

Bundesforschungsinstitut für Kulturpflanzen Federal Research Centre for Cultivated Plants

Preservation of fruit genetic resources in Germany

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ECPGR WG Cryopreservation, Prague 3 - 5 May 2023

www.julius-kuehn.de

JKI Fruit genebank: Ex situ collections





Apple...Pear...Plum...Cherry... Strawberry...Raspberry... Sea buckthorn...Mountain ash... different wild fruit species

1.000 Accessions crop wild relatives of the genera *Malus... Pyrus... Prunus...Fragaria*



2.200 Seedlings

populations from collecting missions to China, Azerbaijan, Georgia, Russia (Northern and Southern Caucasus)



Institute for Breeding Research on Fruit Crops



,GERMAN FRUIT GENEBANK'

- 1. A decentralized gene bank network for fruit genetic resources in Germany
- 2. Central Coordination Institute for Breeding Research on Fruit Crops, Dresden-Pillnitz
- 3. To ensure an effective and long term Conservation and Utilization Cultivars should be preserved at least in duplicate for safety.
- 4. To ensure the genetic resources for research, breeding and pomological and landscaping traits





Apple: Chamber of agriculture North Rhine-Westphalia

CONSERVATION STRATEGY OF WILD SPECIES ACCESSIONS OF VARIOUS FRUIT SPECIES IN **G**ERMANY

- Held as active collections exclusively in the Fruit Genebank in Dresden
- Malus as well as Fragaria collections are one of the largest collections in Europe

Malus-wild species collection:

518 accessions belonging to 26 primary species and 20 hybrid species



- Wild species acc. of the various fruit species are not included in the German Fruit Genebank.
- Single acc. of wild Malus, Pyrus, Prunus or Sorbus species can be found in the inventories of botanical gardens in Germany.
- However, duplication of the whole collection, especially for *Malus*, at a second field site is **not a realistic** solution.
- A seed collection will not represent the true genotypes of clonal, heterozygous accessions.
- Additionally, growing out of genotypes from seed takes too long, as field gene banks are used intensively in breeding and molecular biology





Aim: Establishment of a back up collection for wild species accessions should be preserved at least in duplicate for safety

A safety back-up collection comprises accessions of the active **collection at a different location:**

- > a second field site, a greenhouse,
- culture stock naintained in vitro or
- ➢ in cryopreservation.

Cryopreservation will be used for managing the duplicate collection as a **space and cost efficient method** compared to a second field collection.

The philosophy of the work is to develop a system of methods well adapted to a huge spectrum of genotypes in the genebank.

CRYOPRESERVATION OF MALUS – DORMANT BUD METHOD



developed at Fort Collins, Colorado (Forsline *et al.* 1998) Advantage: No *in vitro* culture – recovery of trees in a very short time

Scion wood containing the season's growth should be collected in winter (after 72 h at -5 °C)

>Stored in plastic bags at $-5 \pm 1^{\circ}$ C for a minimum of 5 d to improve their hardiness

>The scions were cut into single node sections (35 mm long)

Dehydrated to 30% moisture on large-mesh, metallic trays in a -5°C cold chamber (gravimetric measurement)

>When the sections reached the moisture content of 30 % they were placed into cryotubes

Prefreezing in a controlled freezer under the condition of -1 °C/ h to -30 °C

➢Following holding at -30 °C for 24 h



>The cryotubes were **quickly transferred to the vapor phase** over liquid nitrogen

➤After 2 months of cryopreservation vials were rewarmed to +4°C in a refrigerator for 24 h.

> The scion pieces were transferred into moist sand for a 15 d rehydration period at 4 °C.

For chip-budding each rewarmed single bud was grafted onto 1-y-old M9 apple rootstocks planted in the orchard

➢Recovery data was taken 4 months after grafting (during the autumn of the year of grafting and the spring time of the following year).

