
APPLICATION OF MOLECULAR GENETICS METHODS FOR FRUIT CROP GENETIC RESOURCES CHARACTERIZATION IN LATVIA

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FRUIT CROP GENETIC RESOURCES

- ✘ A diversity of fruit crop varieties is maintained at the Latvia State Institute of Fruit-Growing genetic resources collection, which consists of landraces and selections of local breeding as well as germplasm that result from years of scientific exchange and co-operation.
- ✘ Presently germplasm collection comprises about **2500 accessions** of **17 fruit crops**
- ✘ **676 accessions** of fruit crops are designated as **national genetic resources**.
- ✘ **Main activities:**
 - + acquisition
 - + maintenance
 - + characterization
 - + utilization



FRUIT CROP GENETIC RESOURCES

Crop	Number of Accessions	Number of National GR Accessions	Number of Genotyped Accessions
Apple	1061	283	157
Pears	405	101	40
Plums: domestic	151	45	108
diploid	152	25	11
Cherries: sweet	170	40	170
sour	62	33	50
Black currants	144	38	118
Red and white currants	30	16	8
Gooseberries	109	23	26
Raspberries	71	11	43
Sea buckthorn	36	-	36
Strawberries	13	2	23*
Apricots	35	35	-
Peaches	2	2	-
Grapes	26	14	-
Japanese quince	40	6	-
Honeysuckle	2	2	-
Total:	2509	676	790

* Strawberry accessions from Pūre HRC collection included in characterization

FRUIT CROP GENETIC RESOURCES

- ✘ Conservation of germplasm itself has a little value without characterization and further utilization of the stored plant material.
- ✘ Characterization of germplasm:
 - + Phenotypical
 - + Genetical – based on molecular markers
- ✘ **Why DNA (molecular) markers?**
 - + Not subject to environmental influences
 - + Potentially unlimited in number
 - + Objective measure of variation

MOLECULAR MARKERS IN PGR MANAGEMENT

× Acquisition:

- + identifying gaps and improving composition of collection
- + improving sampling strategies

× Maintenance:

- + measuring and reducing genetic drift/shift
- + observing contamination, detection of duplication, control of regeneration quality
- + definition of regeneration priorities

× Characterization:

- + fingerprinting / diversity studies

× Utilization:

- + creation of core collections
- + allele mining – basis for Marker Assisted Selection

MOLECULAR MARKERS IN PGR MANAGEMENT

× Molecular markers utilised for PGR characterization:

+ Non-specific molecular markers:

- × Based on unspecific (not linked to particular trait) sequences of DNA.
- × Provide general genetic characterization.

+ Specific molecular markers:

× Markers are linked to particular gene:

- ★ agronomically important traits,
- ★ resistance to various pathogens.



MOLECULAR MARKERS IN PGR MANAGEMENT

Crop	Implemented marker type	
	Non-specific	Specific
Apple	SSR, MSAP	Scab resistance gene markers: Vd(Rvi13), Vh2(Rvi2), Vh4(Rvi4), Vr2(Rvi15), Vbj(Rvi11), Vf/Vjh(Rvi6), Vb(Rvi12), Vm(Rvi5), Disease Resistance Gene Analogs markers
Pears	SSR, MSAP	Disease Resistance Gene Analogs markers
Plums: domestic	SSR	Self-incompatibility gene (<i>Sf</i>) markers
diploid	SSR	Self-incompatibility gene (<i>Sf</i>) markers
Cherries: sweet	SSR	Self-incompatibility gene (<i>Sf</i>) markers
sour	SSR	-
Black currants	SSR	-
Red and white currants	SSR	-
Gooseberries	SSR	-
Raspberries	SSR	-
Sea buckthorn	SSR, RAPD	-
Strawberries	SSR, RAPD	Disease Resistance Gene Analogs markers

