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Preservation of fruit genetic resources in Germany

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ECPGR WG Cryopreservation, Prague 3 -5 May 2023

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1.500 Cultivars

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)



„GERMAN FRUIT GENE BANK“

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



Cherry: Town Hagen a.T.W:



Apple: Mother tree orchard, Bonn



Apple: Chamber of agriculture North Rhine-Westphalia

CONSERVATION STRATEGY OF WILD SPECIES

ACCESSIONS OF VARIOUS FRUIT SPECIES IN GERMANY

- 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



- Per accession 2 trees
- Reduced pest management
- Replanting – after 20 years

- 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 ~~X~~ site, a greenhouse,
- culture stock ~~X~~ maintained *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 $-5 \pm 1^\circ\text{C}$ 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 $-1^\circ\text{C}/\text{h}$ 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).

Section	Species	No Tested Acc.	No Acc. Recovered	No Acc. Recovery ≥ 40%	% of Acc. Recovery ≥ 40%	Mean of Recovery %	SD. of Recovery	
Malus	<i>M. pumila</i>	2	2	2	100.00	65.00	20.00	
	<i>M. prunifolia</i>	49	48	41	83.67	63.00	25.73	
	<i>M. ×domestica</i>	34	34	25	73.53	57.56	28.86	
	<i>M. orientalis</i>	3	3	2	66.70	46.50	29.80	
	<i>M. sieversii</i>							
	<i>M. sylvestris</i>							
	<i>M. ×moerlandsii</i>	3	2	0	0.00	24.07	17.17	
	<i>M. spectabilis</i>	3	3	0	0.00	5.00	0.00	
Baccatus	<i>M. baccata</i>	4	3	3	75.00	72.50	42.06	
	<i>M. ×robusta</i>	3	3	2	66.70	65.47	22.04	
	<i>M. hupehensis</i>	2	2	1	50.00	38.60	33.60	
	<i>M. sikkimensis</i>	1	1	0	0.00	22.20		
	<i>M. ×hartwigii</i>	1	0	0	0.00	0.00	0.00	
	<i>M. halliana</i>	1	0	0	0.00	0.00	0.00	
		<i>M. ×sublobata</i>	3	3	2	66.70	73.33	27.18
	<i>M. komarovii</i>	3	3	2	66.70	58.33	24.61	
	<i>M. ×zumi</i>	3	3	2	66.70	55.00	24.83	
	<i>M. prattii</i>	1	1	1	100.00	50.00		
Sorbomalus	<i>M. sieboldii</i>	3	3	2	66.70	47.97	24.01	
	<i>M. floribunda</i>	4	4	2	50.00	36.50	31.05	
	<i>M. transitoria</i>	2	2	2	50.00	30.00	25.00	
	<i>M. toringoides</i>	3	3	1	33.30	22.60	12.51	
	<i>M. sargenti</i>	3	3	3	0.00	16.50	15.84	
	<i>M. fusca</i>	3	2	0	0.00	15.73	11.18	
	<i>M. florentina</i>	2	2	0	0.00	14.05	8.15	
	<i>M. yunnanensis</i>	1	1	0	0.00	10.00		
	Chloromeles	<i>M. ×soulardii</i>	1	1	1	100.00	95.00	
		<i>M. ×dawsoniana</i>	1	1	1	100.00	88.90	
<i>M. coronaria</i>		2	2	2	100.00	52.50	12.50	
<i>M. ioensis</i>		2	2	2	100.00	50.00	10.00	
Docyniopsis	<i>M. tschonokii</i>	1	0	0	0.00	0.00	0.00	
Eriolobus	<i>M. trilobata</i>	2	0	0	0.00	0.00	0.00	
Total		180	169	116	64.44	39.00	18.65	

Results: Recovery after cryopreservation using dormant bud wood method

- 180 acc. tested, 161 had living buds (94 %)
- Average of recovery 39%
- 116 acc. reached the 40% survival criterion corresponding to successful treatment (64%).

- Strong genotype effect!**
- Variation between species in one section
 - Variation within one species

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 long-term, 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>

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



JKI Dresden-Pillnitz:

A – Potted plants of the *ex situ* collection;

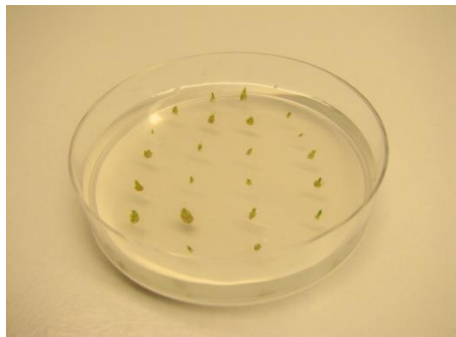
B – virus-free material in an insect-protected screen house

