New Applied Breeding Program of Haskap Berry (*Lonicera caerulea* L.) at the National Institute of Horticultural Research in Skierniewice

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Introduction

Blue honeysuckle *(Lonicera caerulea sp.* L.) is also known as **honey berry/haskap berry** is gaining popularity due to its health benefits and early fruit ripening (late May – beginning of June).

Aims of the applied breeding program is obtain new cultivars with improved fruit quality, disease resistance and tolerant to abiotic stresses like drought and sunburn.

Key points of the haskap berry:

Nutritional Value: rich in vitamins, polyphenols and minerals, beneficial for human health.

Climate Adaptation: Winter hardy, buds & flowers are spring frost tolerant, making it suitable for cultivation in various soil and climate conditions.

Sunburn on the bushes

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Haskap berry breeding goals and strategy

Applied breeding program: started in **2023**, aimed to develop new cultivars with different ripening periods, high yields and better fruit quality and suitable for machine fruit harvesting.

Breeding Goals:

- develop new cultivars suitable for both fresh consumption and processing,
- enhance disease resistance and climate adaptability,
- improve fruit quality, including size, flavor and shelf life + transportability.

Diallel crossing design and seedling production

- In 2023-2024, a total of **51 crossing** combinations were made in **diallel design** using **12 parental forms** (Russian, Canadian and new haskap cultivars).
- In 2023, **470** fruits were obtained, while in 2024, **128** fruits were collected = **598 fruits**.
- A total of **3177** F_1 seedlings were produced and **1136** seedlings were evaluated.
- In 2024, **4** breeding individuals were selected.

Results of breeding program and progress (2023-2024)

Year / Number	2023	2024	TOTAL
Crossin combinations	**36**	**15**	**51**
Parental forms	**6**	**6**	**12**
Pollinated flowers	**1 796**	**980**	**2776**
Obtained fruits	**470**	**128**	**598**
Produced seedlings	**2125**	**1052**	**3177**
Evaluated seedlings	**0**	**1136**	**1136**
Selected individuals	**0**	**4**	**4**

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Evaluation of haskap berry genotypes in the collection

In 2023 and 2024, detailed evaluation was done of genotypes grown in the collection **(36 cultivars**) to assess their suitability for the breeding program. The haskap cvs. were evaluated based on main traits, including flowering intensity, fruit set, yield, fruit weight and fruit quality (chemical compounds). The most promising cultivars identified for their exceptional traits include:

Polish old cvs. 'Wojtek' and 'Zojka'
Canadian cvs. – 'Aurora', 'Boreal Blizzard', 'Boreal Beast', 'Boreal Bueaty'
Russian cvs. – 'Jugana', 'Lawina', 'Usłada', 'Vostorg'
New Haskap cvs. - 'Colin', 'Lori', 'Maxim', 'Tomi' and 'Willa'

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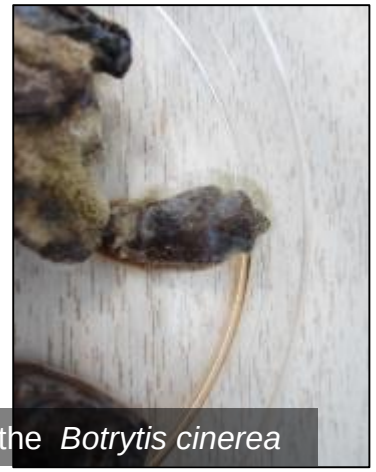
Evaluation of diseases in haskap berry

In 2023 and 2024, observations for bacterial and fungal diseases were conducted on haskap berry cvs, in the field collection. It was identified bacterial pathogens, such as *Pseudomonas* and *Stenotrophomonas*, as well as fungal diseases including *Botrytis cinerea* and *Alternaria*, affecting different plant parts including leaves, flowers and fruits.

- **Bacterial Diseases**: Several genotypes showed symptoms of bacterial canker, with pathogens classified into *Pseudomonas* and *Stenotrophomonas*. Specific isolates from cvs. 'Baczkarskaja', 'Morena' and 'Tomiczka' were identified as pathogenic.
- **Fungal Infections**: *Botrytis cinerea* was found to cause gray mold during fruit storage, and *Alternaria* was present in several cultivars, especially on flowers and shoot tips.

