Virus-free raspberry ´Ninni´ by combined thermotherapy and cryotherapy

NIBIO
NORWEGIAN INSTITUTE OF BIOECONOMY RESEARCH

Peter van der Ende[1], Zhibo Zhang[2], Øyvor Stensbøl[1], Dag-Ragnar Blystad[2]

Background

In Norway, raspberry is an economically important crop of increasing importance the last 10 years. Raspberry is vegetatively propagated, and is often infected with several viruses that cause significant economic losses. A prerequisite for long-term development and production of this crop is virus diagnosis, virus elimination, and preservation of healthy mother stock of important cultivars.

Raspberry bushy dwarf virus (RBDV) is one of the viruses commonly infecting raspberries (*Rubus idaeus* L.). RBDV is effeciently transmitted via seed and pollen (Murant *et al.* 1974). RBDV can induce yellow desease, crumbly fruit, and rapid degeneration and bushy dwarf symptoms (Jones *et al.*, 1996). The aim of the present study was to produce RBDV-free materials of raspberry 'Ninni' by combination of thermotherapy and cryotherapy.

Raspberry with crumbly fruits

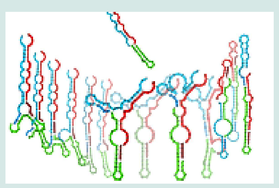
Cultivar 'Veten' was tested in 155 fields in Norway, RBDV were found in more than 50 fields. (Marianne Mittet, master thesis, 1994)

Methods

Next Generation Sequencing (NGS)

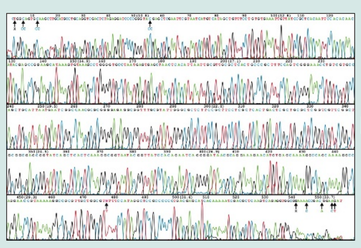
Fresh tissues

Total RNA

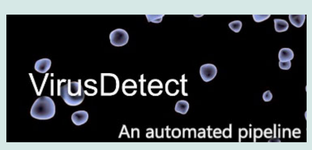


Small RNA sequencing

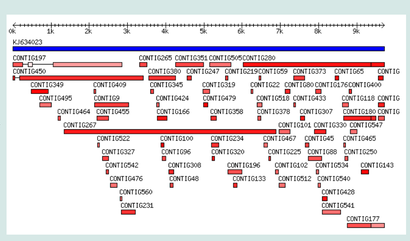
Illumina Hi-Seq 2500



Sequencing data



VirusDetect



Outputs

Thermotherapy and Cryotherapy

4-week-old stock plants
MS+24 mg/l extra Fe^{2+}+0.5 mg/BAP+0.1 mg/L IBA+30 g/L sucrose+6 g/L Agar

Thermotherapy
16-h photoperiod, 38 °C, 30 days

Cryotherapy

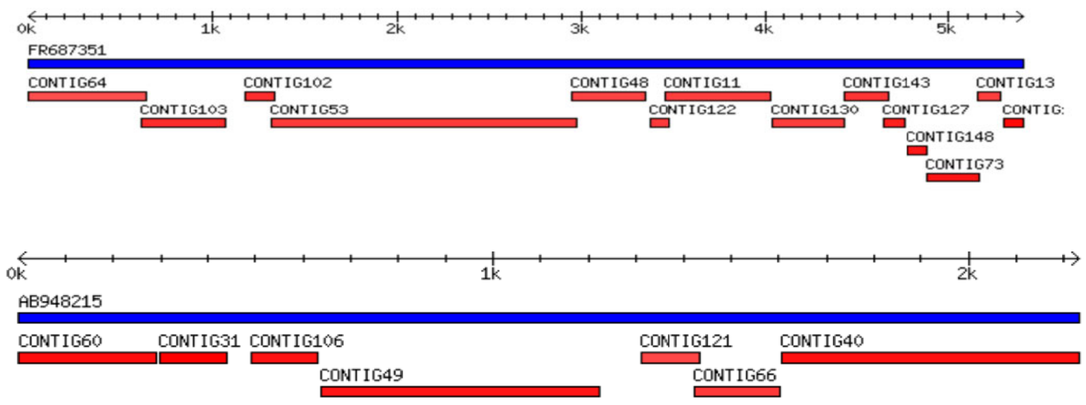
- 1 mm shoot tips stabilized on MS medium containing 2.5 g AC/L for 2-3 days
- Preculture with sucrose concentrations 0.25, 0.5 to 0.75 M, each for 24h
- Loading with 2 M glycerol and 0.5 M sucrose for 20 minutes
- PVS2 for 20min at room temperature
- Liquid nitrogen for 1h
- Thawing with 1.2 M sucrose for 20min
- Culture with MS-medium with 0.75 M sucrose for 2-3 days before regeneration.

Virus detection: Small plants (4 weeks old) of 'Ninni' were transferred directly to soil and grew in a quarantine controlled greenhouse for 6 weeks before virus detection by ELISA and RT-PCR.

Results

Pollen-transmitted RBDV was discoverd by NGS.
DAS-ELISA with antiserum (Bioreba, Switzerland) and RT-PCR with specific primers have been applied to confirm RBDV infection.

Reference	Length	Coverage (%)	#Contig	Depth	Depth (Norm)	% Identity	% Identity Max	% Identity Min	Genus	Description
FR687351	5402	5241 (97)	14	375.1	26.3	95.57	99.07	93.73	Ideaovirus	Raspberry bushy dwarf virus gene for non-structural polyprotein, isolate BY8, genomic RNA.
AB948215	2231	2073 (92.9)	7	186.4	13.1	94.40	100	94.40	Ideaovirus	Raspberry bushy dwarf virus genomic RNA, segment: RNA2, complete sequence, isolate: J1



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

A total of 120 shoot tips were used in thermotherapy and cryotherapy experiment, and 44 (36,7%) of them regenerated afterwards, and 13 (29,5%) of regenerated plants were RBDV-free after testing.

References

Murant, AJ, Chambers, J and Jones AT (1974) Spread of raspberry bushy dwarf virus by pollination, its association with crumbly fruit, and problems of control. Ann. Appl. Biol. 77, 271–281.
Jones AT, Mayo MA, Murant AF (1996) Raspberry bushy dwarf idaeovirus. In: Harrison BD, Murant AF (eds) The plant viruses, vol 5, polyhedral virions and bipartite RNA genomes. Plenum Press, New York, pp 283–301.
Wang QC, Cuellar WJ, Rajamaki M-l, Hirata Y, Velkomen JPT (2008) Combined thermotherapy and cryotherapy for efficient virus eradication: relaion of virus distribution, subcellular changes, cell survival and viral RNA degradation in shoot tips. Molecular Plant Pathology 9(2), 237-250.

Acknowledgement

The financial support of The Norwegian Research Coucil (grant nr 255032), NIBIO, The Norwegian Genetic Resource centre, and private companies are higly appreciated.

nibio.no

PO Box 115, N-1431 Ås, Norway
+47 406 04 100

[1] Sagaplant AS, Prestegardsvegen 17, 3812 Akkerhaugen
[2] Norwegian Institute for Bioeconomy, Norway,

Sagaplant Friske planter