

## „Studies on *Ribes* plants, *Cecidophyopsis* mites and Blackcurrant Reversion virus for sustainable resistance breeding and cultivation of *Ribes*”

1.1.1.1/18/A/026

### Progress of the project (01.09.2020. – 30.11.2020.)

Within **Activity No.1** the scanning electron microscopy (SEM) of *Cecidophyopsis* mites was completed, SEM images obtained and documentation of morphological parameters completed, and the data analysis of morphological parameters was started. Nucleic acid preparations from mite individuals used in SEM were made, multiplex PCR amplification performed for species identification by fragment Length analysis (FLA), ITS/5.8S/28S region amplified and PCR products were prepared for direct sequencing. FLA was performed on new mite multiplex PCR samples on the genetic analyzer ABI PRISM 3130xl for the species determination, using the previously developed automated analysis protocol, and sequencing of ITS/5.8S/28S region of prepared samples by Sanger method was done at BMC. Quality controls of ITS/5.8S/28S sequences were done and alignments prepared for further analysis. *COXI* PCR amplification of all selected mite DNA samples was done and prepared for sequencing. PCR fragments of *COXI* was sequenced by the Sanger method at BMC. The sequence assembly was started. Testing of BRV in single mites after the electron microscopy was continued to study involvement of mite species in BRV transmission. Design and testing of primers for BRV CP amplification was started

Within **Activity No.2** the writing an article on resistance of *Ribes* to *Cecidophyopsis* and BRV, and their interactions was continued, and the manuscript was prepared for submission.

The analysis of the sequences obtained from resistance *Ce* gene-specific amplification fragments was continued. A draft of the publication on the results of SSR marker genotyping of *Ribes* genetic resources has been improved by involving of experts from different project activities. Genotyping of chloroplast molecular markers (cpSSR) for *Ribes* plant material has been completed in order to elucidate the possible species structure of interspecific crosses. Further analysis of the material was performed by BMC, where FLA analysis for 23 chloroplast molecular markers were performed (3812 samples) on the genetic analyzer ABI PRISM 3130xl, and developed automated analysis protocols.

NGS libraries were prepared of *Ribes alpinum* mite-infested and control samples for sequencing on DNBSEQ-G400 (previously known as MGISEq2000). The 12 NGS libraries of the infested and control samples of blackcurrants (cultivar ‘Mara Eglite’) harvested in May 2019 and four NGS libraries of *Ribes alpinum* harvested in May 2019 were sequenced on DNBSEQ-G400. Primary statistical analysis of RNA-Seq data of blackcurrant samples was performed. After data analysis from 1<sup>th</sup> group samples were identified 573 gene transcripts, from 2<sup>nd</sup> group – 769 and from 3<sup>rd</sup> group – 574 gene transcript. We selected base line 3 for logFC and based on this for 1<sup>th</sup> group we selected 10 top upregulated genes (*At3g45310*, *NPC1*, *GOLS2*, *CP*, *ZIP10*, *GOLS1*, *PPDK*, *GOLS4*, *PPD*, *STC*) and three top downregulated genes (*CHSA*, *CHS*, *CHS1*), for 2<sup>nd</sup> group – 14 top upregulated genes (*At3g45310*, *NPC1*, *GOLS2*, *CP*, *ZIP10*, *GOLS1*, *PPDK*, *GOLS4*, *PPD*, *O2390*, *PLT5*, *PDR1*, *CAX3*, *SIGE*) and five downregulated genes (*At1g48100*, *CHSA*, *CHS*, *SKU5*, *P14009*) and for 3<sup>rd</sup> group – 21 top upregulated genes (*Os08g0191100*, *At1g60710*, *At3g45310*, *O23920*, *MSRB1*, *LWD1*, *NPC1*, *GOLS2*, *NPF3.1*, *ABCC10*, *FDH1*, *PECS-2.1*, *AND1*, *APX3*, *SCE1*, *ABCC3*, *PLT5*, *OPR11*, *CP*, *GOS2*, *UBC9*) and 17 top downregulated genes (*At4g15545*, *matK*, *ndhI*, *psaJ*, *ndhE*, *psbK*, *psbW*, *ART2*, *cemA*, *RRT15*, *ndhG*, *PSBS2*, *CHS*, *RRF*, *ATP6*, *RPS17*, *PSRP3*). Work continued

on the preparation of NGS libraries of mite-infested and control samples of red currant (cultivar 'Kodu Suur Valge' ('Hele')) harvested in May 2019 and stored at -80°C.

The work was continued for PCR amplification of TEF 1- $\alpha$ , and HSP70 genes and their possible use for establishment of diagnostic method for *Cecidophyopsis* species on *Ribes* hosts.

After reviewing and compiling the information on the local genetic resources of *Ribes* plants in databases and collections, a consolidated and updated list of *Ribes* genetic resources has been created and submitted for inclusion in the National Genetic Resources System. Evaluation of local genetic resources was continued. A second annual data set has been created on the results of the evaluation of recently obtained, previously did not evaluated local *Ribes* genotypes. The description of genotypes included in genetic resources according to RIBESCO descriptors was continued and their description according to EURISCO descriptors was started. An improved methodology for evaluating the damage by bud mites and BRV in field conditions has been developed. In the Laboratory of Tissue Culture, the propagation and maintaining in *in vitro* the plants required for industrial research and the establishment of genetic resources core collection has continued.

Two popular science publications were published about the project, obtained results and local genetic resources of *Ribes*:

- 1) Moročko-Bičevska I., Stalažs A., Lācis G., Laugale V. (2020) Studies on *Ribes* plants, *Cecidophyopsis* mites and *Blackcurrant Reversion virus* for sustainable resistance breeding and cultivation of *Ribes* [in Latvian]. Proceedings of conference "Sustainable Agriculture 2020", 120-122.

[http://www.lf.llu.lv/sites/lf/files/2020-09/Latvia-lidzsvarota-lauksaimniec\\_rakstu\\_krajums\\_2020.pdf](http://www.lf.llu.lv/sites/lf/files/2020-09/Latvia-lidzsvarota-lauksaimniec_rakstu_krajums_2020.pdf)

- 2) Laugale V. (2020) Genetic resources of *Ribes* in Latvia, research and conservation. [in Latvian]. Professional Horticulture [Profesionālā Dārzkopība], 3 (13): 20-24

[https://fruittechcentre.eu/sites/default/files/2020-11/Profesionala\\_DARZKOPIBA\\_13.pdf](https://fruittechcentre.eu/sites/default/files/2020-11/Profesionala_DARZKOPIBA_13.pdf)

*Information prepared 30.11.2020.*