



**NIBIO**

NORWEGIAN INSTITUTE OF  
BIOECONOMY RESEARCH

# Diagnosis, virus cleaning and cryopreservation of raspberry, blackberry and shallot (Rub&AI)

Zhibo Zhang, Carl Spetz, Erik Lysøe, Dag-Ragnar Blystad

## Background

In Norway, raspberry is an economically important crop; whereas blackberry and shallot are potential crops for increased production and consumption. All of them are vegetatively propagated, and are often infected with several viruses that cause significant economic losses. Prerequisites for long-term development and production of these crops are virus diagnosis, virus elimination, and preservation of healthy mother stock and important cultivars.



Crumbly fruits can be caused by virus infection



Raspberry with virus symptoms probably caused by an aphid transmitted virus

## Goal:

This project will establish and preserve healthy nuclear stock plant material as a basis for innovation.



Symptoms: Raspberry leaf mite / Raspberry leaf blotch virus

### Work packages

- 1) Diagnostic tools for viruses
- 2) Tissue Culture to eradicate shallot viruses
- 3) Cryopreservation
- 4) Survey of virus occurrence and influence
- 5) Implementation

### Benefit for the industry

This project will benefit NIBIO in gaining knowledge and developing diagnostic methods to certify health status of Norwegian production, aid Sagaplant and Gartnerhallen producing certified plant materials and securing nuclear stocks, and ensure a collection and future applications of healthy Norwegian shallots cultivars by Norwegian genetic resource centre.

To achieve the goal we will:

### Develop efficient virus detection methods

Newly developed methods based on nucleic acid detection of plant viruses will be implemented in the diagnosis of relevant viruses.

### Eradicate viruses from mother plants

We will eradicate plant viruses by using a therapy (chemical, cryo or high-temperature treatment) combined with cutting meristems (small shoot tips)

### Preserve clean mother plants with cryopreservation technique

Cryopreservation, storage of living biological samples in liquid nitrogen (LN, -196°C). At this temperature, all cellular activities cease, and theoretically, plant materials can be stored without any changes for an indefinite period. In addition, we keep the plant materials away from any disease or pest, preserving the clean mother stock for future purpose.

## Results in 2016 (Raspberry)

### Tissue culture

Optimized medium [Murashige and Skoog medium supplemented with 24 mg/l extra Fe<sup>2+</sup>, 0.5 mg/BAP, 0.1 mg/L IBA, 30 g/L sucrose and 6 g/L Agar] was developed for efficient tissue culture of three raspberry cultivars: 'Glen Ample', 'Preussen' and 'Ninni'.

Tissue culture of raspberry is the first step to prepare for cryopreservation

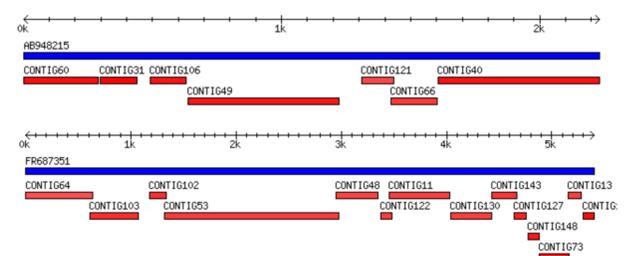


### Cryopreservation

Droplet-vitrification method has been tested on 'Glen Ample', 'Preussen' and 'Ninni', different parameters including pre-treatment and period of loading and vitrification should be further optimised.

## Next Generation Sequencing

Total RNA was extracted with Norgen Plant/Fungi RNA kit (Norgen Biotek, Canada) from fresh young leaf tissues (50 mg) of a new cultivar. Small RNA library was prepared and sequenced with Illumina HiSeq 2500 (Fasteris SA, Switzerland). Sequencing results have been analysed with VirusDetect (<http://bioinfo.bti.cornell.edu/cgi-bin/virusdetect/index.cgi>). Pollen-transmitted Raspberry bushy dwarf virus (RBDV) was discovered. DAS-ELISA with antiserum (Bioreba, Switzerland) has been used to confirm RBDV infection and RT-PCR with specific primers will be applied further on.



Raspberry bushy dwarf virus genomic RNA has been detected with 97% coverage of RNA 1 (top) and 92.9% coverage of RNA 2 (lower), and identity of 95.57% and 98.18%, respectively.

## Survey

Symptomatic raspberry samples (leaves or roots) were collected from fields in separate regions.

Project name:

«Diagnosis, virus cleaning and cryopreservation of raspberry, blackberry and shallot (Rub&AI)»

Years: 2016-2019

Total Budget: NOK 4 300 000

Financed: The Norwegian Research Council (80 %) and Industry (20 %)

Project leader: Dr. Dag-Ragnar Blystad (NIBIO)

Norwegian partners

- NIBIO – Norwegian Institute of Bioeconomy Research
- Sagaplant
- The Norwegian Genetic Resource Centre
- The Norwegian University of Life Sciences
- Plants Forever
- Gartnerhallen
- Gartnerforbundet

International partners:

- James Hutton Institute (JHI), Scotland
- Bioversity International (BI), Belgium
- Crop Research Institute (CRI), Czech Republic
- Northwest Agriculture and Forestry University (NAFU), Yangling, China