CRYOPRESERVATION OF *FRAGARIA* – PVS2 VITRIFICATION



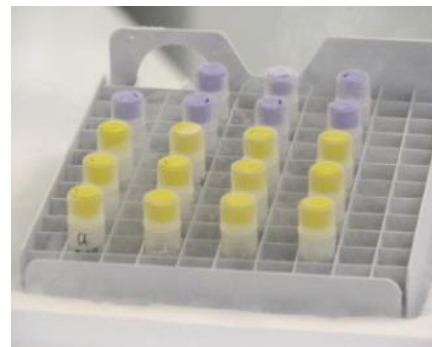
1. Preculture

- 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**



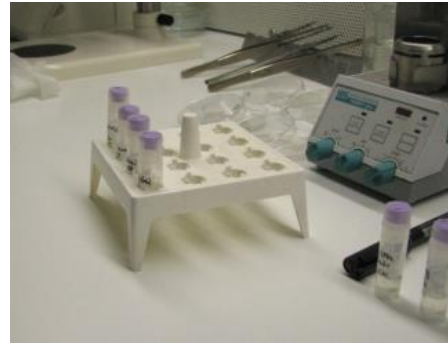
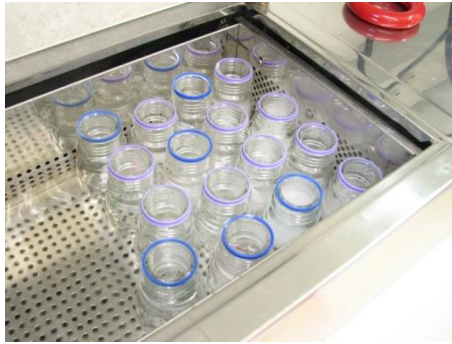
2. Selective dehydration

- 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**



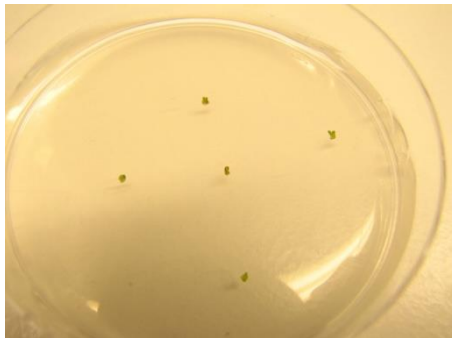
3. Cryo storage

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



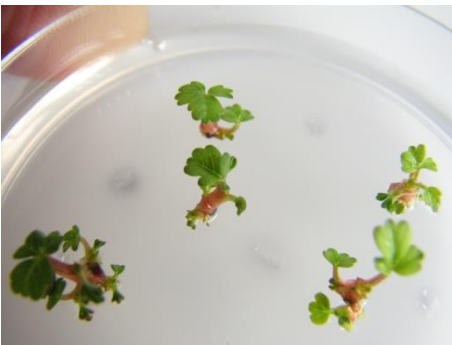
4. Rehydration

- 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

Results: Recovery after cryopreservation using PVS2 vitrification with cold acclimation

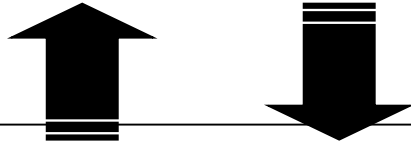
Species	No. accessions	Recovery (%)	SD
<i>Fragaria × ananassa</i>	128	87.31	14.10
<i>Fragaria bucharica</i>	3	80.00	5.00
<i>Fragaria chiloensis</i>	5	82.89	13.90
<i>Fragaria corymbosa</i>	2	86.88	9.72
<i>Fragaria gracilis</i>	2	80.00	0.00
<i>Fragaria iinumae</i>	2	90.00	7.07
<i>Fragaria mandshurica</i>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

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)

German Fruit Genebank

- A **decentralized gene bank network** for fruit genetic resources in Germany under central coordination of the JKI
- **Fruit species specific networks** for apple, cherry, strawberry, plum, *Rubus* and pear **cultivars**

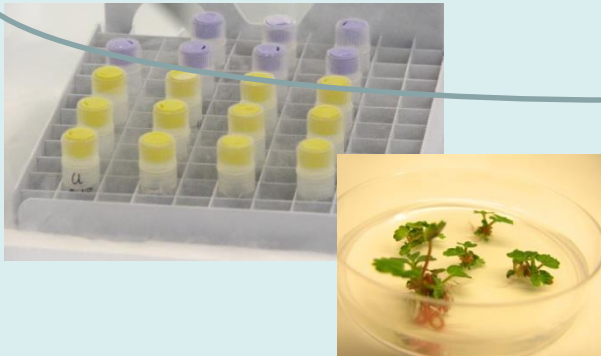


Cultivar collections

Part of the
German Fruit Gene bank



- **Field collections**
1500 cultivars (apple; pear; strawberry, cherry, plum)
- **Cryopreservation**
strawberry cultivars



Wild species collections

- **Field collections**
1,000 wild species accessions (*Malus*, *Fragaria*, *Prunus*, *Pyrus*)
- **Cryopreservation**
Fragaria accessions
Malus accessions
- *Pyrus* accessions



National Programme

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



www.bmelv.de

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

4.1.2 Deutsche Genbank Obst

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. Jahrhunderts zahlreiche



zur Forschungs- und Zuchtungswecke.



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 fruchtartsspezifische 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.



Many thanks for your attention!