

Seed Longevity and Survival of Seed Borne Diseases

after 30 Year's Conservation in Permafrost

REPORT FROM THE 100 YEAR STORAGE EXPERIMENT



Seed longevity and survival of seed borne diseases after 30 years conservation in permafrost. Report from the 100 year storage experiment

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Seed Longevity and Survival of Seed Borne Diseases after 30 Years Conservation in Permafrost

Report from the 100 Year Storage experiment

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Summary

The Nordic Gene bank established the 100 year seed storage experiment in Coal mine no. 3 outside Longyearbyen in 1986. Security duplicate samples of the Nordic seed collection had been deposited in permafrost in the coal mine since 1984.

The experiment was established with the aim to monitor the longevity of seeds in this Nordic back-up seed collection and to gain general knowledge about the longevity of seed stored under permafrost conditions, as well as studying the survival of seed borne plant pathogens.

The experimental set up included in total 41 seed lots of 17 agricultural and horticultural crop species commonly grown in the Nordic countries. The seed germination experiment included two or three varieties of each crop. The experimental part dedicated to studies of pathogen survival included seeds from 11 crops naturally contaminated by pathogens.

The test program comprises germination and pathogen survival tests every 2.5 years during the first 20 years and then every 5 years for the last 80 years. In total 25 identical sets of test seeds placed in sealed glass tubes were packed in wooden boxes, one box for each planned test year.

The tests have been carried out according to schedule and this report sums up the results from the first 30 years of the experiment. All tests have been carried out in accordance with the same ISTA-protocols.

The results show that 9 of the 17 species after 30 years had retained more than 90 percent of their initial germination percentage. Beet (*Beta vulgaris*), Onions (*Allium cepa*), Cucumber (*Cucumis sativus*) and Kentucky bluegrass (*Poa pratensis*) had retained between 97 and 99 percent. At the lower end of the scale, rye (*Secale cereale*) had lost 51 percent of the initial germination percentage. Among the other cereals, barley (*Hordeum vulgare*) showed the highest viability as it had kept 89 percent viability, whilst wheat (*Triticum aestivum*) had kept 79 percent of the initial germination percentage.

Mean germination of all test samples showed a drop from 87.2% at year 0 to 76.9% at year 30. The pathogen tests showed that all pathogens had survived over the 30 years, more or less at the same contamination levels as were detected at the start of the project.

To expand the knowledge about seed longevity under long-term seed storage it is recommended to establish a new more comprehensive experiment with seed materials from a wider selection of crops, and to include more replicates and seeds produced over more years, allowing more in-depth statistical studies of the longevity development. A new experiment should be placed in the Svalbard Global Seed Vault, in order to provide results relevant for optimal gene bank conservation methods at -18°C and giving direct data on the longevity of seeds stored in the Seed Vault.

1. Introduction

The Nordic Gene Bank for plants (NGB, now NordGen) has from its establishment in 1979 been conserving seeds of Nordic agricultural and horticultural crop plant varieties, landraces, wild relatives and genetic stocks. Normal gene bank activities related to conservation and use of plant genetic material have been performed. In 2008, NGB was merged into the Nordic Genetic Resource Centre (NordGen). Soon after the establishment of NGB in 1979, an initiative was taken to find a safe and low-cost storage for a duplicate safety collection of the most valuable material.

1.1. Conserving seeds in permafrost in Svalbard

In 1981, the NGB Board established a Permafrost Working Group to explore options for safety storage of seeds in permafrost. The aims were both to explore options for securing backup copies of the Nordic seed collections and to compare costs between permafrost storage and storage in freezers at NGB headquarters in Alnarp in southern Sweden.

The group consisted of Arne Wold, Director of the Norwegian State Seed Testing Station, Lennart Kåhre, Director of the Swedish Seed Testing and Certification Institute, and Flemming Yndgaard from NGB. It was included in the mandate for the group that possible international interest for cooperation should be explored (NGB Board minutes, July 1982).

Several options were considered, including storage in an inland ice cave in Greenland, mountain caves in the Norwegian mountain area Jotunheimen and a permafrost area located at Torneträsk in northern Sweden.

