have a global impact in shaping modern agriculture.

This visit inspired drafting my own PhD work and reminded me that every little step towards prosperity is significant.

Doctoral research field

The research focus of my PhD is the grapevine: phenotyping and genotyping of wild grape populations in Croatia with emphasis on their disease resistance. Grapevine is one of the world's most important fruit crops and one of the biggest overall pesticide consuments. Wild grape populations, greatly related to cultivated grapevine, exsisting without human intervention, are recently in research focus partly because of concern over their extinction and partly in order to detect traits useful for breeding of modern grapevine. Even though wild grape individuals are plants yielding small and loose clusters, with usually only a few highly acid berries per cluster, there is great potential for using their properties in achieving loose clusters in dense cluster varieties often susceptible to diseases, correcting phenolic components or acidity level in certain varieties or finding resistant traits regarding diseases. In addition to traditional visual techniques for identifying grape individuals and their traits, modern techniques require an additional and reliable genetic approach.

Collaborative efforts

My application to the School of Agriculture, Policy and Development was a pleasant experience. My PhD supervisor in Split, Dr Goran Zdunić, had already made contact with my host supervisor Dr Matthew Ordidge at Reading University through their common involvement in the European Cooperative Programme for Plant Genetic Resources (ECPGR), Grapevine working group. This was an excellent link for our future work.

Moreover, this contact opened up the opportunity to expand our collaboration with another University of Agriculture (Belgrade, Serbia) and we soon the discussed possibility of publishing our future results together.

Establishing a work plan

Since my stay in Reading was planned to take place during the winter period, laboratory work and genetic analysis were agreed to be the main activities. Laboratory research is one of the crucial activities in my doctoral thesis work. So, the draft of my work plan was agreed very early on.

Grapevine collection at the Institute for Adriatic Crops (IAC) in Split (Croatia) contains certain varieties that are yet to be genotyped. Despite the fact my doctoral thesis topic is on wild grapevine populations, cultivated genotypes from the IAC collection will be used in my PhD as a referent material, so it is crucial to have a collection with known genotypes. These results will hopefully contribute to an overall grape collection inventory between the three collections at Split, Reading and Belgrade.

In order to genotype IAC grape varieties, we extracted the DNA from the dormant buds in Split and further analysis was continued at the Reading University.

The plan in action

My work plan was divided into three main sections: 1. Laboratory work, 2. Visit to the collection sites (National Brogdale Fruit Collection and Cocoa Quarantine Centre), 3. Genetic analysis of the results and research work. Once all introductory protocols were completed in terms of Health and Safety information and Lab induction, my next most important task was to check if dormant bud DNA extraction was successful.

1. Laboratory analysis showed satisfying isolation so we continued with DNA amplification using Polymerase Chain Reaction (PCR). PCR is technique used to amplify a segment of DNA, generating thousands to millions of copies of a particular DNA sequence. We then checked whether the amplification had gone well using gel electrophoresis method: a way of separation of macromolecules like DNA and their fragments, based on their size and charge. Medium through which samples are running and separating is agarose gel and all process is visualized using chemical substance etidium bromide in gel preparation, substance that intercalates DNA and enables DNA visualization under the UV light. Electrophoresis results were strong, amplification was successful, so we sent our results to sequencing – determining precise order of nucleotides within a DNA molecule.

We continued with laboratory practice and work on apple samples provided by the Reading University. In addition to practising standard laboratory work, learning a new method brought a new dynamics and knowledge into the work done so far. Purifying DNA from an Agarose Gel is a method that requires very high precision and concentration in work. It is commonly used for molecular cloning. Following electrophoresis, one can cut targeted DNA bands out of the agarose gel and purify DNA. That was the new method we practised next.

2. In addition to the laboratory work, as proposed in my work plan, I visited the National Fruit Collection (NFC) in Brogdale. NFC is the world's biggest fruit collection and Dr Ordidge is NFC's scientific curator. I had the opportunity to see the overall organisation at the site, fruit crops design, different trials set up for different purposes (climate change, training

systems) infrastructure, learn more about collection management system, challenges they are facing with. There was an opportunity to meet and talk with agronomists working on the collection, who have advisory oriented work. Additionally, at the University's campus it was interesting to see and learn about part of apple bud collection being cryopreserved. This is a way of preserving plant material by cooling it to very low temperatures −196 °C using liquid nitrogen. Soon after, we visited the
International Cocoa Quarantine Centre, an organization of global cocoa importance aiming to reduce the amount of disease affecting cocoa plants. It was useful and interesting to see tropical plants growing in specially adapted polytunnels under strict standardized quarantine conditions. Visit to both of the collections gave new ideas for managing our collections of cultivated and wild grapevine in Split.

3. Analyzing the sequencing results. We recieved new results of wild grape sample sent from Croatia to be analysed as part of my doctoral thesis work as well. This was a great opportunity to analyze the results and at the same time gain knowledge and have practice in working with Gene Mapper, a special computer program for genotyping. In the meantime the sequencing results have also arrived, so we managed to analyze them too. We have had the final confirmation that DNA extraction of all our samples was very good. Throughout all that period I had the support of supervisor and research technician who helped with their broad knowledge and great experience in work with this program.

Extra contribution

During my scholarship visit I was invited to participate at some of PhD student presentations where students present their PhD work in progress. They discuss their work with professors and other students in a professional and friendly atmosphere as a kind of guideline for their further PhD work. What better way to experience student life and work at Reading than literally take part in it? This event enriched my overall scholarship experience and served as an incentive to hold my own presentation on the PhD in a similar way. Holding this presentation had immense meaning in terms of the unique experience giving a presentation and having a discussion of my PhD work.

Results and impressions

All the samples that were the subject of the research, and directly relevant to my PhD thesis, showed very good results and successful analysis. The supervisor and research assistant were supportive and always ready to advise and help. Since visit was planned for the winter period, a visit to the fruit collection at first seemed to be an obstacle in terms of lack of vegetation. However, this proved to be an advantage for I had a better insight into the structure and management of the collection, different training systems and trials.

From the beginning I was involved in social events and daily informal meetings of faculty staff where was an excellent mutual communication and collaboration. Administrative and procedural activities were professionally carried out, so the work was completed well and on time.