



„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 (01.09.2019 – 30.11.2019)

Within **Activity No.1**, Multiplex PCR amplification of mite DNA and PCR product preparation for fragment length analyses (FLA) with genetic analyser was continued. FLA was performed for 725 prepared mite DNA multiplex PCR samples on the ABI PRISM 3130xl genetic analyzer for species identification, and an automated analysis protocol for allele identification and selection was developed. Preliminary FLA data analyses and identification of mite species was performed in the collected plant material. Testing of non-destructive electron microscopy for the mite species identification and further isolation of nucleic acids from single mite individuals was continued and the optimal application protocols were elaborated and selected for further use. The testing of methods for viral nucleic acid isolation from mites and BRV PCR amplification was continued and the protocol for testing in the laboratory was established. Theoretical protocols for ITS/5.8S amplification and cloning was elaborated and their practical testing was started in the laboratory.

In **Activity No.2**, further development of methods for the *Ce* and *P* resistance gene identification in *Ribes* plants was performed on the *Ribes* plant material selected in earlier stages of the project, as well as involving additional *Ribes* samples. Modification of molecular marker reaction conditions was continued to ensure stable amplification of gene-specific fragments. In accordance with the project objectives, the collection of existing genetic information on the *Ribes* genetic resources was started, and the evaluation of existing genotyping data for preparation of paper manuscript has been undertaken. The obtained information will provide insight into the diversity and interrelation of genetic resources. To complement the existing genetic data, a review of the scientific literature and selection of molecular markers (cpSSR or chloroplast microsatellites) was done during the reporting period with aim to clarify the inter-specific structure of *Ribes* plant material, considering their complex origin, especially for blackcurrants, that can have the influence to resistance.

Total RNA isolation optimization experiments were continued from the previously harvested samples from genotypes of *Ribes* genus (black currant cultivar ‘Mara Eglite’, red currant cultivar ‘Kodu Suur Valge’ (‘Hele’) and genotype of *Ribes alpinum*, collected in the wild), as well as for the isolated RNA concentration, quality and integrity (RIN) analysis were performed. Comparing the concentration, quality and RIN of the isolated RNA in samples stored under different conditions, it was concluded that the RNA isolated from in *RNAlater* stored samples at +4°C and -20°C is higher in concentration and RIN, which is essential for the preparation of high quality NGS library. Conversely, samples stored at -80 ° C showed the opposite effect. Therefore, *RNAlater* can be used to store plant samples at higher temperatures. The optimal RNA isolation methods have been developed for *Ribes* plant samples by selecting the most appropriate lysis matrix, optimal PVP concentration and isolation kit for each species of the genus. Optimized RNA isolation methods will be used for RNA isolation from inoculation experiment samples for preparation of NGS libraries.

During current period the summarizing of knowledge and scientific information on *Ribes/Cecidophyopsis*/BRV complex interaction and previous observation results in *Ribes* collections of Institute of Horticulture was performed and the preparation of review article was

started. Data collection and description using RIBESCO descriptors of *Ribes* genotypes and inventory of present genetic resources in field collections in Dobele and Pure was continued, to select more valuable genotypes for including in national and international genetic resource databases. Data analysis for the results obtained in season 2019 has begun. Work was continued on the development of evaluation methods. Selected *Ribes* genotypes for further industrial research and valuable local accessions for creating of virus and pest free core collection were propagated and maintained in-vitro.

The project progress and goals were communicated to the society during the European Science Night, where at the Institute of Horticulture (LatHort), Plant Pathology and Entomology laboratories scientists in a popular science format presented poster and provided practical demonstrations about research on *Cecidophyopsis* bud mites, blackcurrant reversion virus and Ribes plants. Information about activities was posted on the LatHort [Facebook](#) site.



Ribes ģints augu, *Cecidophyopsis* pumpurēču un upeņu reversijas vīrusa izpēte ilgtspējīgai *Ribes* ģints ogulāju rezistences selekcijai un audzēšanai

Projekta identifikācijas nr.: 1.1.1.1/18/A/026
 Projekta izpildes termiņš: 2019. gada 1. marts – 2022. gada 28. februāris
 Projekta kopējais finansējums: 522 954,99 EUR
 Projekta zinātniskais vadītājs: Ph.D. Inga Morozko-Bīvēnka (Dārkopības institūts)
 Galvenie izpildītāji: Dr. agr. Arturs Stalāks, Mg. biol. Neda Zuļģe, Ph.D. Guntars Lācis, Dr. agr. Valda Laugale (Dārkopības institūts)
 Projekta partneris: Dr. biol. Ina Balke (Latvijas Biomedicīnas Pētījumu un Studiju Centrs)

Projekta kopšvirskums
 Ilgtspējīgu ģints Ribes augu audzēšanu būtiski ietekmē *Cecidophyopsis* ērces un upeņu reversijas vīrusa (BRV). Selekcijas programmas koncentrējas tikai uz *Cecidophyopsis* ribes un diviem rezistences ģenotiem. Pētījumi parāda, ka arī citās *Cecidophyopsis* sugās ir gēni, kas palīdz, tā ietekmējot atbilsta aizsardzības rezistences preturam.

Projekta mērķis ir skaidrot *Cecidophyopsis*-BRV kompleksa mijiedarbību ar Ribes ģinti augiem:
 1) Iztīrēt *Cecidophyopsis* sugas koncepciju un ģenētisko daudzveidību;
 2) nosaukt katras *Cecidophyopsis* sugas lomu BRV pārnesē;
 3) rekonstruēt rezistences evāciju uz *Cecidophyopsis* un BRV, izmantojot NGS, gēnu ekspresijas un transkripcijas analīzi;
 4) izvērtēt un ieviestarājot vīrojto Ribes ģenofonda.

Iegūtās zināšanas, ievērtētais vīrojtais ģenofonds un vīrojto ģenotipu vīrusbrīvā in vitro kolekcija kalpos par bāzi ilgtspējīgai Ribes augu rezistences selekcijai un audzēšanai, sekmējot uz zināšanu balstītu bioekonomiku.

Projekta īstenošana sniegs jaunus zināšanas ilgtspējīgai Ribes augu rezistences selekcijai un audzēšanai, retot palielinot zinātnisko publikāciju skaitu starptautiskā recenzētos žurnālos un atbilstošu zinātnisko kapacitāti lauksaimniecībā un biotehnoloģijā. Ieguldījumi ir valsts pētniecības institūti, kas garantē rezultātu publicēšanu (zinātniskās publikācijas, zinātniski zinātniskās konferences) un zināšanu izplatīšanu nozarei (populārzinātniskās publikācijas, prezentācijas semināros, informācija publikāciju datubāzēs). Projekta nodrošināšana turpināsies sadarbībā ar *Cecidophyopsis* ērcēm un BRV, un, sadarbojoties abām institūcijām, sasniegt progresīvas pētījumu augu ģenētiskā, patoloģiskā, molekulārā bioloģijā un vīrusu paraugu metagenomiskajā analīzē, nodrošinot pamatu rezistēto publikāciju sagatavošanai.

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