After consultations with the Norwegian state owned mining company Store Norske Spitsbergen Kulkompani (SNSK), abandoned coal mines in Svalbard were considered to be the optimal solution. Reasons for the decision were, despite the remote location, the good infrastructure and accessibility in Svalbard, low establishment and management costs compared to new constructions, lower temperatures than the other options and good structure stability for the storage facility compared to ice caves.

An abandoned transverse passage AT2 in Coal Mine no. 3 on the northern slope of Platåfjellet (Plateau Mountain) three kilometres west of the town of Longyearbyen was chosen for the purpose. The permafrost temperatures in air and rock were measured to be -3.6°C and -3.7°C , respectively (Yndgaard, 1985). The store would be independent of external energy supplies.

A double-pickled steel container, measuring 4.0x1.6x2.0 m, treated against corrosion, was placed in the abandoned transverse passage AT2 and secured for long term storage. To prevent galvanic corrosion the container was installed on a platform made of wood material, isolating the container from the mine floor. The container was placed about 200 metres inside the mine shaft and 70 metres below the soil surface (Yndgaard, 1985).

SNSK assisted with all practical operations, such as preparing the mine gallery for hosting the container and arranging the transport from the airport to AT2. A wooden wall was built to separate the container from the abandoned coal mine area and to protect against the draught of air and gas in the mine during ventilation. The transverse mine gallery allocated for the seed container was named Frøyhall, in honour of Frøy, the Nordic fertility god. *Frø* or *frö* is also the Scandinavian word for seed.

From the start, the cooperation between NGB and SNSK was based on verbal agreements and understanding. A formal agreement, including provisions for future assistance in maintenance and access to the seeds, was signed in 1984. An additional supplementary agreement concerning economic compensation from NordGen to SNSK for rent and assistance was signed in February 2017.

The first seed deposit in Svalbard was put in the steel container in Coal Mine no. 3 on 14th November 1984. Before shipment to Svalbard, seeds were dried to water content between three and six percent (Yndgaard, 1983), placed in sealed glass ampoules and packed in wooden boxes filled with insulation material at NGB. These first seed deposits were safety copies/duplicates of Nordic germplasm conserved in the Nordic Gene Bank seed collection in Alnarp in southern Sweden.

The back-up of the Nordic seed collection in permafrost in the coal mine in Svalbard inspired the discussions and later on the planning process for construction of the Svalbard Global Seed Vault as an international facility for storing safety samples of seeds conserved in gene banks. The experiment described and reported here is related to the storage of seeds in permafrost only, and the results are not relevant for evaluating seed longevity when stored at -18°C , as in the Svalbard Global Seed Vault.

The NGB safety seed collection was transferred from Coal Mine no. 3 to the Svalbard Global Seed Vault when this was opened in 2008. However, the material of the 100 year storage experiment is still kept in the steel container in the transverse AT₂ (Frøyhall) of Coal Mine no. 3.

1.2. The 100 year storage experiment

As a tool to monitor the seed longevity of materials in the safety seed collection, as well as gaining general knowledge about seed longevity under permafrost conditions, it was decided to establish a long term seed storage experiment in the same storage container. The experiment was established in 1986 with the aim to study the storage lifespan and survival of seed borne pathogens of orthodox seeds of common agricultural and horticultural crop species in the Nordic countries, stored under permafrost conditions (Yndgaard, 1985). The experiment was set up for the period until 2086 and was named *The 100 year experiment*. This report describes the background, materials and results from testing of samples after the first 30 years of the experiment.

Seeds for the experiment were packed in sealed glass ampoules, stored in wooden boxes and shipped to Svalbard in December 1986.

Between 1986 and 2016, staff from the Kimen Seed Laboratory (formerly the Norwegian State Seed Testing Station) or Nordic Gene Bank/NordGen visited Svalbard regularly for retrieving seeds for testing. On some occasions, withdrawal of test samples was conducted by SNSK. According to the test plan, seeds were taken out for testing every 2.5 years during the first 20 years. Since 2006, seeds have been taken out every five years.