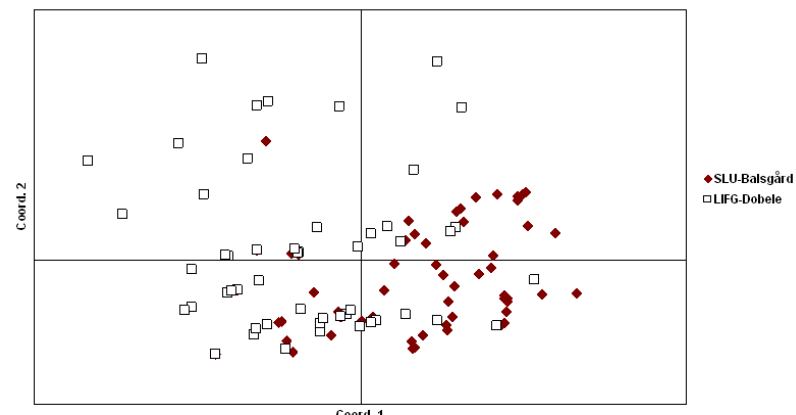
NON-SPECIFIC MOLECULAR MARKERS

- ✘ Description of genetic resources was started by the selection of the most appropriate markers, implementation of methods.
- ✘ ECPGR WG developed crop specific marker lists
- ✘ Adapted non-specific marker methods have been applied in:
 - Identification of genotypes – detection of duplication in the collection
 - Evaluation of collection genetic diversity and structure
- ✘ Collection-wide genotyping using non-specific markers performed for:
 - Sweet and sour cherries
 - Apples
 - Black currants
 - Red and white currants
 - Gooseberries
 - Raspberries
 - Sea buckthorn

SWEET CHERRIES



- ✘ **25 SSR markers**, using ECPGR Prunus WG recommended marker set and additional highly polymorphic markers
- ✘ **170 sweet cherry accessions** have been genotyped
- ✘ obtained data used in the creation of molecular identity profiles
- ✘ This information is **useful in breeding programmes** for the planning of crosses and conservation of alleles.
- ✘ Acquired marker information has been included in the genetic resources data base and could be provided for the **international data exchange** systems.



Distribution of accessions of two sweet cherry collections according to values of two main principal coordinates calculated on the base of allele composition in three SSR loci

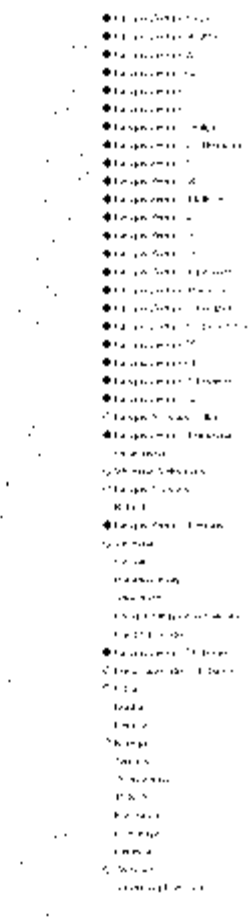
(Lacis et al., 2009)

SOUR CHERRIES

- ✘ **26 SSR markers**, using ECPGR Prunus WG recommended marker set and additional highly polymorphic markers
- ✘ **50 sweet cherry accessions** have been genotyped
- ✘ The ECPGR marker set showed good applicability on new *Prunus* species – tetraploid *Prunus cerasus*.
- ✘ The correspondence of accession grouping based on SSR data and known pedigree confirm the reliability of applied markers.
- ✘ Discrimination of closely related clones of local landraces



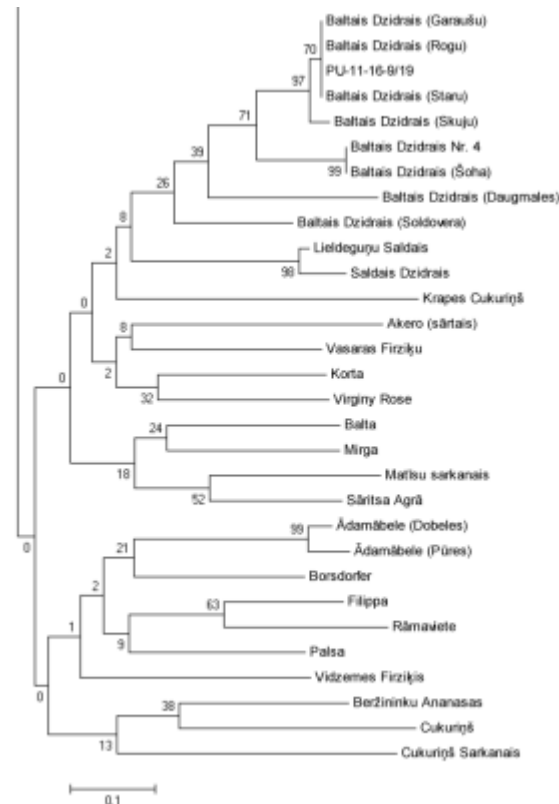
Dendrogram of sour cherry accessions constructed using UPGMA method based on Nei's genetic identity
 Origin: ○ - Russia, □ - Sweden, ◇ - Latvia (◆ - clones of Latvijas Zemais), △ - Germany
 Numbers indicate the percentage of bootstraps in which the branch was observed.
 (Lacis, Kota, 2011)



