

# THE USE OF THE EMBRYO RESCUE TECHNIQUE IN SWEET CHERRY (*PRUNUS AVIUM* L.) BREEDING PROGRAM IN POLAND



## INTRODUCTION

The development of an effective embryo rescue method will increase the efficiency of sweet cherry (*P. avium* L.) breeding programs aimed at obtaining of new early ripening cultivars. The effectiveness of this method depends, among others, on the genotype, maturity stage of the embryo and the composition of the medium.

The goal of the study conducted in 2021-2022 was to assess the effect of four nutrient solutions (Murashige & Skoog 1962; Boxus 1974; Fossard 1977; Stewart and Hsu 1977) differing in the source and concentration of micro- and macroelements and the type of sugar on the development of immature sweet cherry embryos and the number of seedlings obtained.

## MATERIAL AND METHODS

**PLANT MATERIAL:**

In the study the embryos originated from crossing of early ripening parental forms: 'Rita' × 'Kasandra' (420 pcs.) and 'Jacinta' × 'Rita' (490 pcs.).

**STERILIZATION OF PLANT MATERIAL AND ISOLATION OF 5-6 (2021) 7-8 (2022) WEEK EMBRYONS**

- running water (60 min)
- detergent (30 min)
- running water (30 min)
- 15% Clorox (30 min)
- 3 × rinsing in sterile water (30 min)

**REMOVAL OF THE ENDOCARP**

**MEDIUM:**

CHEMICAL COMPOUND	MEDIUM (mg/l)			
	MS	SH	Fossard	Boxus
MgSO <sub>4</sub> × 7H <sub>2</sub> O	370	490	370	510
CaCl <sub>2</sub> × 6H <sub>2</sub> O	332.2	1300	491.76	-
KNO <sub>3</sub>	1900	5055	1011	250
Ca(NO <sub>3</sub> ) <sub>2</sub> × 4H <sub>2</sub> O	-	-	-	1440
NH <sub>4</sub> NO <sub>3</sub>	1650	-	800	-
KH <sub>2</sub> PO <sub>4</sub>	170	272	-	250
K <sub>2</sub> SO <sub>4</sub>	-	-	-	-
NaH <sub>2</sub> PO <sub>4</sub>	-	-	138	-
KCl	-	-	-	120
FeSO <sub>4</sub> × 7H <sub>2</sub> O	27.8	27.8	10.7	27.8
Na <sub>2</sub> EDTA	37.3	37.3	18.61	37.3
FeCl <sub>3</sub>	-	-	-	5
MnSO <sub>4</sub> × H <sub>2</sub> O	16.9	15	8.45	12.76
ZnSO <sub>4</sub> × 7H <sub>2</sub> O	8.6	4.8	5.75	8.6
CuSO <sub>4</sub> × 5H <sub>2</sub> O	0.025	0.016	0.024	0.025
CoCl <sub>2</sub> × 6H <sub>2</sub> O	0.025	0.02	0.118	0.025
KI	0.83	0.8	0.415	0.83
H <sub>3</sub> BO <sub>3</sub>	6.2	6	3.09	6.2
Na <sub>2</sub> MoO <sub>4</sub> × 2H <sub>2</sub> O	0.25	0.2	0.024	0.25
Na <sub>2</sub> SO <sub>4</sub>	-	-	144.99	-

Tab. 1 The media composition used in the study.

**EMBRYON/PLANT CULTURE - NUTRIENT MEDIA:**

**THE STAGES OF CULTURING IMMATURE CHERRY EMBRYOS INCLUDED:**

- warm seed stratification carried out at 25°C
- cold embryo stratification carried out 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 conditions

## RESULTS

CROSSED PARENTAL FORMS	EMBRYON CONDITION	MS	SH	BOXUS	FOSSARD
		SHARE OF EMBRYOS (%) IN RELATION TO THE OF LINED			
Rita × Kasandra	hard (non-injected)	49	30	47	67
	infected	8	35	29	6
	soft	43	35	24	27
Jacinta × Rita	hard (non-injected)	80	53	62	73
	infected	10	25	13	19
	soft	10	22	25	11

Tab. 2. Condition of the embryos on the studied media after 12 weeks of cooling at 4°C (Skierniewice, 2021-2022).