2. Background and practical implementation of the 100 year experiment

The storage potential of seeds is influenced by pre- and post-harvest conditions, such as weather during seed maturation, seed dormancy and maturity at harvest, mechanical damage, moisture and vigour of seeds at the time of storage, in addition to genetic effects, e.g. variation among crop species and cultivars. However, seed moisture content and storage temperature are the most important factors (Justice and Bass, 1978).

Fungi infecting seeds when they are developing on the plants in the field (field fungi) may reduce the seed quality, and storage fungi can cause serious loss of viability when seeds are conserved in the seed store. Dry and cool conditions during storage may reduce such risks (Christensen, 1972). In order to obtain maximum seed quality, harvesting should be carried out after the seeds are fully mature (Probert *et al.*, 2007).

Low moisture and low temperature in storage will slow down the deterioration processes, and for maximum storage life, these two conditions must be kept low and carefully controlled. However, the storage time will still be limited. Cromarty *et al.* (1982) and Rao *et al.* (2017) discuss thoroughly the theories of seed longevity and give valuable references.

Low temperature is achieved in freezers and cold storage rooms; however, such facilities are vulnerable to failure in energy supplies. The coal mine in Svalbard was chosen for the NGB security backup collection and for the 100 year experiment because it provided permafrost independent of any energy supply and ensured the same physical conditions during a long storage period.

The NGB Board decided on August 7th 1985 to approve the experiment plan for a 100 year experiment prepared by the *Permafrost Working Group*.

It was agreed that all germination and disease analyses should be carried out in Norway in order to avoid possible problems with cross border trans-

fers of seed samples from the permafrost storage to the analysis institute since Norway has had the sovereignty of Svalbard since August 14th 1925. Hence, the seeds have been analysed for germination capacity, moisture and diseases at Kimen Seed Laboratory in Norway. The analyses of lettuce mosaic virus have been conducted at the Norwegian Institute of Bioeconomy Research in Ås.

2.1. Experimental design and materials

In total, samples from 41 seed lots of 17 agricultural and horticultural crop species commonly grown in the Nordic countries were included in the experiment. The study was divided into two series. Series A is for studying the development of germination capacity and moisture content in some of the seed lots during the first 10 years and comprises 15 species with two cultivars of each, except for pea which is represented by three cultivars, i.e. altogether 31 seed lots/cultivars (Table 1).

Series B considers the survival of 14 common seed borne pathogens found in Nordic Crops, in addition to development of germination capacity, in ten crop species, each represented by one seed lot/cultivar (Table 2). It was considered most appropriate to use naturally infected seeds. In addition, one sample of fungal survival structures (*sclerotia*) was included in series B.

Most materials for series B were selected among Norwegian seed lots. These were identified in routine seed health tests at the Norwegian State Seed Testing Station. In addition, seed laboratories in the Netherlands and United Kingdom provided infected seed of lettuce and carrot (seed lot B-7 and B-9), and beet (B-10), respectively. Agriculture Canada's Research Station in Winnipeg, Manitoba supplied wheat seed (B-2) infected by loose smut (*Ustilago nuda* f sp *tritici*). To study longevity of sclerotia, one sample of sclerotia of stem rot (*Sclerotinia sclerotiorum*) cleaned out from a Norwegian *Brassica oleracea* seed lot was included (B-3).

To ensure that the same methods of estimating the germination capacity and the detection of seed borne pathogens are practiced over the whole 100-year period, a complete paper copy description of the experimental

layout and procedures is kept along with the stored material in each of the wooden boxes with ampoules. Following the same procedures and test methods through the whole experiment is crucial for the scientific value of the results.

Table 1. Crop species, cultivars and country of origin of seed materials included in the seed longevity investigations in the 100 years storage experiment in permafrost (Series A). Each crop is represented by two cultivars, but with three cultivars for pea (*Pisum sativum*).

