OECD's CO-OPERATIVE RESEARCH PROGRAMME THEME 3: THE FOOD CHAIN

Title:

Increasing hardiness of cereal crops against fungal pathogens through the CRISPR/CAS gene editing system

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Host Institution:

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Host supervisor:

Prof. Shahryar Kianian

Term:

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The report is free to be posted on the Co-operative Research Programme website.

1. Relevance:

The fellowship is relevant to main objective Research Fellowships: sponsorship of scientists to conduct research projects in a different Member country with a view to strengthening the international exchange of ideas and increasing international mobility and co-operation at OECD's CO-OPERATIVE RESEARCH PROGRAMME THEME 3: THE FOOD CHAIN.

The fellowship was focussed on applying of the newest gene editing system (CRISPR/Cas9) to narrowing down or identification of powdery mildew APR resistance gene introgressed to hexaploid wheat from related *T. militinae*. Additional aim was use of the editing system to modify conserved domains of resistance gene in order to identify and validate gene active sites. These could be used for study of interaction of the resistance genes with the AVR genes of pathogen and. As examples the *Mla* genes of barley were considered as pilot targets.

2. Main objectives of the fellowship:

The fellowship had two main objectives, development of base and knowhow transfer of gene editing and cereal transformation systems for R gene editing for creation and study of new resistance alleles and functional validation of APR gene using newest genomic tools (CRISPR/Cas9 gene editing system) and resources (cloned resistance and virulence genes). Since duration of the fellowship do not allow finishing all experiments in the host laboratory (wheat and barley plant transformation, regeneration and seeds production takes more than six months) the uniting aim of the fellowship was to transfer the CARISP/Cas9 technology to the applicant laboratory and establish close collaboration with the host laboratory.

3. Major Achievements (up to three)

The first part of the fellowship was devoted to detailed annotation and validation of candidate genes. 73 genes were annotated and validated. Three of them were discarded as transposon related. 46 of the genes were identified as potential pseudogenes based on premature stop codon presence. From the remaining 24 genes, nine were related to the LRR like genes involved in hypersensitive resistance response. However, gene 69 comprises "Malectin" conserved domain (pfam11721), recently shown to be involved in race nonspecific resistance similar to APR phenotype we have observed in this material.

To narrow down the number of candidate gens for the APR resistance a six candidate gRNAs were designed. To construct the CRISPR/Cas9 construct suitable for transformation or infiltration of wheat and barley plants and induce deletions and/or knock out of the genes. The gRNAs were selected from the intergenic low copy regions to induce deletions in the APR region. Such small number of genes could be efficiently validated by knock out. Also, for gene 69, as the most probable candidate, three gRNAs were designed to induce deletions and/or frameshift to knock out the gene.

In the last part of the fellowship the applicant was trained in protoplast assay to verify functionality of the CRISPR/Cas9 constructs in the target plant line. Additionally, he was trained in the embryo preparation, transformation using agrobacterium infiltration and validation of the process through GUS reporter system.

4. Follow-up

We envisage several publications from this work including cloning and identification of the powdery mildew APR resistance gene from *T. militinae*, characterization and validation of the

gene, study of the gene functional domains, validation of barley *Mla* functional domains and their interaction with identified AVR genes.

The collaboration established by this fellowship is expected to expand in the future, as much of the research work is ongoing. We expect this to be a long-term partnership in a highly important and productive research area. This research can lead to protected IP for as long as they confirm to IEB and USDA-ARS policies. We expect new and novel approaches and germplasm to result from our work that will be made available to the public for further use in development of new cultivars more resistant to fungal pathogens.

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?**

Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

Identification, editing and successful transfer or resistance genes is important for stabile food production with decreasing of pressure on environment due to use of chemical protectants as fungicides and pesticides. Additionally, the stable yield and decreased number of field sprays have positive effect on profitability of farming.

6. **Satisfaction**

The fellowship was an amazing experience. It more than confirmed to expectation and it came at exactly the right time. I wish OECD would shorten the duration of repeat application for these fellowships from 5 years to at least every 2 years. The fellowship allowed establishment of a much stronger collaboration and interaction that would otherwise be possible. Even though we didn't fulfil all planned tasks; the stay was very fruitful for its duration. Actually, the cereals as a scientific objects are still a challenge and I found the troubles in the work as very beneficial for it allowed me to go through all troubleshooting in the environment more than suitable for solving the problems. With the experiences of the host laboratory and laboratory of prof. D. Voytas (one of leading labs. in the area of gene editing) I fell well prepared to finis the remaining tasks in collaboration with the host lab. This work will lead to many important findings in our area of research that are relevant to agricultural production and food security.

7. **Advertising the Co-operative Research Programme**

I first learned about this program from Prof. Kianian. Upon further investigation I learned about this amazing opportunity and jumped at the chance at the first possible moment. During my visit to the University of Minnesota I visited several other institutions and at each advertised this program in discussion with other scientists. I also sent several emails to colleagues that could benefit from this experience. I believe that the only way to make this program more visible is through advertising by members of scientific community and especially by the former alumni of this fellowship.