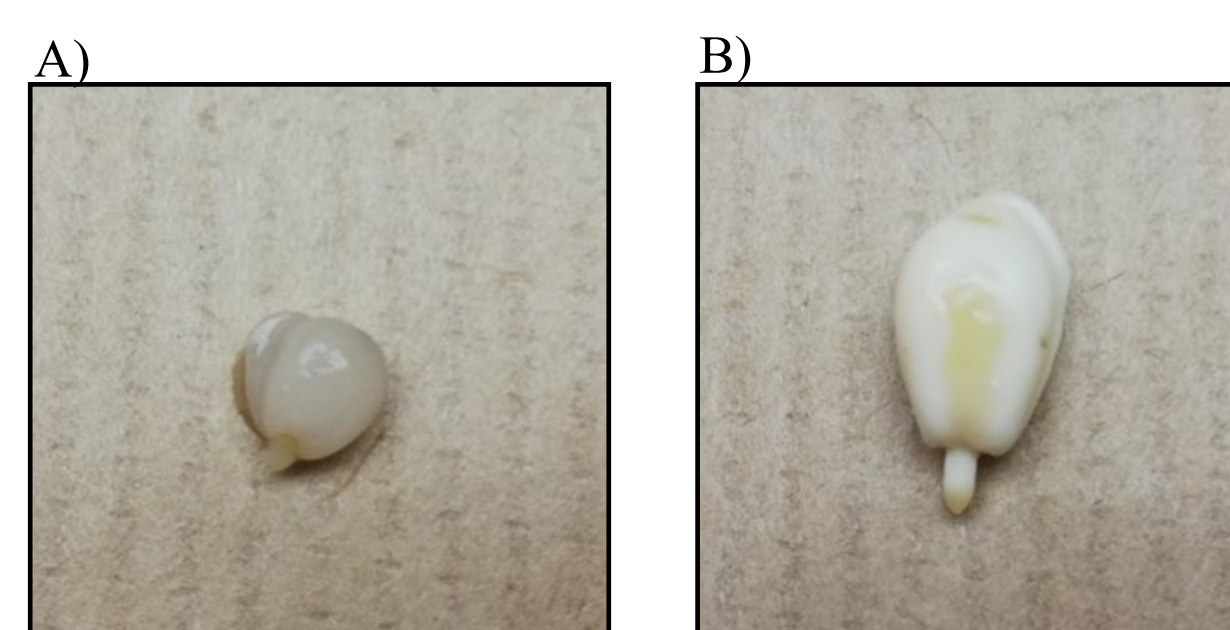


Fig. 1. The embryo after cooling period in 4°C A) soft B) hard

CROSSED PARENTAL FORMS	PLANT ORGANS	MS	SH	BOXUS	FOSSARD
Rita × Kasandra	plant	34	17	7	9
	only shoot	7	54	38	36
	only root	10	7	0	0
Jacinta × Rita	plant	42	17	37	45
	only shoot	20	49	21	50
	only root	13	0	0	3

Tab. 3. 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, 2021-2022).

- For the combination 'Jacinta' × 'Rita', from 17% of plants were obtained on SH medium to 42% on MS and 47% Fossard medium.
- The most shoot-forming embryos were observed on SH and Fossard media, 54% (Rita × Kasandra), 49% (Jacinta × Rita) and 36% (Rita × Kasandra), 50% (Jacinta × Rita), respectively.
- There was no influence of the medium nutrients on the length of the seedlings' roots.
- Plants that developed shoots and roots (a total of 159 seedlings) were planted in pots and subjected to acclimatization in a greenhouse.

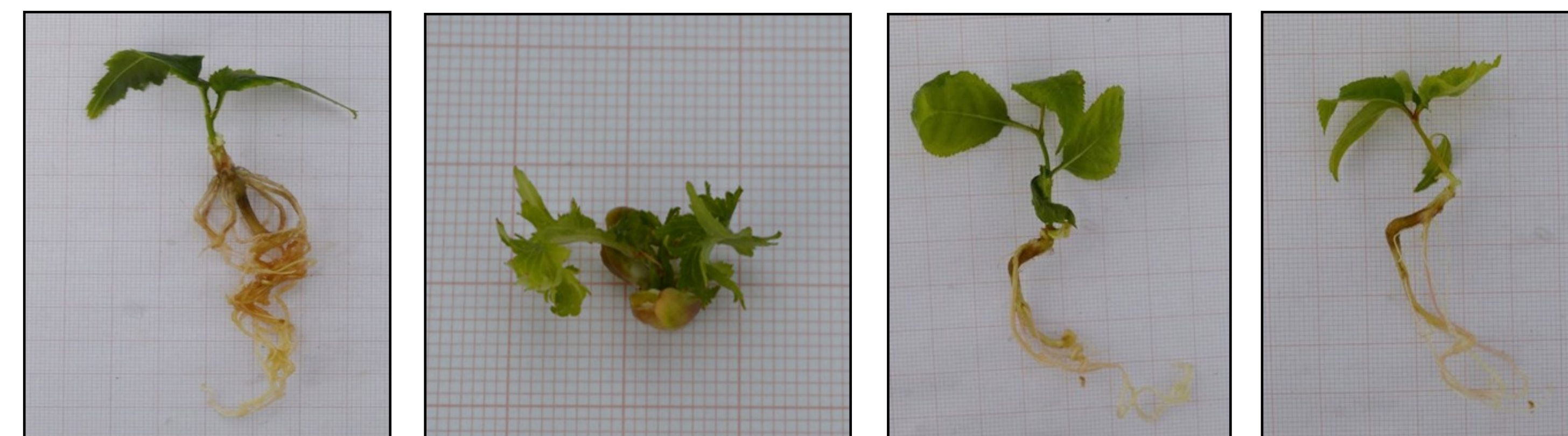


Fig. 2. Plant obtained from embryos from the combination 'Rita' × 'Kasandra' on medium according to Boxus (1974) with fully developed shoot and root.

Fig. 3. Non fully developed plant with 2 leaves without developed root obtained on a medium according to Fossard (1977).

Fig. 4. Plants obtained from embryos from the combination 'Jacinta' × 'Rita' on the medium according to Stewart and Hsu (1977).

## SUMMARY

1. Our results showed that the embryos obtained from the cross 'Jacinta' × 'Rita' had a greater ability to form seedlings than the embryos from the combination 'Rita' × 'Kasandra'.
2. Embryos combination 'Jacinta' × 'Rita' on Fossard and MS media formed more fully developed seedlings in relation to the number of bryos placed on this medium 45% and 42%.
3. The most shoot-forming embryos were observed on SH and Fossard media, 54% (Rita × Kasandra), 49% ('Jacinta' × 'Rita') and 36% ('Rita' × 'Kasandra'), 50% ('Jacinta' × 'Rita'), respectively.
4. Embryos combination 'Rita' × 'Kasandra' on MS media formed more fully developed seedlings in relation to the number of bryos placed on this medium 34%.