Symptoms of gray mold on fruit



Symptoms of gray mold caused by the *Botrytis cinerea*

Pest infestation in haskap berry

In 2023 and 2024, monitoring of pests were conducted on haskap berry genotypes during May and June. The surveys aimed to monitor pest infestation levels and identify potential threats.

Key Findings:

- **2023**: During the inspection (May 12th), **larvae of the Pink Bollworm (*Archips rosana*)** from the Tortricidae family were observed in low numbers (3 larvaes).
- **2024**: In both of inspections (May and June), **caterpillars of the Pink Bollworm** were again observed, in small numbers. Additionally, a **plum scale (*Parthenolecanium corni*)** was found on one plant during the second inspection. No aphids or other pests were detected.

Larvae of the Pink Bollworm

Plum scale (*Parthenolecanium corni*)

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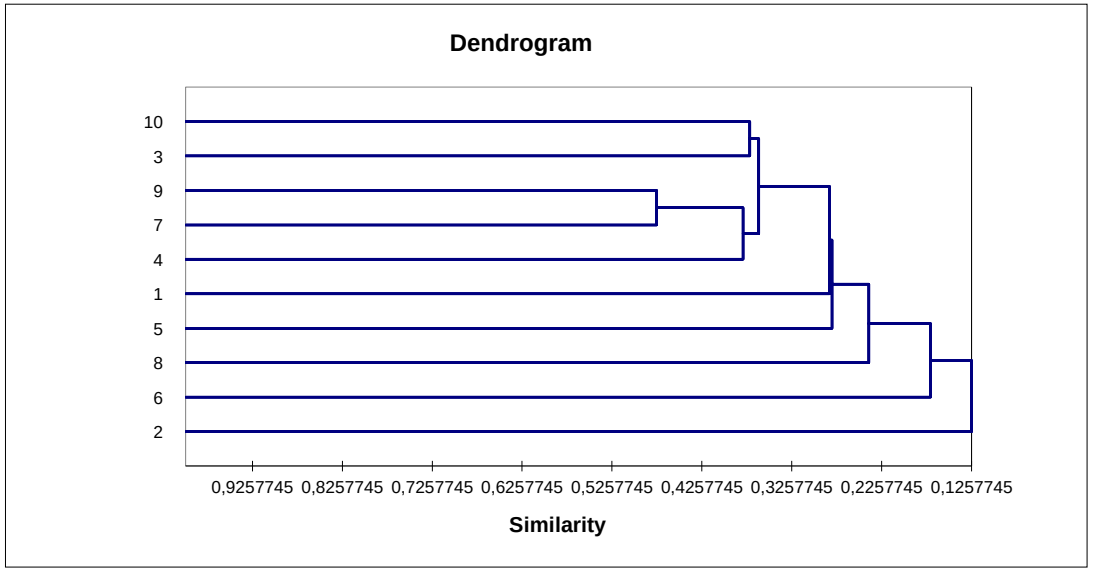
Molecular studies to suport breeding work

In 2023 and 2024, molecular analyses were conducted to determine the genetic relationships among the parental genotypes of haskap berry used in the applied breeding program. DNA was isolated from young leaves, followed by polymerase chain reaction (PCR) and SSR (Simple Sequence Repeat) analysis to study microsatellite regions of the genome.

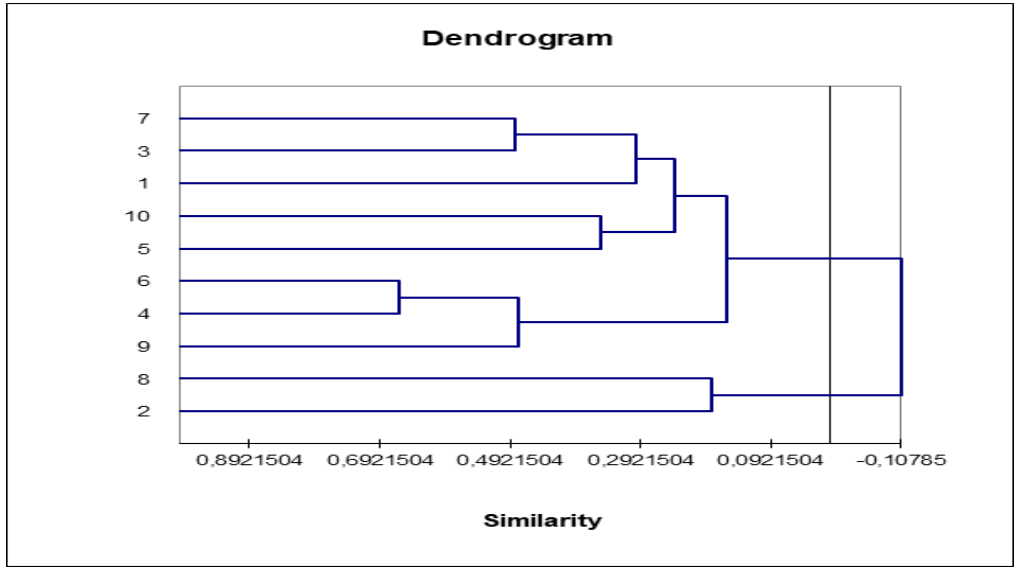
Results:

The study included 10 parental forms and genetic polymorphism (similarity) ranged from **12% to 47%** in 2023 (dendrogram left) and from **10% to 65%** in 2024 (dendrogram right)

Dendrogram (2023) of the genetic similarity of the Haskap berry genotypes based on SSR data with 16 pairs of primers. **No. 1-10** (Jugana', 'Sinij Utes', 'Boreal Blizzard', 'Boreal Beast', Nr 5, Nr 6, Nr 44, 'Rebeka', 'Wojtek', 'Indigo Gem').



Dendrogram (2024) the genetic similaryty of the Haskap berry genotypes based on SSR data with 16 pairs of primers. **No. 1-10** ('Amphora', 'Aurora', 'Bakczarskaja', 'Bakczarskaja Jubilejnaja', 'Baczkarskij Velika', 'Czułymskaja', 'Docz Velikan', 'Duet', 'Gordost Bukczara'.

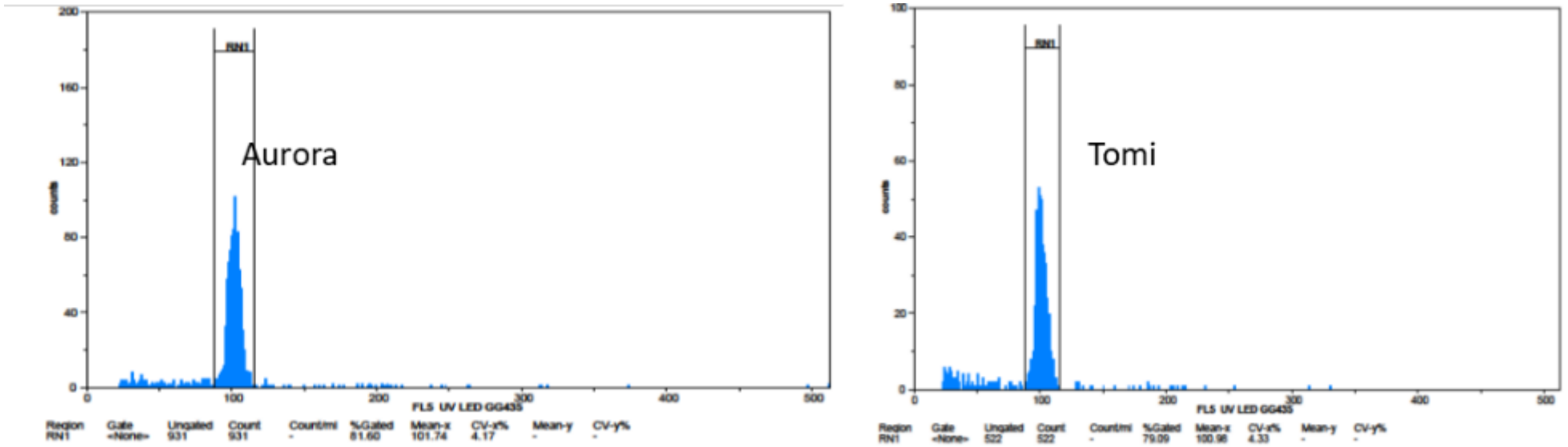


Cytometric ploidy analysis of Haskap berry genotypes

In 2023 and 2024, cytometric analysis was conducted on haskap berry genotypes to assess their ploidy levels. Young leaf samples were collected from the tested genotypes in spring and analyzed using a CyFlow PA flow cytometer (Partec, Germany). The DNA content was stained with DAPI, and the fluorescence level was analyzed based on the position of the fluorescence peaks on the X-axis, with reference to the tetraploid standard cv. **'Aurora'** (***2n = 4x = 36 chromosones***).

