



**ANALYSIS OF GENETIC DIVERSITY
OF SELECTED *RIBES* GENOTYPES IN THEIR
RESISTANCE TO BLACKCURRANT GALL MITE
USING SSR MARKERS**



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RIBES – SCIENTIFIC CLASYFICATION

DOMANIAN: EUKARYA
KINGDOM: PLANTAE

SUPERPHYLUM: SPERMATOPHYTE
PHYLUM: MAGNOLIOPHYTA

FAMILY: GROSSULARIACEAE
GENUS: ***RIBES***

SPECIES: *Ribes nigrum* (Blackcurrant)
Ribes odotatum (Red Currant)
Ribes uva-crispa (Goosberry)

.....



HEALTH BENEFITS OF *RIBES*

RICH IN

**VALUABLE FOR HEALTH PHYTO-NUTRIENTS
AND ANTI-OXIDANTS**



- **ANTHOCYANINS**
- **VITAMIN-C**
- **VITAMIN-A**
- **B-VITAMINS** (PANTOTHENIC ACID – VITAMIN B5,
PYRIDOXINE – VITAMIN B6, THIAMIN – VITAMIN B1)
-

***RIBES* CULTIVATED FOR:**

**FRESH FRUITS
AND
PROCESSING INTO**

**JUICE AND BEVERAGE, JAMS
JELLIES, YOGHURTS, PUREES, TEAS**

FUNCTIONAL FOOD PRODUCTS



***RIBES* PRODUCTION IS VERY IMPORTANT**



BLACKCURRANT BUD GALL MITE

Cecidophyopsis ribis (Westw.)

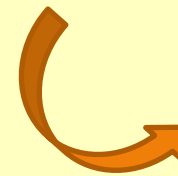
ONE OF THE MOST IMPORTANT PEST OF CULTIVATED *RIBES*

- **INFECTS NEW BUDS THROUGHOUT THE BLOSSOM PERIOD**
- **CAUSES BUD DAMAGING ('BIG BUD')**



LIVES INSIDE THE DORMANT BUDS SUCK SAP FROM EMBRYONIC LEAVES

CAUSES INFESTED BUDS TO BECOME ABNORMALLY SWOLLEN AND ROUNDED



CECIDOPHYOPSIS RIBIS* – VECTOR OF *BRV **(BLACKCURRANT REVERSION VIRUS)**

**EUROPEAN
form (E)**

**RUSSIAN
form (R)
(more severe than E)**



**STERILITY OF
FLOWERS**

**REDUCE YIELD
of blackcurrant plants**

VARIETIES RESISTANT TO *BRV*
R. nigrum sibiricum, R. bracteosum
R. cereum, R. carrierieriei
R. dikuscha

**CULTIVARS WHICH ARE RESISTANT TO *BRV* ARE NOT ALWAYS
RESISTANT TO GALL MITE**
(e.g *Ribes dikuscha*)

CURRENT KNOWLEDGE - GALL MITE RESISTANCE

TWO GENES OF RESISTANCE & THE SOURCE OF THEM:

Ce – *R. grossularia*

P – *R. nigrum sibiricum*

?????????

PYRAMIDING OF RESISTANCE

Ce RESISTANCE IS MUCH MORE EFFECTIVE AGAINST GALL MITE INFESTATION THAN **P RESISTANCE**

TRAITS INTRODUCED BY INTERSPECIFIC
HYBRIDIZATION MAY BE LOST
(BACKCROSSES WITH *R. nigrum* CULTIVARS)

THERE ARE NOT EFFECTIVE CONTROL OF THE PEST
RESISTANT CULTIVARS ARE DESIRED

IDENTIFICATION OF MITE-RESISTANT PLANTS

**PHENOTYPIC
DATA**

(from field observations
or infestation plots)

TIME-CONSUMING!



MOLECULAR METHODS – MARKERS

EARLY DIAGNOSTIC SYSTEM



**SELECTION OF RESISTANT
GENOTYPES**

- **IN ALL PHASES OF PLANT
DEVELOPMENT**
- **WITHOUT INFESTATION**



THE AIM OF STUDY

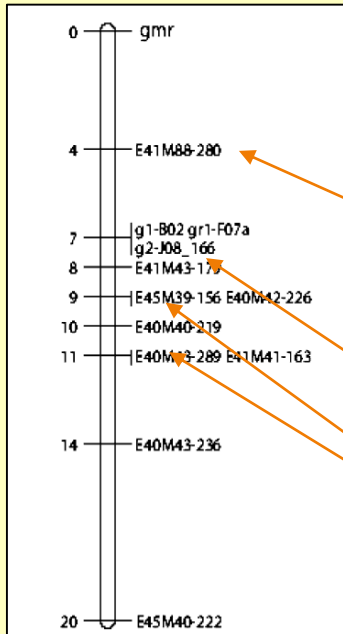
SSR-BASED COMPARISON OF *RIBES* GENOTYPES DIVERSED IN THEIR RESISTANT TO GALL MITE TO SELECT ALL FRAGMENTS LINKED TO ANALYSED TRAIT

LINKAGE MAPS IN OUR STUDY...