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	Total		180	169	116	64.44	39.00	18.65	
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Summary: Cryopreservation in *Malus*

- The general protocol was adapted to Central European weather and laboratory conditions.
- Yearly weather variations are common, requiring at least 2 year of testing for the selected *Malus* accessions.
- For accessions with recovery rates below 40%, other possibilities should be considered:
 - Increase the number of cryopreserved buds of each accession
 - Viable shoot tips can be excised from cryopreserved buds and recovered under in vitro conditions;
 - Cryopreservation of isolated shoot tips
- In the future, the dormant bud method should be used for establishing a longterm, duplicate *Malus* collection held exclusively at the Fruit Genebank Dresden-Pillnitz

Höfer M, (2015) Cryopreservation of winter-dormant apple buds – Establishment of a duplicate collection of Malus germplasm' (Journal Plant Cell, Tissue and Organ Culture, 121: 647 – 656; DOI 10.1007/s11240-015-0735-1).

Höfer M. and Flachowsky H (2023) Cryopreservation of Malus and Pyrus Wild Species in the 'Fruit Genebank' in Dresden-Pillnitz, Germany. Biology 2023, 12, 200. https://doi.org/10.3390/biology12020200 Institute for Breeding Research on Fruit Crops



Fragaria-wild species collection: 286 accessions belonging to 22 diff. species

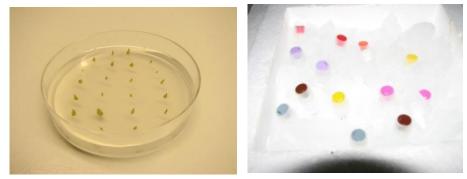


JKI Dresden-Pillnitz:

- A Potted plants of the *ex situ* collection;
- B virus-free material in an insect-protected screen house

CRYOPRESERVATION OF FRAGARIA – PVS2 VITRIFICATION









1. Preculture

- After the last subculture (2 week-old shoots) cultivation of *in vitro* plants for 14d (16h at -1°C darkness and 8h at 22°C light)
- Dissection of shoot tips and cultivation for 48h on MS medium with 5% DMSO

2. Selective dehydration

- Incubation in Loading solution (2 M glycerol + 0.5 M sucrose) for 15 min at 25°C in cryo vials
- Vitrification: PVS2 for 2.5 h on ice

3. Cryo storage

 Rapid transfer directly into liquid nitrogen (minimum 1 day).





4. Rehydration

- Rewarming by plunging in water bath at 40°C for 1-2 minutes.
- Removing the PVS2 and treating with Unloading 1.2 M sucrose for 20 min

5. Recovery

- Incubation in dark at 23°C for 7 days
- Incubation in light at 23°C.

Evaluation of regrowth after 6-8 weeks





Institute for Breeding Research on Fruit Crops

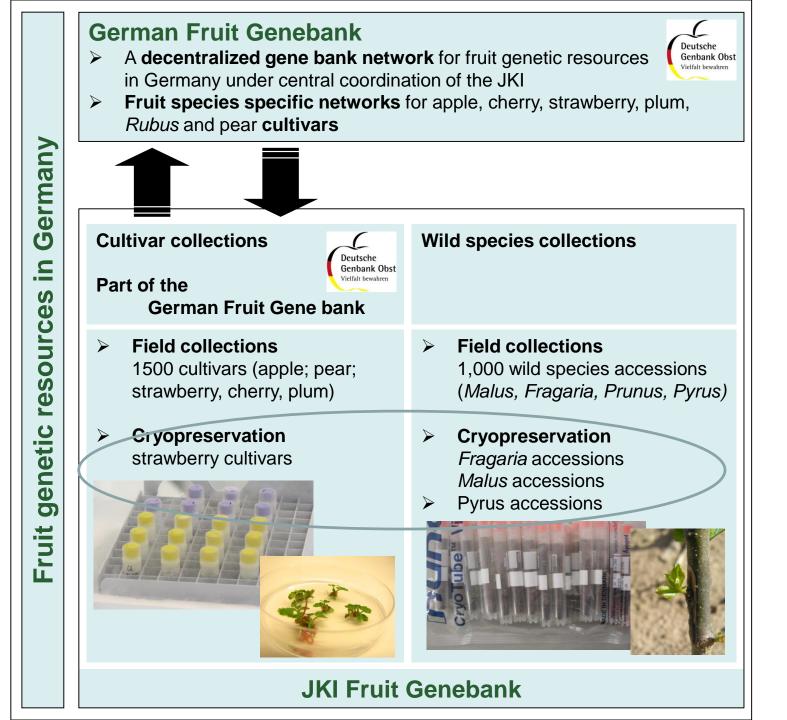
Species	No. accessions	Recovery (%)	SD	
Fragaria × ananassa	128	87.31	14.10	
Fragaria bucharica	3	80.00	5.00	
Fragaria chiloensis	5	82.89	13.90	
Fragaria corymbosa	2	86.88	9.72	
Fragaria gracilis	2	80.00	0.00	
Fragaria iinumae	2	90.00	7.07	
Fragaria mandshurica	2	95.00	7.07	
Fragaria mosch. × Fragaria viridis	1	100.00	0.00	
Fragaria moschata	6	85.00	12.65	
Fragaria moupinensis	1	100.00	0.00	
Fragaria nilgerrensis	2	75.84	12.96	
Fragaria nipponica	2	92.50	10.61	
Fragaria nubicola	2	80.60	6.23	
Fragaria orientalis	2	100.00	0.00	
Fragaria pentaphylla	2	82.50	10.61	
Fragaria species	1	84.21	0.00	
Fragaria tibetica	2	95.00	7.07	
Fragaria vesca	18	80.78	17.97	
Fragaria virginiana	4	88.75	10.31	
Fragaria viridis	1	80.00	0.00	
Fragaria × ananassa ssp. cun.	2	77.50	24.75	
Fragaria × bifera	2	75.00	21.21	
Fragaria yezoensis	2	80.00	0.00	
Fragaria spp.	66	86.27 🔨	13.92	