Results:

In 2023 it was found that tested 32 genotypes in the breeding collection were **tetraploids**. In 2024, three new cvs. **('Tomi', 'Maxim'**, and **'Willa**') were also analyzed, confirming their **tetraploidy** level with fluorescence peaks similar to 'Aurora'.



Example histograms from the FCM/DAPI cytometric analysis for the reference variety 'Aurora' and the cv. 'Tomi'.

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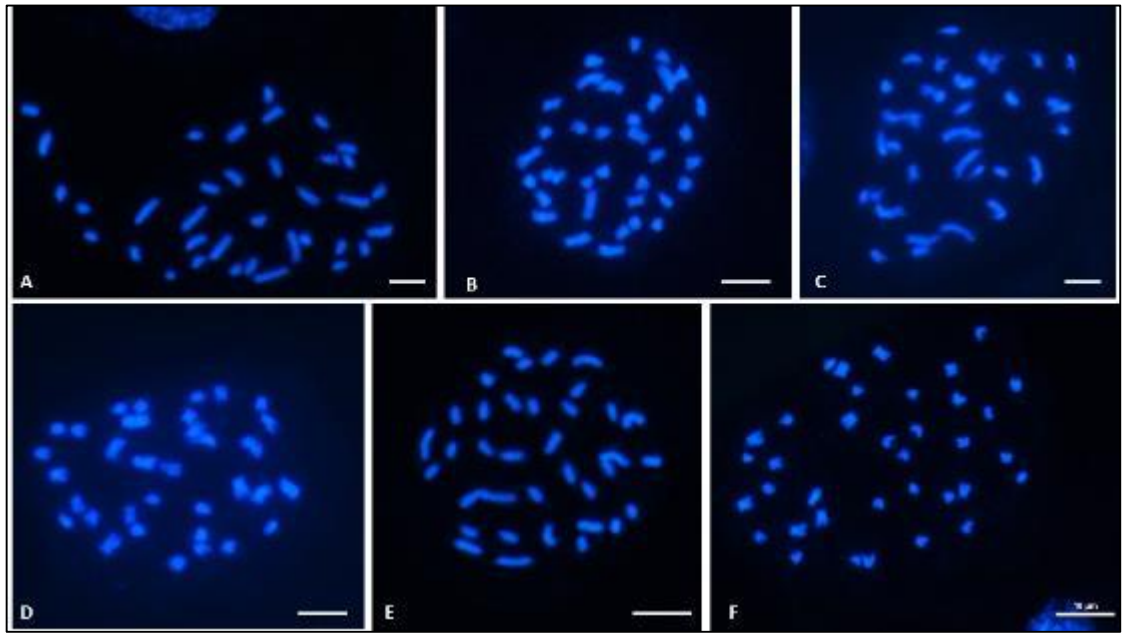
Chromosome number analysis in Haskap berry genotypes

In 2023 for chromosome number analysis, microscope slides were prepared from the root apex meristems treated with 8-hydroxyquinoline and stained with DAPI. The microscope analysis confirmed that all analyzed cultivars were **tetraploids** with ***2n = 4x = 36 chromosomes***.

In 2024, chromosome number assessments were performed for 10 additional cvs. from the collection. Tested cvs. 'Aurora', 'Czułymskaja', 'Baczkarskaja', 'Indigo Gen', 'Kamczadalka', 'Tomiczka', 'Tundra', 'Wojtek', 'Vostorg', and 'Zojka' were also found to be **tetraploids**, with ***2n = 4x = 36 chromosomes***.



Microscopic observations of chromosome numbers in root apex meristems of selected cvs:. (**A**) 'Jugana', (**B**) 'Sinji Utes, (**C**) 'Boreal Blizzard', (**D**) 'Boreal Beast', (**E**) 'Colin', (**F**) 'Lori' confirming tetraploid level (***2n = 4x = 36***). Scale = 10 µm.