SCOTTISH

Brennan et al. 2008

(SCRI S36/1/100 × EMRS B1834)



**Ce RESISTANCE
MAPED TO LG2**

AFLP
(linked to gall
mite resistance)

SSR

EST



GMresa (130 bp)

**AMPLIFIED
ONLY IN THE
RESISTANT PLANTS**

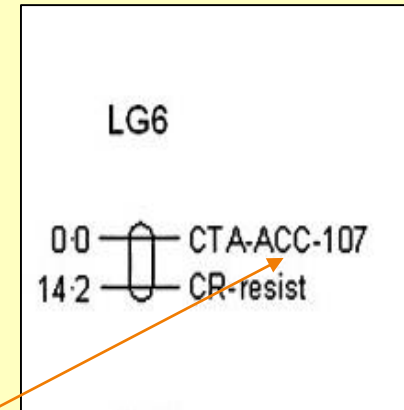


LITHUANIAN

Mazeikine et al. 2012

(No 93-169-2 × 'Dainiai')

**P RESISTANCE
MAPED TO LG6**



AFLP

(directly used to identify
resistance in blackcurrant
germplasm)

MATERIAL

15 *RIBES* GENOTYPES (young leaves)

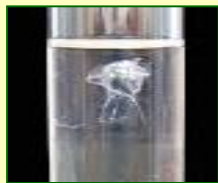


Lp.	VARIETY	PEDIGREE	COUNTRY OF ORIGIN
1.	Tisel	Titania x selfed	Poland
2.	Ruben	Bieloruskaja Słodkaja x Ben Lomond	Poland
3.	Ben Hope	Westra x (SCRI 238/36 x EM 21/15)	Scotland
4.	Ben Gairn	Ben Alder x Golubka	Scotland
5.	Kroma	unknown	Germany
6.	Gofert	Golubka x Fertodi 1	Poland
7.	Lentaj	Brodtorp x Minai Shmyrev	Russia
8.	Dlinnokistnaja	Primorsky Champion x Boskoop Giant	Russia
9.	Tihope	Titania x P9/11/14	Poland
10.	Vir	unknown	Russia
11.	Blizgiai	Öjebyn' x 'Minaj Shmyriov	Lithuania
12.	Kerry	Black Naples x selfed	Canada
13.	Ben Sarek	(Goliath x Ojebyn) O.P.	Scotland
14.	Ben Alder	Ben More x Ben Lomond	Scotland
15.	Ben Kilbreck	(Ben More x C2/13/15) X (Ben More x RI-74020-16)	Scotland

METHODS

DNA EXTRACTION

(Doyle & Doyle, 1990)



DNA QUANTIFICATION

(Amersham Pharmacia Biotech's Gene Quant pro Spectrofotometer)



PCR AMPLIFICATION (25 SSR PRIMERS)

Datebase mapping: Brennan *et al.*
2008, Mazaikine *et al.* 2012)



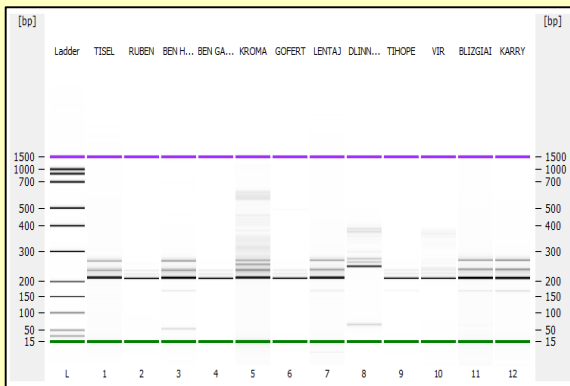
WISUALIZATION OF AMPLIFIED PRODUCTS

(Bioanalyzer Agilent 2100)

