FELLOWSHIP SUMMARY REPORTS

Developing genomic resources for African nightshade (*Solanum scabrum*) breeding

Dr Peter Poczai

Host: Prof Dr Celestina Mariani
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Herby, I give my consent that the report can be posted in the Co-operative Research Programme's website.

24.08.2019. Geneva, Switzerland

1. What were the objectives of the research project? Why is the research project important?

In this project, we aimed to provide the complete sequences of the plastid and mitochondrial genomes of *S. scabrum* and all 19 Old World species. We aimed to analyze the organization and variation of the organellar genomes and compare it with currently available sequences of important solanaceous crops. We hope that the organellar genome sequences of African nightshades will initiate a holistic breeding approach in this underutilized crop and stimulate further breeding programs concerning traditional African vegetables (TAVs). We will also assess the phenotypic evaluation under controlled stress conditions: heat/drought tolerance of selected *Solanum scabrum* germplasm and collect transcriptomic data to further investigate genes involved in these conditions.

2. Were the objectives of the fellowship achieved?

During the granted period we have collected samples of 21 species of black nightshades (*Solanum* sect. *Solanum*) including: *S. alpinum, S. americanum, S. annuum, S. chenopodioides, S. emulans (=S. ptychanthum), S. hirtulum, S. memphiticum, S. nigrescens, S. nigrum, S. nitidibaccatum, S. opacum, S. pseudospinosum, S. pygmaeum, S. retroflexum, S. salamanceae, S. salicifolium, S. scabrum, S. tarderemotum, S. tweedianum, S. umalilaense* and *S. villosum.* Leaf samples were rinsed with deionized water and 70% ethanol, and total genomic DNA was isolated using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany). All work was carried out in a dedicated laboratory with UV sterilized equipment; blank samples were processed during DNA extraction. DNA concentration was measured with a Qubit fluorometer (Invitrogen) and verified on an 0.8% agarose gel. A paired-end genomic library was constructed using the Nextera DNA library preparation kit (Illumina, San Diego, CA, USA). Fragment analysis was conducted with an Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip. Sequencing is currently performed on an Illumina MiSeq platform from both ends with 150 bp read length and expected to end in September 2019. Final reads will be processed, plastid genomes analysed and annotated by the end of the year. Preliminary results were presented as a poster. The generate illumina read based DNA sequence data will be distributed to international repositories (NCBI SRA) and available for wide dissemination. DNA sequences will be deposited in openly accessible genetic databases (NCBI GenBank) and made freely available.

We also carried out green house experiments with 20 selected accession of *S. scabrum* collected from the distributional range of the species in Africa. The accessions are maintain in the collection of Radboud University and duplicates are shared between Wageningen University of Technology and University of Helsinki. Morphological parameters such as plant height, leaf size, root mass, number of shoots etc were scored among the control and water stress groups of plants. Plants were watered according to a certain weight defined for the drought stress group receiving 50 mL of water. The experiment was carried out through 12 weeks, and 180 plants were screened under induced drought stress conditions.

Fig.1. An example of drought susceptible and tolerant individuals of African nightshade (*Solanum scabrum*) identified during greenhouse experimental conditions.

At the end of the experiment morphological parameters were evaluated again among the two groups, and leaf samples were collected in liquid nitrogen and stored under -80°C for further RNA extractions and transcriptome

sequencing. Based on our preliminary results we have identified 3 accessions of drought tolerant breeding lines of *S. scabrum*, which could be used in further breeding of the crop species.

3. What were the major achievements of the fellowship? (up to three)

- generation of high-throughput sequencing data for 21 species of *Solanum* sect. *Solanum*
- identification of three drought tolerant African nightshade accessions
- collection of RNAseq data from drought tolerant and sensitive *S. scabrum*

4. Will there be any follow-up work?

o Is a publication envisaged? Will this be in a journal or a publication? When will it appear?

Currently preliminary results from partial data of the program were presented in a form of a poster. Bioinformatic tools were developed for the evaluation of drought indices. From the plastid genomic data collected we are expecting to publish a comprehensive phylogenomic paper, while RNAseq data will be used in a separate publication.

SABLOK G, OREJUELA A, HE X, HYVÖNEN J, SÄRKINEN T, **POCZAI P** (2019) Complete plastid genome sequence of African nightshade (Solanum scabrum) and its comparative plastomics across Solanales. The 2019 Congress of the European Society for Evolutionary Biology (ESEB), Turku Finland. DOI: 10.13140/RG.2.2.30592.38402

POUR-ABOUGHADAREH A, YOUSEFIAN M, MORADKHANI H, VAHED MM, **POCZAI P**, SIDDIQUE KHM (2019) iPASTIC: an online toolkit to calculate plant abiotic stress indices. Applications in Plant Sciences 7: e11278

Other papers written and/or submitted/published during the fellowship period in various topics:

SZABÓ TA, **POCZAI P** (2019) The emergence of genetics from Festetics' sheep through Mendel's peas to Bateson's chickens. Journal of Genetics 98: 63

AHMADI J, POUR-ABOUGHADAREH A, FABRIKI-OURANG S, KHALILI P, **POCZAI P** (2019) Unravelling salinity stress responses in ancestral and neglected wheat species: a baseline for conservation and utilization in future wheat improvement programs. Physiology and Molecular Biology of Plants, submitted

WOOD RJ, SZABÓ TA, **POCZAI P** (2019) Festetics' 'Genetic laws of Nature' considered in relation to the prevailing concept of blood inheritance. Journal of the History of Biology, submitted, HIST-D-19-00034

POCZAI P, SEKERÁK J, BARISKA I, SZABÓ TA (2019) The Good, the Coachman and the Sheep Breeders' Society of Moravia: how political repression stifled the nascent foundations of heredity research prior to Mendel. Journal of the History of Biology, in revision, HIST-D-19-00027

o Is your fellowship likely to be the start of collaboration between your home institution and your host?