Sample identity	Crop / species	Cultivars (country of origin) ¹
A-1-1/A-1-2	Barley (<i>Hordeum vulgare</i>)	Inga Abed (DNK), Tunga (NOR)
A-2-1/A-2-2	Wheat (<i>Triticum aestivum</i>)	Vakka (FIN), Solid (SWE)
A-3-1/A-3-2	Rye (<i>Secale cereale</i>)	Petkus II (DNK), Voima (DNK)
A-4-1/A-4-2	English ryegrass (<i>Lolium perenne</i>)	Pippin (DNK), Riikka (FIN)
A-5-1/A-5-2	Timothy (<i>Phleum pratense</i>)	Tammisto (FIN), Bodin (NOR)
A-6-1/A-6-2	Kentucky bluegrass (<i>Poa pratensis</i>)	Hankkijan Kyösti (FIN), Annika (DNK)
A-7-1/A-7-2	Red clover (<i>Trifolium pratense</i>)	Jokioinen (FIN), Molstad (NOR)
A-8-1/A-8-2/ A-8-3	Pea (<i>Pisum sativum</i>)	Weitor (SWE), Hankkijan Hemmo (FIN), Weitor pt. 10468 (SWE)
A-9-1/A-9-2	Beet (<i>Beta vulgaris</i>)	311 N typ (SWE), 70500 (DNK) ²
A-10-1/A-10-2	Oilseed rape (<i>Brassica napus</i>)	Jupiter (SWE), Linrama (DNK)
A-11-1/A-11-2	Bulb onion (<i>Allium cepa</i>)	Hamund (SWE), Owa (DNK)

A-12-1/A-12-2	Lettuce (<i>Lactuca sativa</i>)	Attraktion Sana (DNK), Hilro (SWE)
A-13-1/A-13-2	Cucumber (<i>Cucumis sativus</i>)	Langelands gigant (DNK), Rhensk Druv (SWE)
A-14-1/A-14-2	Carrot (<i>Daucus carota</i>)	Nantes Fancy (DNK), Regulus (SWE)
A-15-1/A-15-2	Cauliflower (<i>Brassica oleracea v. botrytis</i>)	Svavit (SWE), Pari (DNK)

¹ Country codes according to ISO 3166-1 alpha-3 (Wikipedia 2019)

² Material from Nordic sugar beet breeding programmes

Table 2. Plant pathogens tested for survival in the 100 years storage experiment in permafrost (Series B). The table shows the respective crop species, cultivars and the country of origin of seed materials.

Sample identity	Pathogen species	Crop species	Cultivar (country of origin) ¹
B-1	<i>Septoria nodorum</i> , <i>Fusarium spp.</i>	Wheat (<i>Triticum aestivum</i>)	Runar (NOR)
B-2	<i>Ustilago nuda f.sp. tritici</i>	Wheat (<i>Triticum aestivum</i>)	Line 79 CBW "A" No 72 (CAN)
B-3	<i>Sclerotinia sclerotiorum</i>	Sclerotia from <i>Brassica/Cabbage</i> ²	(NOR)
B-4	<i>Drechslera spp.</i> / <i>Fusarium spp.</i>	Barley (<i>Hordeum vulgare</i>)	Bamse (NOR)
B-5	<i>Drechslera dictyoides</i>	Meadow fescue (<i>Festuca pratensis</i>) ³	Salten (NOR)
B-6	<i>Drechslera phlei</i>	Timothy (<i>Phleum pratense</i>)	Forus (NOR)
B-7	Lettuce mosaic virus	Lettuce (<i>Lactuca sativa</i>)	Attractie (NLD)
B-8	<i>Botrytis allii</i> / <i>Fusarium spp.</i>	Bulb onion (<i>Allium cepa</i>)	Laskala (NOR)

B-9	<i>Alternaria radicina</i> <i>Alternaria dauci</i>	Carrot (<i>Daucus carota</i>)	Forto Nantes (NLD)
B-10	<i>Phoma betae</i>	Beet (<i>Beta vulgaris</i>)	Hilma (GBR)
B-11	<i>Alternaria brassicicola</i>	Cabbage (<i>Brassica oleracea ssp. capitata f. alba</i>)	Trønder Lunde (NOR)

¹ Country codes according to ISO 3166-1 alpha-3 (Wikipedia 2019)

² *Sclerotia* infecting the genus *Brassica* were collected from cabbage (*Brassica oleracea*) and conserved separately in the ampoules, without any seed material

³ According to current taxonomy, the name is *Schedonorus pratensis* (Huds.) P.Beauv.

