Breeding for fruit quality of highbush blueberry (*Vaccinium corymbosum* L.) at the National Institute of Horticultural Research, Skierniewice, Poland

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Applied breeding of highbush blueberry in Poland

Department of Breeding of Horticultural Crops (ZHRO)
Fruit Plant Breeding Laboratory
- the only public breeding program in Poland financed by the Ministry of Agriculture and Rural Development

Fruit Plant Breeding Laboratory

- breeding/core collection of highbush blueberry genotypes (>50 parental forms)
- high plastic tunnels, glasshouse and labs.
- selection fields and cultivar trials

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HIGHBUSH BLUEBERRY BREEDING

– InHort, SKIERNIEWICE (2009)

- **75% dessert cultivars** (hand picked fruits)
- **25% processing cultivars** (machine fruit harvest

Maternal form

X

Paternal form

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Aims and breeding directions

Breeding for resistance

to main pests and diseases

Breeding for fruit quality

for fresh market and suitability for processing and freezing

Good adaptation

to local environmental conditions (winter hardiness, spring frost tolerance, chilling requirements and machine fruit harvest).

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Breeding goals

I. Fresh fruit (hand harvest)

Fruit: large size, firm, good flavor, attractive light blue color, small dry scar, long storage (shell life)

Plant: erect habit, productive, loose cluster, easy to pick, disease free, fruit ripening: very early – late.

II. Processed fruit

(machine harvest)

Erect habit, productive, easy to harvest, uniformity of ripening, no pedicel adherence

Genetic and environmental control

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Conventional Breeding

Crossing programs are mainly done under cover (high-plastic tunnel)

Classical, hybridization breeding methods, ~~no GMO~~

1. Crossing of selected parental forms (according to DNA polyphormism, phenotypic evaluation in the collection and genetic studies
2. Evaluation of F_1 seedling progenies
3. Selection of breeding material (best individual are selected) and propagated
4. Further evaluation and selecting of best clones

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CROSSING PROGRAM

Traditional breeding – crossing of parental forms, production of F_1 seedlings and selection of valuable individual and clones

X

Maternal form X Paternal form

- 25-50 flower buds are emasculated and pollinated with pollen, labeling and bag isolation,
- Ripening of fruits, collecting and seed extraction,
- Sawing of seeds immediately or stratification of seeds, germination of seeds in the glasshouse conditions,
- Production of F_1 seedlings in glasshouse (tunnel) for 8-12 months,
- Planting of seedlings in breeding/selection fields at the Experimental Orchard at Dąbrowice for further evaluation and selection the best materials.

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Crossing program – plastic tunnel

Selection field at the Experimental Station

Advanced selections – examples

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Crossing combinations and number of seedlings – highbush blueberry breeding program

at InHort, Skierniewice, **2008-2022**

Lp.	Years	No. of cross combinations	No. of seedlings produced
1.	2008/2009	30	775
2.	2009/2010	50	995
3.	2010/2011	102	5000
4.	2011/2012	78	5500
5.	2012/2013	83	10140
6.	2013/2014	75	5100
7.	2014/2015	24	4890
8.	2015/2016	60	1800
9.	2016/2017	75	2100
10.	2019/2020[A]	53	1570
11.	2021-2022	40	2000
	Total	**670**	**39870**

[A] – from 2020 highbush blueberry crossing programs are carried out every other year

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Molecular evaluation of selected blueberry plants

PLANT MATERIAL FOR MOLECULAR EVALUATION

Seven highbush blueberry cv. differ in regard to fruit wax coating

,


'Aurora’


'Rubel’


'Bonifacy’


'Bluegold’


' Toro’


'Liberty’


'Jorma’

Differ in regard to intensity of fruit wax coating

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METODS APPLIED IN THE STUDY I.

1. Plant genotyping

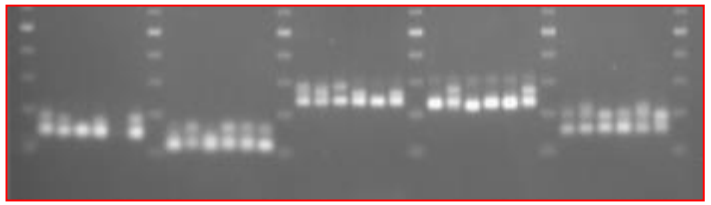
Analysis of DNA polymorphism of parental genomes selected for breeding programs

DNA isolation - young leaves (2g of plant tissue) (Doyle & Doyle; CTAB method).