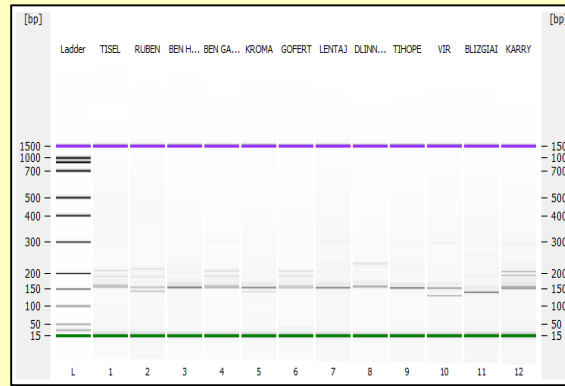


RESULTS

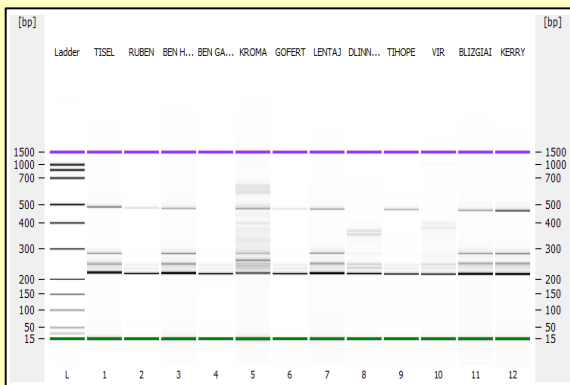
PCR PRODUCTS DIFFERENTIATING THE VARIETIES - examples



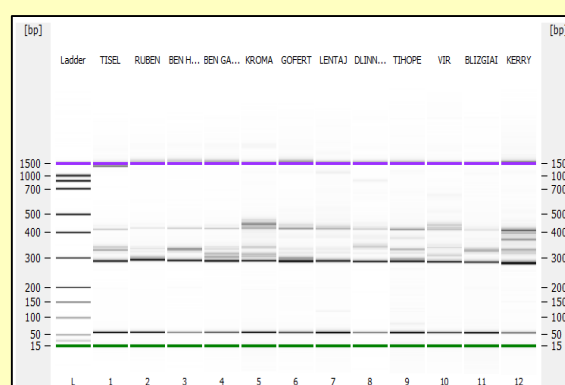
Primer g1-A01



Primer e1-O21



Primer Fa11



Primer M32

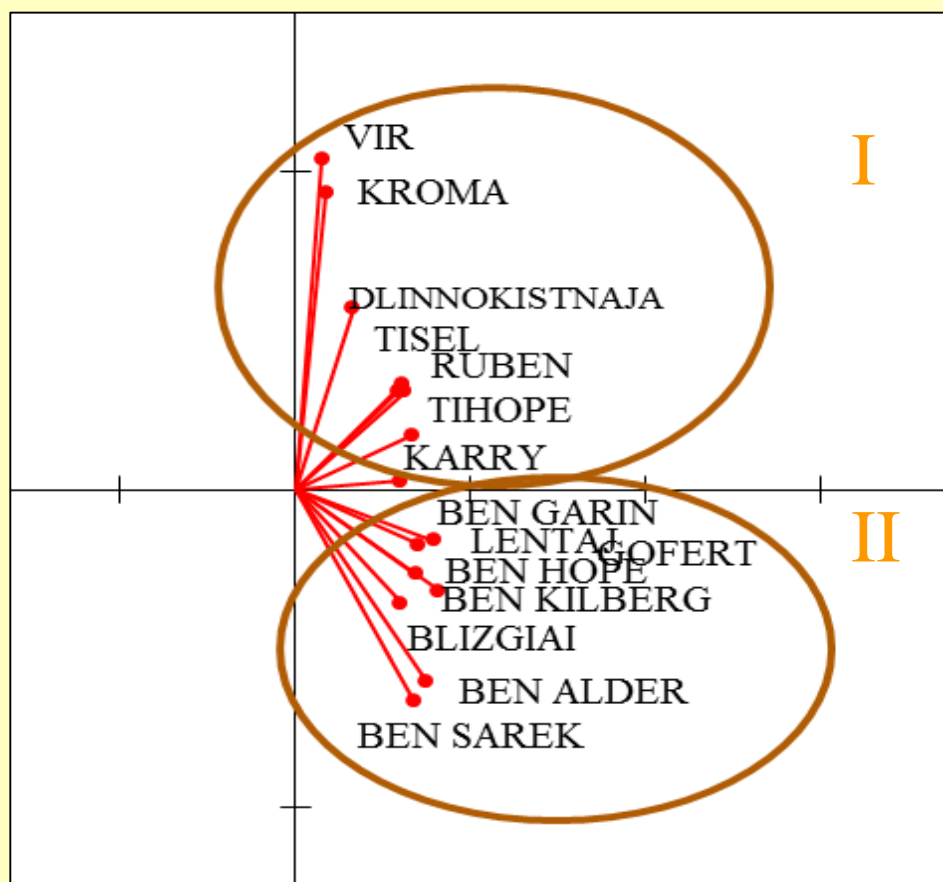
Tested 25 SSR primers



**with 13 primers:
93 polymorphic
amplikons
(130-820 bp)**

RESULTS

MULTIPLE CORRESPONDENCE ANALYZE (MCA)

