

„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 (01.03.2020. – 31.05.2020.)

Within **Activity No.1**, the amplification and cloning of the ITS/5.8S region of mites were continued, and the next samples sent for sequencing to the project partner BMC where sequencing was performed by the Sanger method. additional blackcurrant samples were received from UK, mite DNA isolated and samples prepared for for the species detection by FLA. FLA was performed by the project partner on the new mite multiplex PCR samples on the genetic analyzer ABI PRISM 3130xl for species determination using the previously developed automated analysis protocol. The testing of BRV in single mites after electron microscopy was continued.

Within **Activity No.2** the collecting of information and writing an article on resistance of *Ribes* to *Cecidophyopsis* and BRV, and their interactions was continued. The analysis of existing *Ribes* genetic resources genotyping information for preparation of a publication manuscript was continued by performing additional literature and data analysis. Adaptation of the methodology of application of chloroplast molecular markers (cpSSR) selected in the previous stages of the project has been performed, the set of the most informative markers has been selected to explain the structure of interspecific crossings of *Ribes* plant material. DNA extraction was performed using the existing *Ribes* sample collection. In order to ensure the representation of *Ribes* species, additional samples were collected in the LatHort collection and their DNA extraction and quality assessment was performed. PCR of resistance *Ce* gene-specific amplification fragments was performed for further cloning and sequencing, and analysis of the obtained sequences was started. In BMC the sequencing of cloned amplification fragment of *Ce* genes samples transferred by the Leading partner was performed by the Sanger method.

Work continued on the preparation of NGS libraries of blackcurrant (cultivar ‘Mara Eglite’) mite-infested and control samples for sequencing on MGISEq2000, as well as the isolation of total RNA from *Ribes Alpinum* and red currant (cultivar ‘Kodu Suur Valge’ (‘Hele’)) for mite-infested and control samples sampled in May and August and stored at -80°C has been initiated by BMC following the previously optimized RNA isolation protocols. The work has been undertaken on the preparation of NGS libraries for sequencing.

Primers based on the literature search were selected and new primers developed for amplification of COX1, TEF 1- α , HSP70 gene fragments and sequencing to develop mite detection method.

The evaluation of *Ribes* genetic resources field collections was continued according to RIBESCO descriptors. The second year data set on plant phenological and vegetative development, frost resistance was collected. The collections in Püre and Dobeles were assessed for the prevalence and symptoms of BRV. Samples were collected from the red and white currants for the determination of BRV to develop a disease evaluation methodology. In the tissue culture laboratory, 9 *Ribes* genotypes from different species were propagated and rooted in the substrate, which will be used for resistance studies, and 9 blackcurrant and 4 gooseberry genotypes for a creating of virus and pest free core collection, which will be tested for healthiness. In parallel, samples of all genotypes are maintained and preserved in vitro.