DNA quality - (Gene Quant Pro Amersham Pharmacia Biotech).

DNA fragment amplification – SSR-PCR

96°C – 30”
60°C – 90” } **10x (-0,5°C /cycle)**
72°C – 60”
96°C – 30”
55°C – 60” } **35x**
72°C – 60”



Electroforesis in2 % high resolution agarose gels /ethidium bromide solution (50 mg/L) / UV light.

- DNA fragments differentiating blueberry cultivars = binary (01) matrices.
- Cluster analysis by plotting the genetic distance dendrogram (Unweighted pair group method, UPGMA) (XLStat 2009 software).

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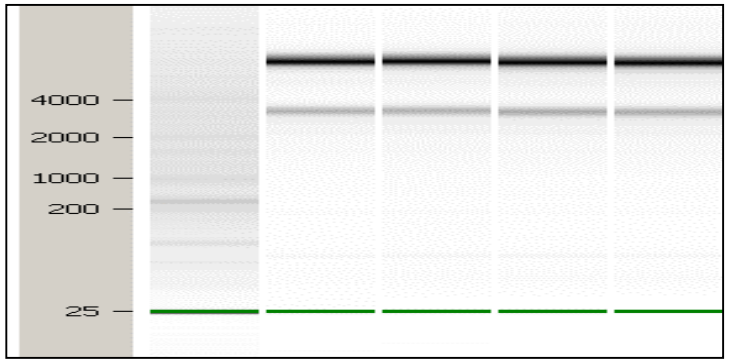
METODS APPLIED IN THE STUDY II.

2. Expression profiling of genes involved in plant wax biosynthesis

Fruit skin and fruit flesh collected from 7 cvs.:
'Aurora', 'Bluegold', 'Bonifacy', ‘Jorma’, 'Liberty', 'Rubel’, 'Toro'

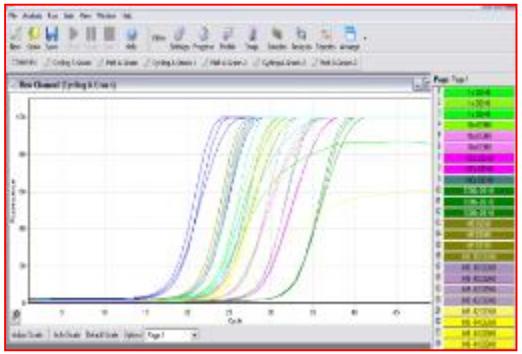
- RNA isolation (Qiagen column – kit applied protocol)

RNA concentration and integration (2100 Agilent Bioanalyzer)



- Amplification of RT-qPCR products, number of gene transcript calculation (Rotor-Gene 6000 Series Software v. 1.7 (Corbett).

95°C – 5min
95°C – 20s
60°C – 30s 40x
72°C – 20s



Standard curve comparison (ΔΔCt method):
Target gene vs. GADPH (glyceraldehyde-3-phophate dehydrogenaze) ref. gene

