

Use of biotechnological methods in sweet cherry (*Prunus avium* L.) breeding at the National Institute of Horticultural Research, Skierniewice, Poland

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- A major problem in sweet cherry breeding aimed at obtaining early ripening genotypes is the limited possibility of use varieties with early fruit ripening as mother forms.
- The fruits of such varieties sometimes ripen even 6-7 weeks after flower pollination and the embryos contain in fruit do not have enough time to reach full physiological maturity and are unable to germinate in conditions of traditional stratification.
- Therefore, the research was undertaken to optimize the embryo culture method ensuring the proper development of immature sweet cherry embryos.
- In order to eliminate closely related parental genotypes from the cross combinations and to verify the genetic identity of the best individuals selected from the evaluated seedling populations, the Simple Sequence Repeats method is used.



2. Material and Methods



EMBRYO RESCUE:

PLANT MATERIAL: The embryos originated from crossing of early ripening parental forms: Rita × Kasandra (240 pcs.) and Jacinta × Rita (240 pcs.).



STERILIZATION OF PLANT MATERIAL AND isolation of 7-8 week embryos

MEDIA: MS, Boxus, Fossard, SH



THE STAGES OF CULTURING IMMATURE CHERRY EMBRYOS INCLUDED:

- warm seed stratification at 25°C
- cold embryo stratification at 4°C
- development of plant organs in a phytotron with a photoperiod of 16/8 day/night at 24°C
- acclimatization of obtained plants to greenhouse.

MOLECULAR ANALYSIS:

PLANT MATERIAL: 15 genotypes

POLYMERASE CHAIN REACTIONS: SSR, 45 primers

MOLECULAR DATABASE ANALYSIS: Genetic similarity coefficient, UPGMA



3. Results and Discussions

EMBRYO RESCUE METHODS:

The influence of the medium in the initial stage of embryo culture on the number of obtained plants and embryo forming only shoot or root after 4 weeks of growth in the phytotron in relation to the viable embryos obtained, expressed in % (Skierniewice, 2023).

| PARENTAL FORMS | PLANT ORGANS | MS | SH | BOXUS | FOSSARD |
|-----------------|--------------|----|----|-------|---------|
| Rita × Kasandra | plant | 23 | 10 | 5 | 5 |
| | only shoot | 2 | 24 | 21 | 17 |
| | only root | 5 | 3 | 0 | 2 |
| Jacinta × Rita | plant | 11 | 7 | 28 | 24 |
| | only shoot | 15 | 8 | 13 | 26 |
| | only root | 0 | 0 | 0 | 0 |

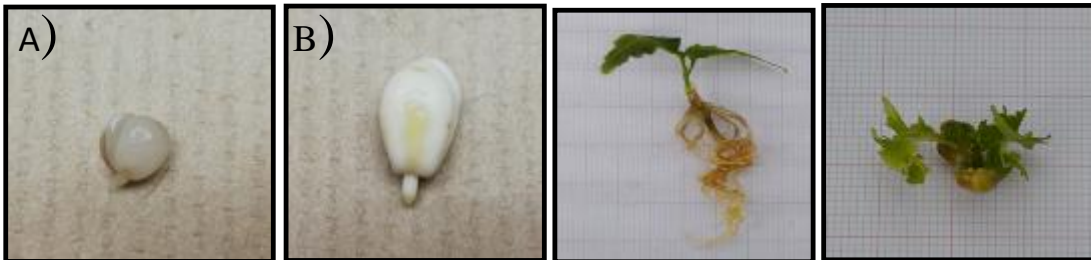


Fig. 1. The embryo after cooling period in 4°C: A) soft, B) hard

Fig. 2. Plant fully developed obtained from 'Rita' × 'Kasandra', medium Boxus (1974).

Fig. 3. Non fully developed plant (without root), the combination 'Rita' × 'Kasandra' on medium to Fossard (1977).

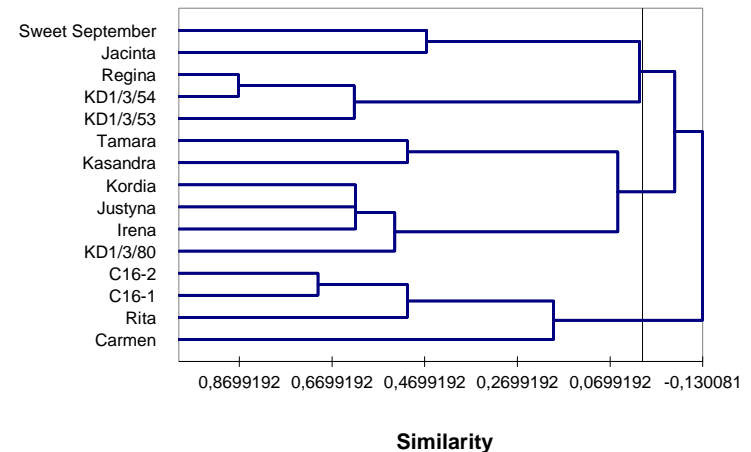
MOLECULAR ANALYSIS:

- plant identification
- assessment of genetic diversity and determination of varietal identity
- assessment of genetic distance

POLYMORPHISM:

- Among 45 primers applied in the tests only 31 generated clear and reproducible polymorphic bands.
- In total, 137 alleles (97%) with size 60-400 bp were obtained.
- The mean value of polymorphism information content (PIC) was determined as 0.874.
- Each of the 15 genotypes of *Prunus* have been characterized on the basis of 19-35 alleles.

Dendrogram



4. Conclusions and Perspectives

EMBRYO RESCUE TECHNIQUE

- It is possible to obtain seedlings from seeds of early ripening cherry varieties using the embryo rescue technique.
- For the culture of embryos obtained from 'Rita' × 'Kassandra' crosses, the influence of MS medium was beneficial, while for the culture of embryos obtained from 'Jacinta' × 'Rita' crosses, Boxus and Fossard media had a positive effect.
- The number of seedlings obtained from immature embryos depends on the medium used and the parental forms used in the crossbreeding program (seeds from the 'Jacinta' × 'Rita' crossbreeding combination had a greater ability to develop plant organs than from the 'Rita' x 'Kassandra' combination).

MOLECULAR ANALYSIS

- The pool of sweet cherry genotypes used in breeding programs at the National Institute of Horticultural Research, Skierniewice, Poland has enough amount of genetic variation. Regarding germplasm management, our results show that the genotypes in collection are variable and may be important for future sweet cherry breeding program.