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Pollen viability assessment in Haskap berry cultivars

In 2024, the viability of pollen was evaluated for haskap berry genotypes used in the breeding program, including Russian ('**Jugana', 'Sinij Utes**'), Canadian (**'Boreal Blizzard', 'Boreal Beast'**) and new haskap cvs. ('**Colin', 'Lori**'). Pollen viability was assessed using two methods: **(1)** cytoplasmic staining of pollen grains with Alexander's stain, according to the procedure by Niles and Quesenberry (1992); **(2)** pollen grain germination on a 15% sucrose medium.

Results:

It was found no correlation between the cytoplasmic staining of pollen grains and their germination rates. Cytoplasmic staining exceeded **97%**, while germination rates varied from only **8.3%** in 'Jugana' to **73.8%** in 'Boreal Beast'. These findings provide important information of these parental forms (genotypes) used in the breeding program.

Pollen viability based on cytoplasmic staining with Alexander's reagent and germination on 15% sucrose medium.

Method/ cultivar	Jugana	Sinij Utes	Boreal Blizzard	Boreal Beast	Colin	Lori
Cytoplasmic Staining (%)	97.1	97.5	99.0	99.8	98.6	97.2
Germination (%)	8.3	44.1	61.7	73.8	13.3	72.7

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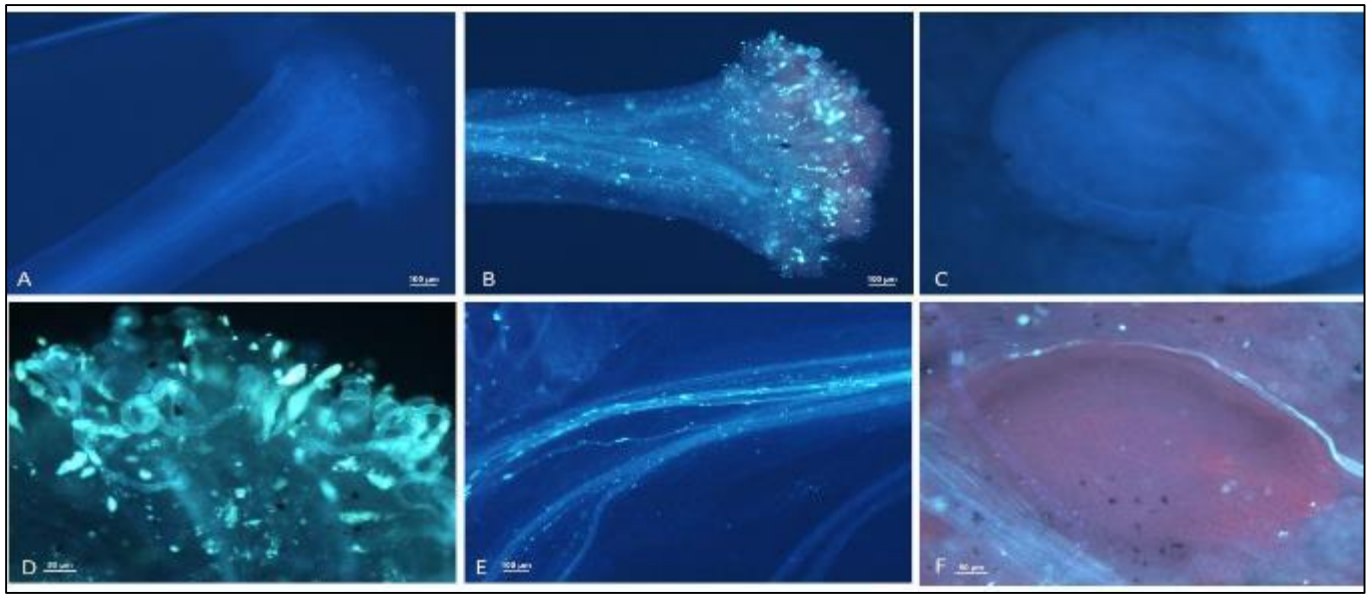
Pollen germination and pollen tube penetration

In 2023 and 2024, pollen viability and compatibility were assessed by observations of pollen germination on the stigma and the penetration of pollen tubes through the styles of 12 crossing combinations. The analysis involved pollen from each combination observed at 24, 48, and 72 hours after pollination.