The seed materials for the experiment were dried to 3–5 % moisture content according to seed material storage procedures in the Nordic Gene Bank. Each seed lot was divided into 25 sub-samples, one for each testing date during the 100 years (Table 3). Each sub-sample consisting of 1,000 seeds were divided and encapsulated in two glass ampoules with 500 seeds in each.

A set of sub-samples from each seed lot was placed in a wooden box (50x25x25 cm) together with packing materials to protect the glass ampoules during transport. Altogether, 25 boxes were prepared. Each box was labelled with the date for withdrawal.

The boxes were transported by air to Longyearbyen, and placed in the steel container in the abandoned gallery of Cole Mine no 3, where NGB already had its safety collection of Nordic seeds.

Every two and a half year during the first 20 years and every five years over the next 80 years, a series of samples (one wooden box) is taken out for analyses. The first series of samples were analysed in December 1986 (year0 = y0). The last box will be analysed in December 2086 (y100). During the first 30 years of the experiment eleven boxes have been retrieved and seeds analysed at Kimen Seed Laboratory in Ås (Table 3).

Table 3. *Dates for retrieval of samples and types of analyses.*

Year	Date	Germination (series A and B)	Seed health (series B)	Moisture (series A)
y0	December 1st 1986	X	X	X
y2.5	June 1st 1989	X	X	X
y5	December 1st 1991	X	X	X
y7.5	June 1st 1994	X	X	X
y10	December 1st 1996	X	X	X
y12.5	June 1st 1999	X	X	
y15	December 1st 2001	X	X	
y17.5	June 1st 2004	X	X	
y20	December 1st 2006	X	X	
y25	December 1st 2011	X	X	
y30	December 1st 2016	X	X	
y35	December 1st 2021	X	X	
y40	December 1st 2026	X	X	
y45	December 1st 2031	X	X	
y50	December 1st 2036	X	X	

y55	December 1st 2041	X	X	
y60	December 1st 2046	X	X	
y65	December 1st 2051	X	X	
y70	December 1st 2056	X	X	
y75	December 1st 2061	X	X	
y80	December 1st 2066	X	X	
y85	December 1st 2071	X	X	
y90	December 1st 2076	X	X	
y95	December 1st 2081	X	X	
y100	December 1st 2086	X	X	

Upon arrival at the laboratory, the samples were placed at 10°C for a few days. Then the glass ampoules were broken with a hammer and the seeds exposed to room temperature and air moisture for a few days, before the start of the germination and seed health analysis.

The methods for germination and seed health analysis follow the methods established by the International Seed Testing Association (ISTA 1985, 2017). ISTA was established in 1924 and plays an important role in establishing internationally agreed methods for seed sampling and testing. ISTA has more than 130 accredited member laboratories worldwide, and Kimen Seed Laboratory has been a member since the foundation of ISTA.

2.2. Germination test methods

Analysis of the germination potential of seeds is performed under controlled standardised conditions, which are found to be optimal for a given species (Table 4). The first step in germination analysis is to facilitate the conditions for the seed to absorb water.

Table 4. Germination conditions specified in the experiment protocol.

Species	Germination substrate	Temperature °C	First count (days)	Final count (days)
<i>Hordeum vulgare</i>	BP ¹	20	4	7
<i>Triticum aestivum</i>	BP	20	4	8
<i>Secale cereale</i>	BP	20	4	7
<i>Lolium perenne</i>	TP ¹	20<=>30 ²	5	14
<i>Phleum pratense</i>	TP	15<=>25 ²	7	10
<i>Poa pratensis</i>	TP	15<=>25 ²	10	28
<i>Trifolium pratense</i>	TP	20	4	10
<i>Festuca pratensis</i>	TP	20<=>30	7	14
<i>Pisum sativum</i>	S ¹	20	5	8
<i>Beta vulgaris</i>	PP ¹	20	4	14
<i>Brassica napus</i>	TP	20	5	10 ³
<i>Allium cepa</i>	TP	15	6	14 ⁴
<i>Lactuca sativa</i>	TP	20-light	4	7
<i>Cucumis sativus</i>	TP	20<=>30 ²	4	8
<i>Daucus carota</i>	TP	20	7	14
<i>Brassica oleracea v. botrytis</i>	TP	20<=>30 ²	5	10