APPLES



- ✘ **7 SSR markers**, using ECPGR Prunus WG recommended marker set
- ✘ **129 sweet cherry accessions** have been genotyped
- ✘ Cluster analysis based on the SSR genotyping data did not reveal a clear pattern with well-defined cultivar groups, but confirmed some relationships based on known or putative pedigrees, as well as suggesting the possible parentage of some cultivars.
- ✘ Genotyping results, together with known pedigree data, helped to suggest the possible parentage of several local cultivars.



Dendrogram of relationships discovered by SSR markers in early apple cultivars and 'Baltais Dzidrais' clone group constructed using Nei and Li/Dice similarity index and Neighbour-Joining clustering method

(Ikase, Lācis, 2011)

BERRY CROPS (Ribes)



Core collection of Northern European gene pool of Ribes – RIBESCO

- ✘ **8 Northern European countries**
- ✘ **846 Ribes accessions:**
 - ✘ 400 black currants (*Ribes nigrum*),
 - ✘ 202 red and white currants (*Ribes rubrum* group),
 - ✘ 242 gooseberries (*Ribes uva-crispa*),
 - ✘ 2 jostaberries (*R. nigrum* x *R. uva-crispa*).
- ✘ **6 SSR markers**
- ✘ **257 Ribes accessions from Latvia**
- ✘ **Core collection:**
 - ✘ 64% of black currant alleles
 - ✘ 80% of red and white currants alleles
 - ✘ 61% of gooseberries alleles

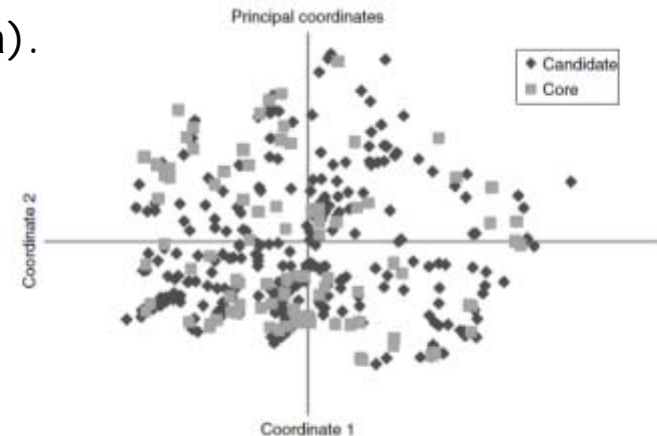
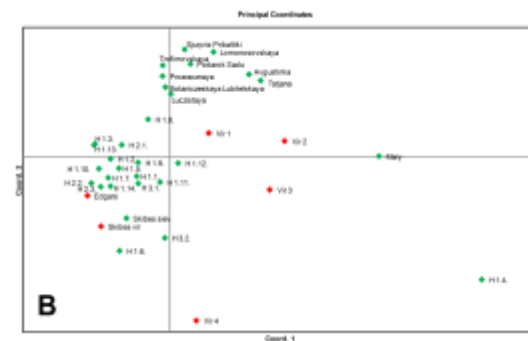
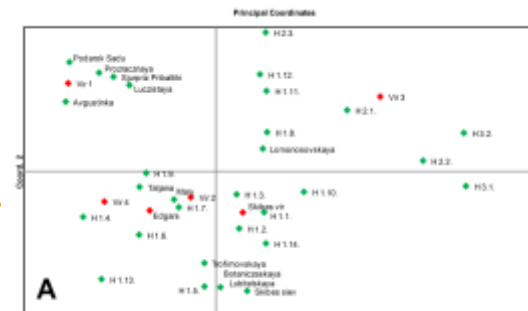


Fig. 1. PCoA of the RIBESCO black currant core collection (Core) and the non-selected candidate cultivars (Candidate).
(Antonius et al., 2012)

SEA BUCKTHORN



- ✘ **36 accessions, 8 SSR** and **16 RAPD** markers
- ✘ Selected sets of SSR and RAPD markers ensured high level of polymorphism and are suitable for application in different *H. rhamnoides* subspecies, as well as in crosses among *H. rhamnoides ssp. mongolica*, *ssp. rhamnoides* and *ssp. fluviatilis*.
- ✘ More adequate grouping of sea buckthorn accessions according geographical origin and gender was for RAPD markers.
- ✘ Further sea buckthorn plant material evaluation should be performed using both marker types, because SSR's are species sequence specific markers, which ensure higher repeatability and transformability of data.



