Prebreeding for future challenges in Nordic apples ‘Nordapp’

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Prebreeding for future challenges in Nordic apples-Nordapp in 2012–2014

Prebreeding for future challenges in Nordic apples – 2\textsuperscript{nd} step in 2015–2017

SLU (Se) + Graminor (No) + LUKE (Fi)

..... improve the development of apple varieties that are suitable for Nordic conditions.....

..... good winter hardiness combined with high quality, consistent yields, resistance to pests and diseases, and good storage performance ..... 

..... a common platform, with sharing of methods and plant material among Nordic breeders and scientists .....
**WP 1: Nordic-Baltic Platform** (LUKE)
Nordic-Baltic platform webpage, joint workshops.

**WP 2: Information - networking** (Graminor)
Survey about diseases, yearly project workshops (incl. also external experts and other colleagues), NordApp webpage.

**WP 3: Canker** (SLU)
Inoculations of shoots and trees with spores of *Neonectria ditissima*, qPCR analyses of wood samples, crosses with canker-tolerant germplasm.

**WP 4: Storage rots** (SLU)
Inoculations with *Penicillium* and other fungi, crosses with storage-disease tolerant germplasm.

**WP 5: DNA markers** (SLU)
Germplasm identification and estimation of relatedness with SSR analyses, information on candidate genes for traits of interest (co-operation with e.g. Fruitbreedomics).

**WP 6: Information dissemination** (Graminor)
Papers and posters for scientists, meetings and workshops for stakeholders
WP 1: Nordic-Baltic Platform (LUKE)
Nordic-Baltic platform webpage

WP 2: Information - networking (Graminor)
NordApp webpage https://sites.google.com/a/nordgen.org/ppp-apples/

WP 6: Information dissemination (Graminor)
Papers and posters for scientists, meetings and workshops for stakeholders: check out our web pages!
Caused by *Neonectria ditissima*.

One of the most devastating diseases in modern apple orchards in Southern Sweden, Norway and Åland.

Attacks the fruit and – most importantly – can kill the whole tree.
Phenotyping of apple canker susceptibility: screening of inoculated cut shoots (SLU, Graminor) and young trees (SLU, LUKE) in greenhouse, using old Nordic cvs as well as modern breeding material and some advanced selections.

Study how histopathology of infected tissue varies between cultivars with different levels of susceptibility.

Analyse genetic diversity in *Neonectria ditissima* using SSR and AFLP markers.

International workshop on apple canker and storage rots at Alnarp, SLU in October 2014.

Within-project workshops/meetings about establishing production of *Neonectria* spores, inoculation procedures, symptom evaluations.

Making crosses with superior genotypes.
Resistance against fruit tree canker in apple
Evaluation of Disease Symptoms, Histopathological and RNA-Seq Analyses in Different Cultivars, Genetic Variation of Neonectria ditissima

PhD student: Marjan Ghasemkhani

Main supervisor: Prof. Hilde Nybom
Co-Supervisors: Prof. Erland Liljeroth
Dr. Larisa Gustavsson
Dr. Salla Marttila
Phenotyping by inoculation of manually inflicted ‘leaf scars’ on cut shoots. Resulting lesions are measured every 5 days during 6–8 weeks.
Canker tolerance estimated in cvs inoculated at SLU
One-year-old tree: cortex and pith cells, 110 dai

Susceptible cultivar

Healthy tissue

Resistant cultivar

From Marjan Ghasemkhani’s dissertation presentation
A principal coordinate analysis (PCoA) was performed with SSR data. The isolates were loosely grouped and there was no association with the geographic origin. Similar results were obtained when a PCoA was calculated with AFLP data. Isolates from different trees in the same orchard, or from trees grown in geographically close orchards were not grouped together.

From Marjan Ghasemkhani’s dissertation presentation
WP 4: Storage rots

Fungal diseases attack fruit, in the field and during harvest. Damage is not visible until during or after storage, causing serious economical losses.

Many fungal species, e.g. *Penicillium expansum* (produces patulin), *Neofabraea alba* and *N. perennans*, *Colletotrichum acutatum*. 
Phenotyping of storage rot susceptibility: inoculation of fresh fruit and evaluation of resulting damage after storage, by all three partners, using a wide set of germplasm (old Nordic cvs as well as modern breeding material and advanced selections).