Key findings:

Germination and tube penetration: The study showed variation in the number of pollen grains germinating on the stigma and the extent of pollen tube growth through the style. **The highest compatibility was observed in crosses of parental forms originated from the same geographical region, with lowest compatibility in self-pollinations.**



Microscopic observations of pollen germination on the stigma and pollen tube penetration through the style of Haskap berries:

- (**A**) Non-germinating pollen grains on the stigma after 24 hours in cross #2.
- (**B**) Single pollen grains germinating on the stigma after 24 hours in cross #8.
- (**C**) Ovule section showing unfertilized ovule from cross #8 at 24 hours post-pollination.
- (**D**) Germinating pollen grains on the stigma in cross #10, 72 hours after pollination.
- (**E**) Pollen tubes penetrating the style at 72 hours post-pollination in cross #10.
- (**F**) Presence of pollen tubes in the ovule at 72 hours post-pollination in cross #10

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Summary & results of the Haskap berry breeding program

✓ **Program Goals**:

The main objectives are to develop new Haskap cultivars with improved fruit quality, disease resistance and tolerance to abiotic stresses such as drought and sunburn.

✓ **Progress of the breeding program (2023-2024)**:

51 crossing combinations were made in diallel design using 12 parental forms.

A total of **598 fruits** were obtained from pollination; **3,177 seedlings** were produced and **4 valuable individuals** were selected for further evaluation.

✓ **Disease and Pest Findings**:

Identified bacterial pathogens (*Pseudomonas*, *Stenotrophomonas*) and fungal diseases (*Botrytis cinerea*, *Alternaria*).

Pest inspection found in low numbers: Pink Bollworm larvae and Plum scale.

✓ **Molecular and Cytometric Studies**:

Genetic relationships among parental forms showed the polymorphism ranged from **12% to 47%** (2023) and from **10% to 65%** (2024).

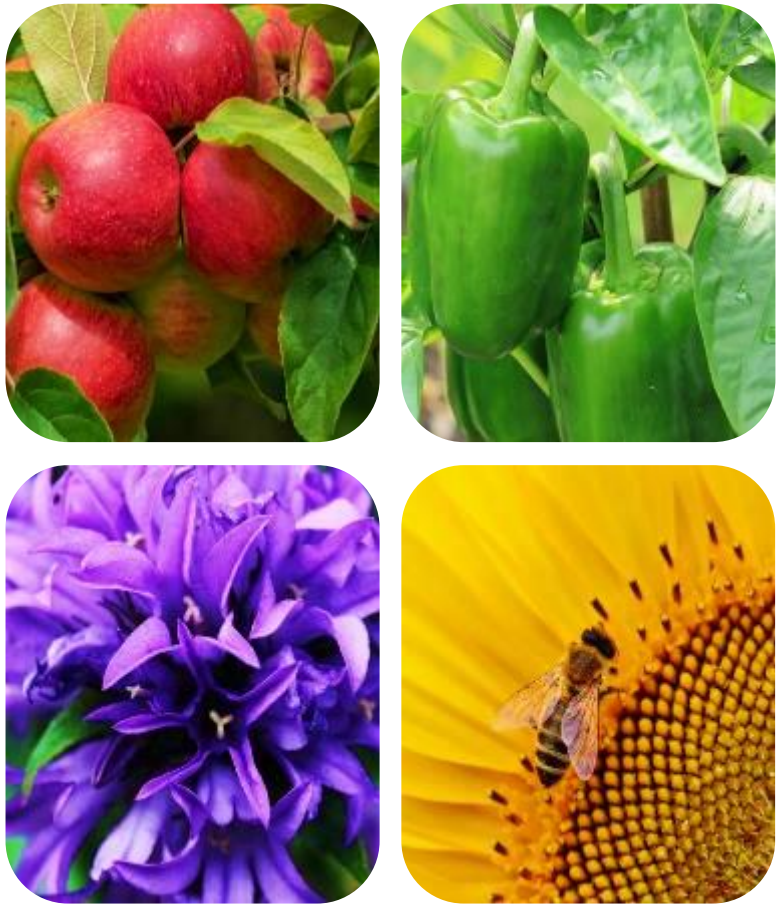
All analyzed genotypes were **tetraploids** (***2n = 4x = 36 chromosomes***).

✓ **Pollen Viability**:

Cytoplasmic staining exceeded **97%**, while germination rates varied from **8.3%** in ‘Jugana’ to **73.8%** in ‘Boreal Beast’.

THANK YOU

FOR ATTENTION!



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