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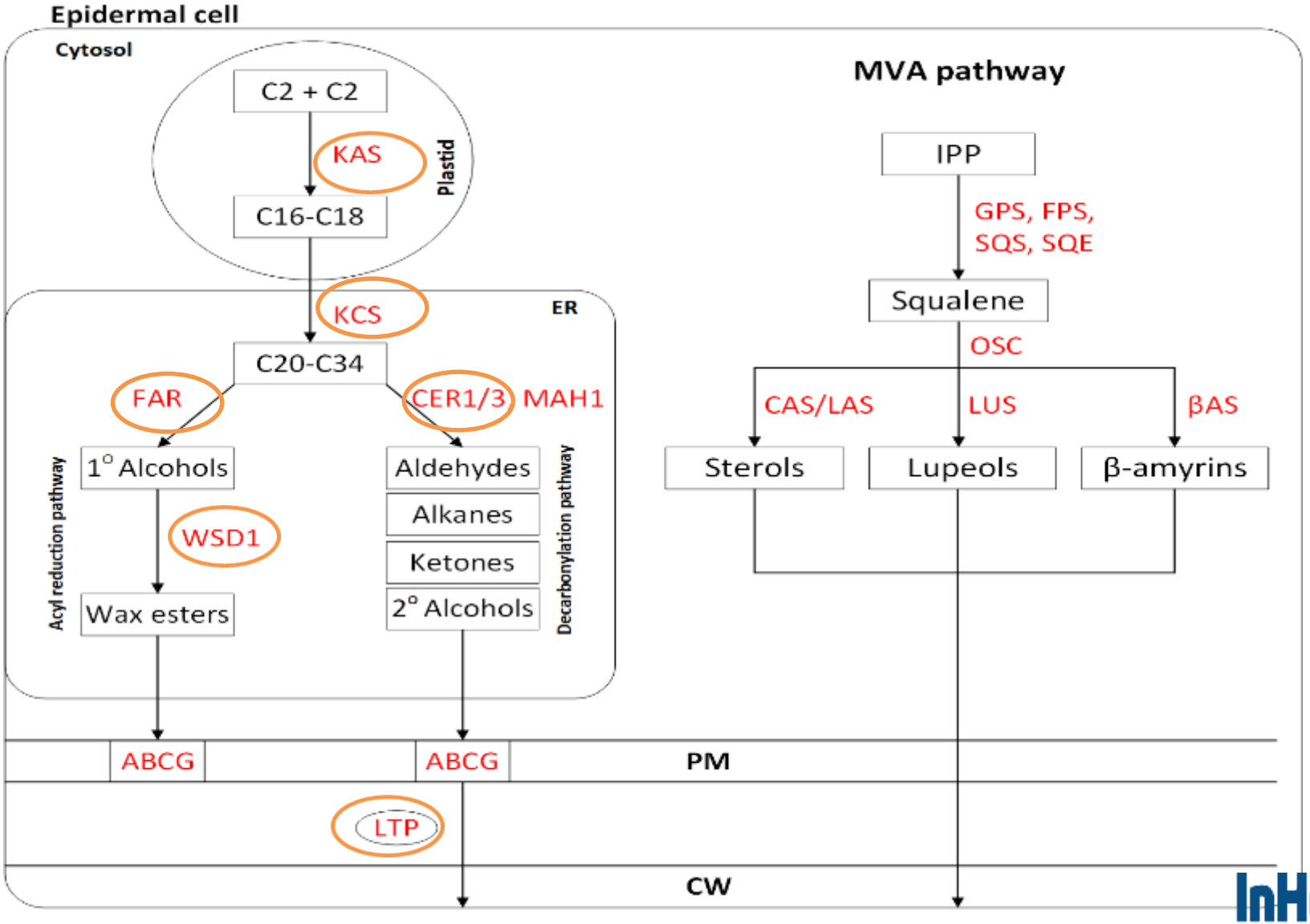
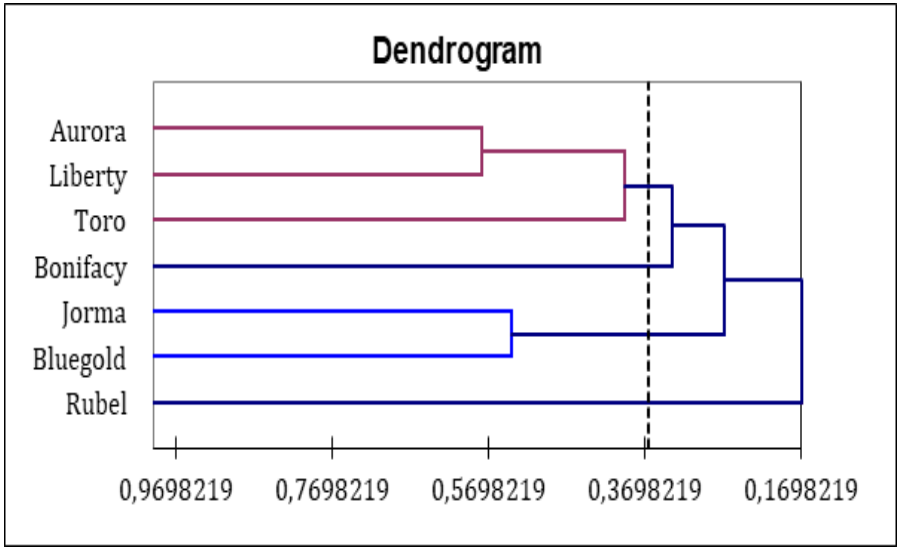
Outline of the genes involved in cuticular wax biosynthesis pathway, by Trivedi et al. 2021