Distribution of sea buckthorn accessions according to two main principal coordinates.

- A - SSR genotyping data
- B - RAPD genotyping data
- C - SSR and RAPD genotyping data
- ◆ - sea buckthorn male plants
- ◇ - sea buckthorn female plants

SPECIFIC MOLEULAR MARKERS

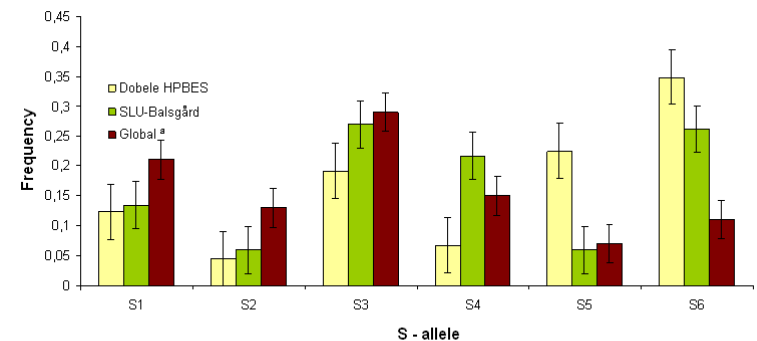
- × **Molecular markers specific for particular traits:**
 - + **Self-incompatibility in *Prunus*:**
 - × sweet cherries
 - × plums
 - + **Disease resistance:**
 - × apple and pear scab (caused by *Venturia inaequalis* and *Venturia pirina*)
 - × strawberry root rot and petiole blight (caused by *Gnomonia fragariae*)



SELF-INCOMPATIBILITY GENE (*Sf*)

Sweet cherries

- ✘ **Self-incompatibility gene (*Sf*) genotyping:**
 - + Research on *Sf* gene allele distribution, their inheritance,
 - + Additional identification tool,
 - + Basis for MAS (Marker Assisted Selection) – application in the self-compatible sweet cherry cultivar breeding.
- ✘ **147** sweet cherry accessions have been *S*-genotyped based on consensus and allele specific markers.
- ✘ Markers of self-compatibility allele *S4'* implemented in Marker Assisted Breeding).



S-allele frequencies in accessions of the Dobele HPBES and the SLU-Balsgård sweet cherry collections (a Allele frequencies calculated from S-allele identification data published by Tobutt et al. (2004). Standard error bars shows the 0.95 confidence level ($P < 0.05$))

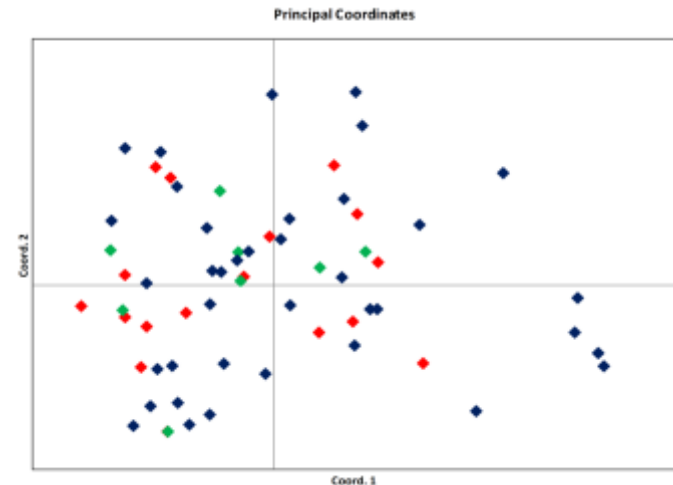
(Lacis et al., 2008)



SELF-INCOMPATIBILITY GENE (*Sf*)

Plums

- ✘ Application of molecular markers developed in different *Prunus* species for plum *Sf* genotyping.
- ✘ Consensus primers flanking the first and second intron of the *S*-RNase gene and the SFB intron specific primers have good applicability.
- ✘ **99 plum accessions** genotyped: 7 diploid and 92 hexaploid plums
- ✘ Future development - detection of plum self-incompatibility groups by allele specific genotyping.



PCA distribution of plum cultivars according to self-compatibility, based on *S*-genotyping.