¹ BP=Between paper, TP=top of paper, S=sand, PP=Pleated paper

² <=> indicates alternating temperatures, low temperature for 16 hours, high temperature for 8 hours

³ ISTA rules 1985 describe 7 days for final count of *Brassica napus*

⁴ ISTA rules 1985 describe 12 days for final count of *Allium cepa*

The assumed optimal conditions for germination may change with new knowledge. However, the germination methods specified by ISTA for all the sixteen species included in this experiment have been the same since commencement in 1986 (ISTA, 1985, ISTA 2017). In the case of *Brassica napus* and *Allium cepa* the number of days to final count specified in the experiment protocol is higher than the number of days described in the ISTA-rules (Table 4).

The standard ISTA-method is to germinate 400 seeds from one sample, as replicates of 4x100 or 8x50 seeds. The percentage germinated seeds from each replicate are compared to the mean of all replicates. If one replicate shows unacceptable discrepancy from the mean, according to established tolerance limits in the described method, the sample is retested.

In this study, four core conditions for germination have been followed according to the ISTA-methods:

- i) Germination substrate
- ii) Temperature
- iii) Light
- iv) Germination time and assessment of seedlings

2.2.1. Germination substrate

In germination analysis the seeds are placed on a substrate, usually filter paper or sand, where the seed imbibe and germinate. Some species may be germinated in both sand and paper. In general, paper is preferred. In this study, *Pisum sativum* is germinated in sand whereas specially developed germination test paper is used for the other species.

Three different types of filter paper have been applied in this study;

- a) Paper towels; seeds are spread on a moist paper towel with another towel on top. The towels are rolled together and the roll is placed vertically within a plastic bag. Inside the roll, the seeds will germinate and the seedlings develop. During the germination period the roll does not need any supplement of water. This method is used for cereal seeds.

- b) On top of a circular piece of filter paper; the piece is laid on a specially designed water table, called a Jacobsen germination table. The table has a pool of water underneath a benchtop with slots, allowing a paper strip attached to each germination paper to act as a wick from the water pool to the seeds on top. Red clover, all grass and vegetable species, except beet, are germinated in this way.
- c) Pleated filter paper; the seeds germinate in the pleats, used for seeds of *Beta vulgaris*.

2.2.2. Temperature

For some species, constant ambient temperature is optimal, whereas other species respond to alternating temperatures, for instance 20°C for 16 hours and 30°C for 8 hours (Table 4). In Jacobsen germination tables, the temperature is controlled by adjusting the water temperature.

2.2.3. Light

The only species included in the study, requiring light for germination is *Lactuca sativa*. Light is for most species not required. However, illumination is recommended for better-developed seedlings and the tested seeds are therefore given eight hours light. In the absence of light, the seedlings will become etiolated.

2.2.4. Germination time and assessment of seedlings

After a certain period essential morphological structures have developed and the seedlings are categorized into normal seedlings, abnormal seedlings, dead seeds, fresh healthy un-germinated seeds and hard seeds. Normal seedlings have the potential for development into a vigorous plant, if grown under favourable conditions in the field. Abnormal seedlings have developed certain structures but do not show the potential for continuing development into vigorous plants. Dead seeds have a dead embryo and no structures have developed. Dormancy, either expressed as fresh healthy un-germinated seeds or as un-germinated hard seeds has in this experiment been observed in red clover and in a very few cases in pea, where hard seeds have been encountered.

Preceding the final assessment (final count), the numbers of normal seedlings emerging at an early stage are counted (first count). A germination analysis is a test of germination potential under optimal conditions. However, the number of normal seedlings at first count may serve as an indicator of the seed vigour and how long seeds will retain a high germination rate. At the beginning of the study, the figures at first count were not reported. From test y30 results from first count have been registered, however, not included in this report.

2.3. Seed health test methods

The seed borne pathogens of the samples in series B were analysed either by incubation of seeds on moist paper (blotters), on artificial growth substrate (agar plates) in petri dishes, or by recording symptoms of disease on plants grown in soil. Unless otherwise specified a sample of 400 seeds was analysed.