RESULTS

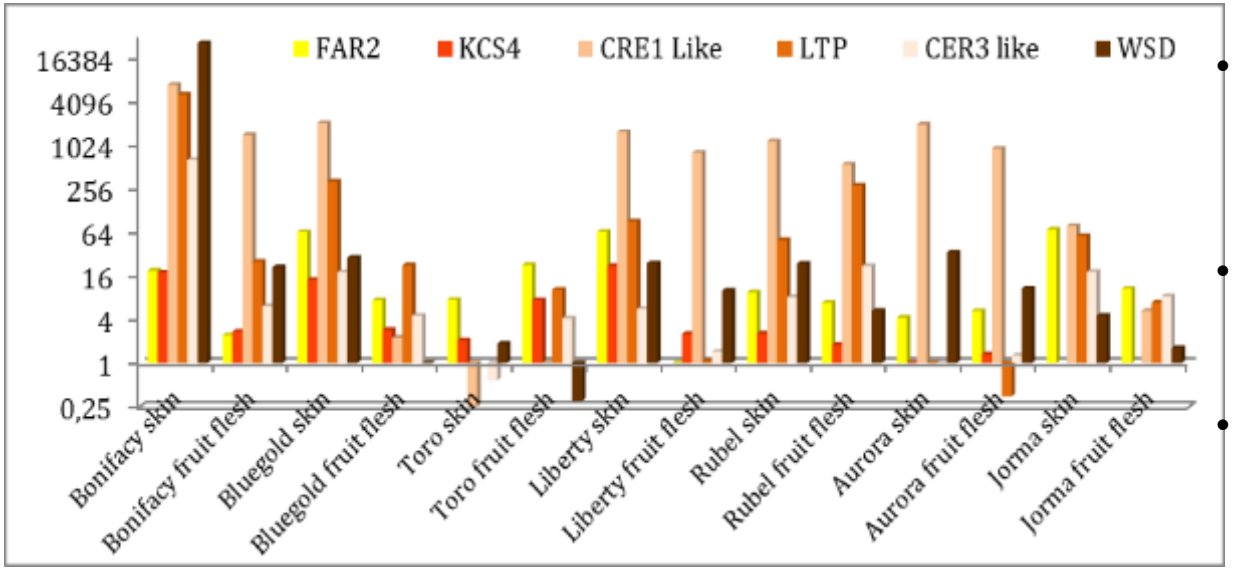
Genetic similarity between blueberry cvs., SSR allele diversity among the genome region of interest (ROI).



Grouping clusters:

1. Low intensity waxy coating fruits cvs. such as: ‘**Aurora’, ‘Liberty’, ‘Toro’, ‘Bonifacy’.**
2. Slight cuticule coating cv. ‘**Jorma’** and intensive cuticule coating cv. **‘Bluegold’.**
3. Lowest similarity - cv. **‘Rubel’** (17%) (significantly excluded from the other clusters).
4. Highest similarity (57%) - cvs. **‘Aurora’, ‘Liberty’**

Expression profiling of the genes of interests.



- WSD, CER-like, KCS, LPT - high expr. in fruit skin of cvs. **'Bonifacy', ‘Bluegold’, 'Liberty**’ - wax coated berries
- **'Rubel’** fruit skin and flesh - high activity of analyzed genes (different way of trait regulation).
- CRE1-like - high expression in fruit skin and flesh of tested cultivars, excluding **‘Toro’.**

CONCLUSIONS

1. The highbush blueberry breeding program at InHort in Skierniewice, Poland is already bringing the first effects in the form of selected valuable individuals and breeding clones.
2. Molecular characteristics of genetic diversity within the blueberry breeding collection will enable for precise and targeted selection of plant initial forms in the crossing programs.
3. The analysis of specific genes activity allows initial recognition of the mechanism of trait regulation of the blueberry cultivars from the breeding collection.

ACKNOWLEDGEMENT:
The research was carried out under the special-purpose subsidy of the Polish Ministry of Agriculture and Rural Development, Task 3.5: "***Production of highbush blueberry (Vaccinium corymbosum* L.*) initial materials with high-quality fruit and molecular analysis of specific genomic fragments***".

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QUESTIONS ?

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