Results: Recovery after cryopreservation using PVS2 vitrification with cold acclimation

All tested *Fragaria* genotypes passed the baseline for storage minimum recovery of 40 %.

A very large genotype spectrum was screened, the average recovery was 86 %. (239 acc)

Höfer M, (2016) Cryopreservation of in vitro shoot tips of strawberry by the vitrification method – Establishment of a duplicate collection of Fragaria germplasm. CryoLetters 37 (3), 163-172 (2016)



Federal Ministry of Food, Agriculture and Consumer Protection

National Programme

for the Conservation and Sustainable Use of Plant Genetic Resources of Agricultural and Horticultural Crops



4 Schwerpunkte des Arbeitsprogramms

In diesem Kapitel werden die zur Erreichung der in Kapitel 1 genannten Ziele der Agrobiodiversitätsstrategie und des vorliegenden Nationalen Fachprogramms notwendigen Schwerpunkte des Arbeitsprogramms festgelegt, das bisher Erreichte beschrieben sowie die weiteren notwendigen Maßnahmen ausgeführt.

4.1 Ex-situ-Erhaltung



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Im Laufe der Jahrhunderte hat sich im Obstbau eine große Obstarten und -sortenvielfalt entwickelt. Es wird geschätzt, dass dabei ca. 40 Arten und zwischen 5.000 und 6.000 Sorten oder Herkünfte genutzt wurden, davon allein rund 2.000 Apfelsorten. Die Erhaltung von heimischen obstgenetischen Ressourcen ist eine Grundlage für die dauerhafte Sicherung des Obstbaus in Deutschland. Aus diesem Grund werden bereits seit Beginn des 20. lahrhunderts zahlreiche



iui i oischungs- unu zuchtungszwecke.



Handlungsbedarf

- → Sicherstellung einer hohen Qualität der in der Deutschen Genbank Obst erhaltenen Sortimente und ihrer Erhaltungsstandards.
- → Erhebungen zur Sortenechtheit (pomologisch und molekularbiologisch), Dokumentation und Charakterisierung der Akzessionen.
- → Ausbau um weitere fruchtartspezifische Netzwerke.
- Sicherung aller Akzessionen an mindestens zwei Standorten (Sicherheitsduplikat) innerhalb der Deutschen Genbank Obst.
- → Aufnahme von unterstützenden Partnern in die Deutsche Genbank Obst.
- Ausbau der Kryokonservierung der Fragaria- und Malus-Sammlung des JKI.

Institute for Breeding Research on Fruit Crops

Many thanks for your attention!