(Analyses of chemical contents in flesh and peel of inoculated and control fruits of 20 cvs).

(Gene expression study of 2 susceptible and 2 resistant cvs).

International workshop on apple canker and storage rots at Alnarp, SLU in October 2014.

Within-project workshops/meetings with practical demonstrations of establishing spore colonies, inoculation procedures, symptom evaluations.

Making crosses with superior genotypes.
Inoculation with *P. expansum* at Graminor

Medium sensitive cv ‘Zonga’

Very resistant cv ‘Fu Shuai’

Crosses with ‘Fu Shuai’ will be made in 2017.
Lesion diameter/storage week after inoculation with *Penicillium expansum* at SLU.

The most resistant cultivars were, overall, late-ripening and had firm fruit flesh.
Cultivars analysed for susceptibility to *Penicillium* at Graminor

DI = decay index: lesion diameter/weeks in storage
Soft = loss of fruit firmness during storage
Fir = fruit firmness at harvest
Cultivars analysed at SLU

DI = decay index: lesion diameter/weeks in storage
Soft = loss of fruit firmness during storage
Fir = fruit firmness at harvest
PhD student: Masoud Ahmadi-Afzadi

Main supervisor: Prof. Hilde Nybom
Co-Supervisors: Dr. Kimmo Rumpunen, Dr. Ibrahim Tahir, Dr. Anna Holefors
Results
Biochemical analysis of 20 cvs

- Total titratable acidity (TTA) and malic acid (MA)
- Total phenolic compounds (TPH)
- Individual polyphenolic compounds:
  - 10 individual compounds: CHL, PB2, EPI, QRU, QGA, QGL, QXY, QAR, QRH, PHL

Most important correlations:
TPH, PB2 and all the quercetins were negatively correlated with lesion diameter in the peel of inoculated fruit = protection?
Results
Gene expression analysis: 2 susceptible and 2 resistant cvs inoculated with *Penicillium* – genes expressed 1 week or 6 weeks later in inoculated tissue and in control tissue were compared.

Validation of a candidate gene that induces protection against *Penicillium* by production of the plant hormone methyljasmonate

From Masoud Ahmadi-Afzadi’s dissertation presentation
Neofabraea (bull’s eye rot, Pezicula-rot)

Often the most common storage rot in all Nordic countries.

Usually attacks through the lenticels.
Wound inoculation (deposit spores in a hole or mycelium under the skin) detects spreading within fruit – but how can we determine infection ability?
WP 5: DNA markers

Identification of sampled material – Swedish and Norwegian cvs used within NORDAPP have been genotyped with 10 SSR loci, profiles have been verified by comparison with other databases. Samples of Finnish cultivars will be analysed in 2017.

Co-operation with Fruitbreedomics: samples of c. 140 Nordic apple cultivars included in large SSR-based study of genetic variation in 1859 cultivars from 14 gene banks. Parentage analysis.
Three somewhat differentiated gene pools were identified: Northeast (blue), West (green) and South (red).
Step 1: Identification of valuable germplasm
Step 2: Identification of valuable genes

Which are the potentially important genes in e.g. ‘Särsö’?
Co-operation with Fruitbreedomics: 160 apple cultivars in the Balsgård-SLU gene bank were included among 1168 cultivars from 6 gene banks, analysed with 480,000 SNPs on a microarray.

Genome Wide Association Studies of flowering and fruiting phenology, fruit quality and resistance to *Penicillium*.

Adaptation to climate: average flowering and fruiting is much earlier for SLU cultivars compared to other gene banks (Angers in France being the latest). Associated chromosomal locations determined and candidate genes suggested.

GWAS also attempted for *Penicillium* resistance.

Analyses (in NZ) with selected loci for genes of potential value for *Penicillium* resistance.
SLU: Ibrahim Tahir, Jasna Sehic, Hilde Nybom, Larisa Gustavsson

Graminor: Dag Røen

LUKE: Tuuli Haikonen, Saila Karhu