- ◆ - self-compatible plums
- ◆ - partly self-compatible plums
- ◆ - self-incompatible plums

(Kota, Lācis, 2012)

RESISTANCE TO PATHOGENS

- × **Resistance gene specific molecular markers:**
 - + Scab resistance genes in apple.
- × **Markers linked to general plant defence mechanisms:**
 - + Resistance Gene Analogs:
 - × Pears – resistance to pear scab (*Venturia pirina*)
 - × Strawberries – resistance to root rot and petiole blight (caused by *Gnomonia fragariae*)

RESISTANCE TO PATHOGENS

✗ Resistance gene specific molecular markers:

+ Scab resistance *Vf* gene in apple:

✗ **109 apple accessions,**

✗ ***Vf* gene marker,**

✗ 1, 6 and 102 genotypes of *VfVf*, *Vfvf*, *vfvf* detected.

+ Scab resistance genes in apple:

✗ *Vh2*(*Rvi2*), *Vh4*(*Rvi4*),
Vm(*Rvi5*), *Vf/Vjh*(*Rvi6*),
Vbj(*Rvi11*), *Vb*(*Rvi12*),
Vd(*Rvi13*), *Vr2*(*Rvi15*)

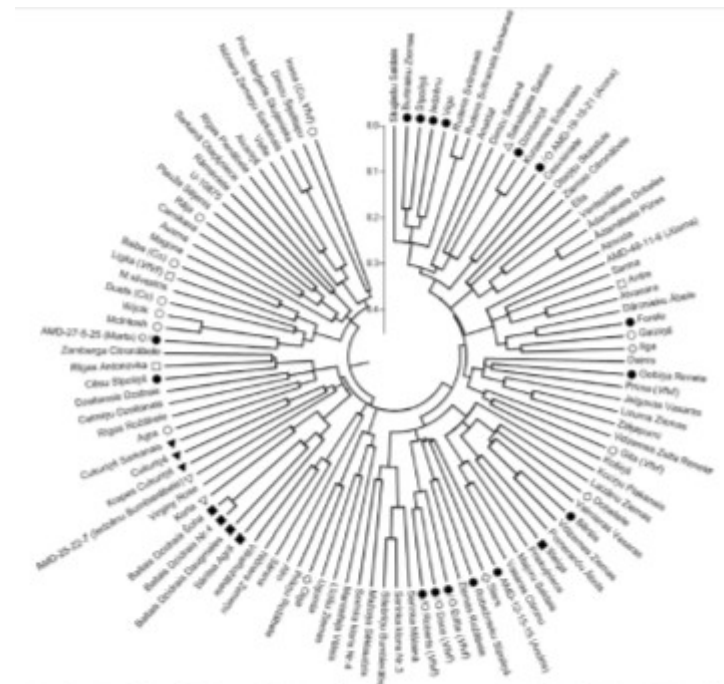


Fig. 1. Genetic relatedness dendrogram of Latvian apple genetic resources accessions. Group of origin: ○, 'McIntosh'; ●, 'Stipolst'; ■, 'McIntosh' and 'Stipolst'; □, 'Antonovka'; ▲, 'Baltas Dzūlais' (Yellow transparent); △, 'Revels Bumberstiefele' ('Kessler Bismarck'); ▲, 'Cakung' ('Korobonka'); ○, 'Delicious'.

(Lacis et al., 2011)

RESISTANCE TO PATHOGENS

- × **Markers linked to general plant defence mechanisms:**
 - + Resistance Gene Analogs:
 - × **Nucleotide Binding Site – Leucine-rich Repeat (NBS-LRR)** proteins genes
- × **Scab resistance in pears:**
 - × Selection of resistant and susceptible pear accessions
 - × Application of molecular markers specific to NBS-LRR conservative sequences
 - × Analysis of variability
- × **Resistance to root rot and petiole blight in strawberries:**
 - × Selection of resistant and susceptible strawberry accessions
 - × Development of mapping population
 - × Application of molecular markers specific to NBS-LRR conservative sequences
 - × Analysis of variability

CONCLUSIONS

- ✘ **Molecular genetic methods provide effective fruit genetic resources collection characterization**
- ✘ **Obtained genotyping information is important for successful and optimal maintenance of genetic resources collections, dissemination of information about Latvian fruit crop genetic resources internationally, as well as to improve and intensify the breeding program**
- ✘ **Developed and implemented by molecular genetics methods provide data for international information exchange, their deployment in genetic resources databases**
- ✘ **Molecular marker technology has a great potential to improve PGR management and utilization in future**

THANK YOU FOR YOUR ATTENTION!