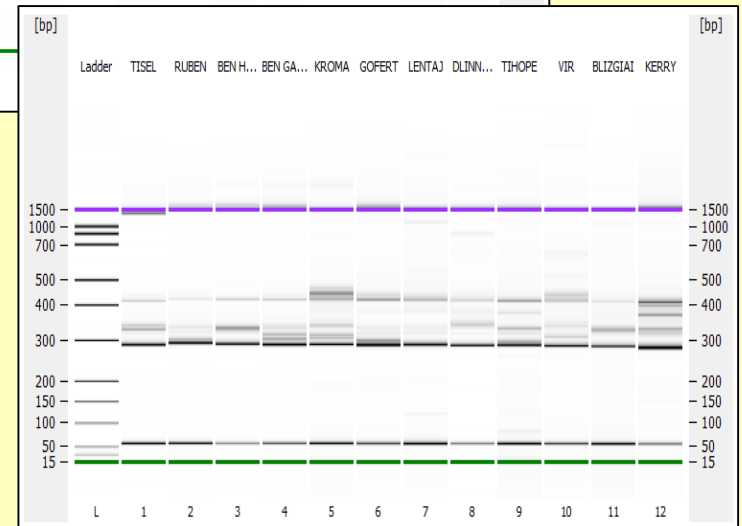
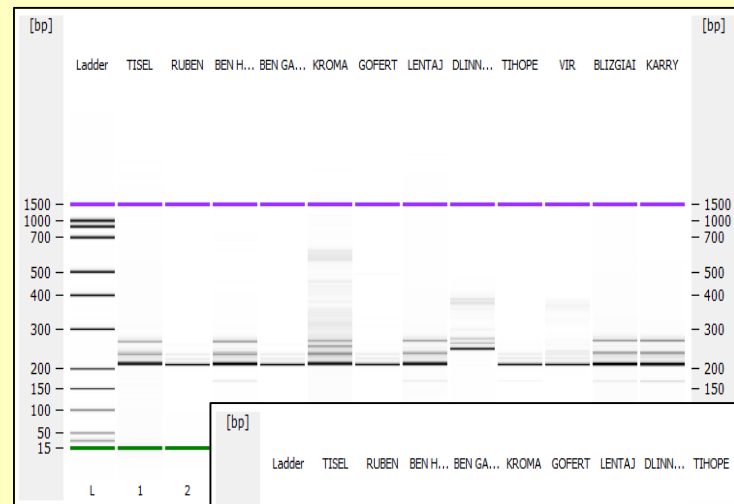


**I – RESISTANT
GENOTYPES**

**II – SUSCEPTIBLE
GENOTYPES**

CORRELATION BETWEEN PHENOTYPING & GENOTYPING RESULTS

Lp.	VARIETY	RESISTANT/ SENSITIVE ¹
1.	Tisel	resistant
2.	Ruben	susceptible
3.	Ben Hope	susceptible
4.	Ben Gairn	susceptible
5.	Kroma	resistant
6.	Gofert	susceptible
7.	Lentaj	susceptible
8.	Dlinnokistnaja	susceptible
9.	Tihope	resistant
10.	Vir	resistant
11.	Blizgiai	resistant
12.	Kerry	resistant
13.	Ben Sarek	susceptible
14.	Ben Alder	susceptible
15.	Ben Kilbreck	susceptible



RESULTS

IDENTIFICATION OF ALLELES **POTENTIALY** LINKED TO GALL MITE RESISTANCE



GENOTYPE	ALLEL (bp)/ MARKER/ LG		
	270bp/ g2-j08/LG2*	230bp/ g2-17/LG4*, LG1**	200bp/ RJL-5/LG2*, LG2**
‘Tisel’	+	+	+
‘Ruben’	+	+	+
‘Kroma’	+	+	+
‘Dlinnokistnaja’	+	+	+
‘Tihope’	+	-	-
‘Vir’	+	+	+
‘Karry’	+	-	+

* Scottish map; ** Lithuanina map

SUMMARY



Correlation between the DNA polymorphism and plants resistance to gall mite, evaluated by multiple correspondence analyzes (MCA), allowed to group tested genotypes in two clusters: I) resistant ('Vir', 'Kroma', 'Dlinnokistnaja', 'Tisel', 'Ruben', 'Tihope', 'Karry') and II) susceptible ('Ben Sarek', 'Ben Alder', 'Blizgiai', 'Ben Kilbreg', 'Gofert', 'Ben Hope', 'Lentaj', 'Ben Garin') to gall mite.

In the genomes of tested plants, identified alleles linked to gall mite resistance, derived from LG2 (seven genotypes) and LG4 (five genotypes), corresponding to Scottish map, as well as from LG1 (five genotypes) and LG2 (six genotypes), corresponding to Lithuanian genetic *Ribes* map.



Study are running.....

THANK YOU

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