The fellowship was highly beneficial to start active collaboration between Radboud University and other Dutch institutions and between the University of Helsinki. These extend to further topics in Solanaceae including: i) eggplants investigating the S. melongena complex and populations of S. incanum/S. insanum; ii) bittersweet nightshade (Solanum dulcamara) genome sequencing. We have also outlined plans for further grant submissions to encourage collaboration between the two institutions from both national and EU funding schemes. Further experiments are underway sequencing the entire genome of Solanum scabrum, thanks to the trigger funding from the OECD CRP programme.

o Is your research likely to result in protected intellectual property, novel products or processes?

Highly unlikely.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

A growing number of households are turning to markets for their food supply. In urban areas, almost all food is bought on the market with an average of 93% of household food consumption supplied through various distribution channels. At the same time, increasingly diversified rural economies and the spread of urban products and lifestyles mean that the share of rural food supply from markets is also growing. Overall, markets provide at least two-thirds of household food consumption at the regional level (OECD, 2013). Urbanisation and urban lifestyles are also accompanied by shifts in dietary patterns, which are spreading beyond the frontiers of towns and cities. More fruits, vegetables and processed foods are being consumed, while the share of cereals and pulses is declining. Urban consumers are clearly moving towards higher value food products. Fruits and vegetables, and meat and fish now account for half of the total food expenditure by urban households. The demand for convenience is an overarching trend across income groups and area. This is reflected in the strong demand for processed and prepared foods and in the expansion of street food. In urban areas, processed foods represent 41% of food budgets (OECD-FAO Agricultural Outlook 2016-2025). The overall aim of our project is to improve food and nutrition security and household incomes of smallholder farmers in sub-Saharan Africa, particularly Kenya, Uganda and Cameroon. The project builds upon past OECD policy directives (OECD 2013 and OECD-FAO 2016) to addresses two major constraints, i.e., a lack of high yielding varieties and quality seed that are resistant against heat/drought stress.

6. How was this research relevant to:

- The objectives of the CRP?
  The current project was related to innovation in agriculture, as it applies to plant sciences for the OECD's priorities in advancing agricultural policies based on the experiences and perspectives of agricultural technology developers. Under CRP's theme III Transformational technologies and innovation. Advanced breeding tools/Genetic and genomic technologies. Plant breeding has been very successful in developing improved varieties using conventional tools and methodologies. To breed improved African nightshade varieties, a better understanding of their genetics and origins (eolvution) is necessary. With the advent of next-generation DNA sequencing technologies, many important crop genomes have been sequenced. Primary importance has been given to food crops, including cereals, tuber crops, vegetables, and fruits in Solanaceae to potato and tomato. However, underutilized crop species belonging to Solanaceae are lagging behind. The DNA sequence information in these species just like *S. scabrum* and related species are extremely valuable for identifying key genes controlling important agronomic traits and for identifying genetic variability among the cultivars. For these plastid, mitochondrial genome sequences and full transcriptomes could provide the first step to decipher the complete genomic information of these plants substantially improving our understanding of the genetics of these crops. Application of the knowledge obtained from the plastid genomes and transcriptomes, expression studies of this project will enable the development of improved varieties in line with CRP's Theme III.

- The CRP research theme?
  With these objectives outlined above our project directly targeted the environmentally healthier, "sustainable intensification" focus of CRP research theme which recognizes that food security and nutrition of the world will need to balance food production and food demand for a population of at least nine billion in 2050. This principle of sustainability in the world's natural capital as well as its food production systems has become the key element for the CRP investments. According to OECD report prepared by Staatz and Hillinger (2016) the African food system has the challenge of producing more and nutritious food for growing populations and external international markets while reducing the environmental impact of food production systems, and that subsistence farmers must efficiently produce nutritious food for their families and conquer the market as commercial farmers to increase income through enhanced land productivity. Specifically, the current project links to the following two research areas: 1) The identification and breeding of crops to maintain/increase productivity and resilience under conditions of limited external inputs and increased abiotic and biotic stresses, 2) Models and indicators aiming at measuring simultaneously production, environment and socio-economic issues, variables and parameters in order to compare situations and dynamics. Furthermore, the project has reverse benefits to the OECD community, as knowledge on the traits under study is relevant for agricultural production in OECD members and it seems likely that, in time, *Solanum* crops like the African nightshade vegetables, currently not common on the OECD members, will

grow into a viable African export product and become part of citizens of OECD countries listed in the co-operative research programme.

7. Satisfaction

- Did your fellowship conform to your expectations?
  Yes, absolutely. I could widely recommend to any expert to apply. I have benefited greatly from the fellowship.
- Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.
  Yes. The fellowship served as good trigger funding to move forward with my carrier and broaden my research network. The work carried out will highly likely have longer term effects on my carrier and also obtaining research funding.
- Did you encounter any practical problems?
  None.
- Please suggest any improvements in the Fellowship Programme.
  I have enjoyed the wonderful opportunity what the fellowship has provided during the granted period.

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme?
  From a former colleague who has been an OECD research fellow.
- What would you suggest to make it more "visible"?
  Maybe a campaign on social media channels, and relevant mailing lists would improve the visibility of the programme.
- Are there any issues you would like to record?
  No, there are none.