Samples of species *T. aestivum*, *H. vulgare*, *F. pratensis*, *P. pratense*, *A. cepa*, *D. carota* and *B. oleracea* were analysed for their respective seed borne pathogens using the "freezing blotter method" modified after the ISTA descriptions at the time when the experiment was started (ISTA 1985). In brief, seeds are placed on moist filter paper in plastic dishes with a transparent lid, for 24 hours in room temperature to allow imbibition, followed by 24 hours at -20°C, and subsequently incubated five days at 20 +/- 10°C with alternating 12 hours near ultraviolet (NUV) light and 12 hours darkness.

After incubation, the seeds were examined for the presence of fungi under a stereo microscope at 6-50X magnification. When examination at higher magnifications (400X) was necessary for identification, microscope slides were prepared. Identification of the fungi was based on the morphological characters of spores and fruiting bodies developed on seed. Results were recorded by counting seeds infected by the investigated pathogens. The percent infection by each fungus in a sample was calculated from the number of seeds tested.

The sample of *B. vulgaris* was analysed by incubation of seeds on water agar (WA) with cotton blue staining (ISTA, 1985). Seeds showing characteristic mycelium structures from the fungus *Phoma betae* were recorded as infected.

The sample of *L. sativa* was analysed for lettuce mosaic virus (LMV) by sowing the seeds in soil in trays. The trays were placed in a greenhouse at approximately 18–20°C. When emerged plants had 2–3 leaves, the plants were evaluated for symptoms of lettuce mosaic virus. Each plant showing characteristic symptoms as well as plants with weak signs of infection was tested separately for LMV using the ELISA method (Clarke and Adams, 1977). Plants testing positive with this method were recorded as infected. Percent infection was calculated from the number of emerged plants.

The sample of *T. aestivum* was analysed for loose smut (*Ustilago nuda*) by sowing 1,000 seeds in soil in trays. The trays were placed in a greenhouse at 10°C until emergence. Thereafter the trays were moved to 15°C under light during daytime (16 hours) and darkness during night (8 hours). At heading time the number of plants showing smut symptoms were recorded and percent infection was calculated from the number of emerged plants.

The viability of *S. sclerotiorum* sclerotia was analysed by placing them on agar plates (potato dextrose agar) in petri dishes, after surface disinfection in NaOCl (1 % available Cl) for 10 minutes. One sclerotium was placed in each dish. The number of sclerotia out of 20 in each sub-sample showing mycelial growth after incubation at 20°C for one week was recorded.

3. Results

Germination results are obtained from both series A and B. The results are presented as average germination percentage from four analysed parallels carried out on the same seed sample. If test results from the four parallels vary over limits stated in the applied ISTA-protocols the results are not approved and the tests have to be repeated. Test results from the survival of seed born plant diseases are obtained from series B.

3.1. Germination

Seed germination results are presented below for six crop groups; cereals (Figure 1), grasses (Figure 2), legumes (Figure 3), *Beta* and *Brassica* cultivars (Figure 4), bulb onion and lettuce (Figure 5) and cucumber and carrot (Figure 6).

After 30 years the 100 year experiment has revealed considerable differences between crop groups, but also differences between species and cultivars within the same species were observed. The most long-lived seeds in this experiment, displayed as minimal loss of germination percentage over 30 years, have been the vegetable seeds: cucumber, lettuce, onion, beet, cauliflower and carrots.

The picture is more varied within cereal, grass and legume species. Among cereals, barley seeds have maintained a high level of seed germination over 30 years. Wheat and rye have declined significantly in viability, in particular during the last ten years. Among the grasses some species and some cultivars have maintained viability while others have declined. Among legumes, red clover has maintained germination ability very well whereas the three cultivars of pea show mixed results.

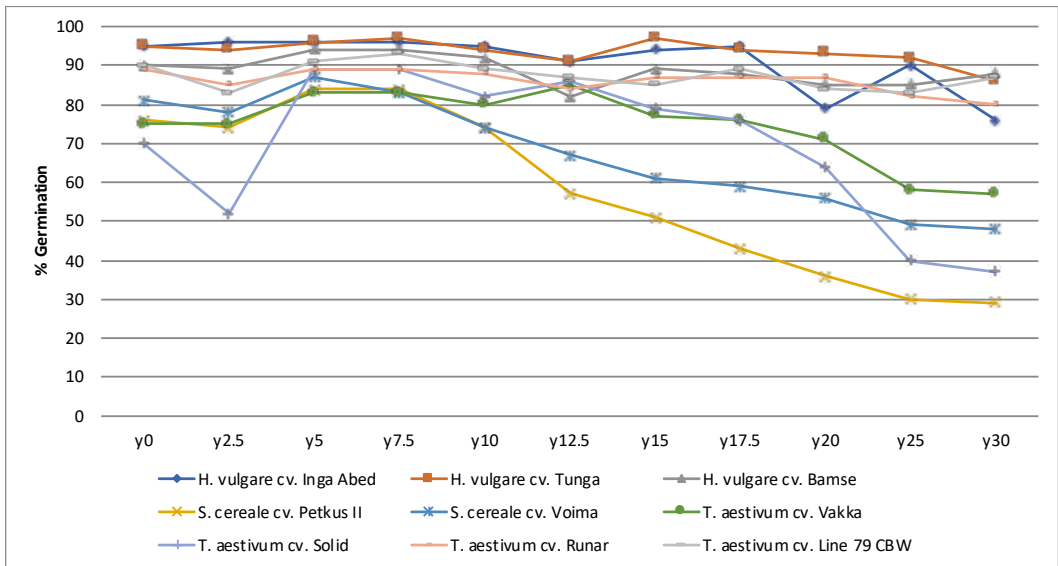


Figure 1. The germination percentages for cereal crops (samples from Series A and Series B); three cultivars of barley (*Hordeum vulgare*), two cultivars of rye (*Secale cereale*) and three cultivars of wheat (*Triticum aestivum*) conserved in permafrost over 30 years.

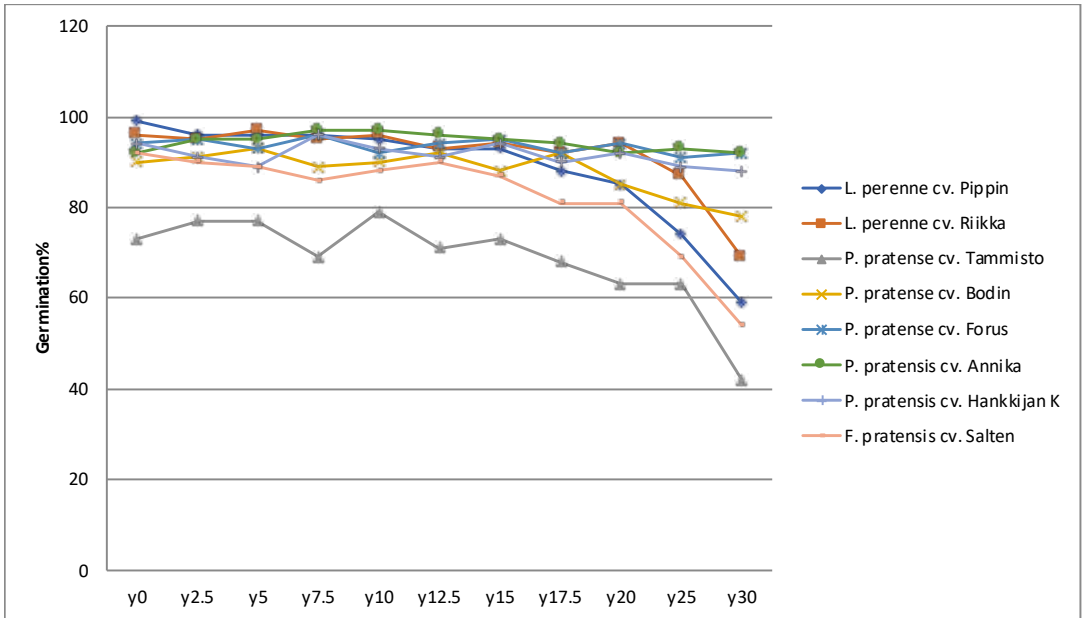


Figure 2. The germination percentages for grass species (samples from Series A and Series B); two cultivars of perennial ryegrass (*Lolium perenne*), three cultivars of timothy (*Phleum pratense*), two cultivars of Kentucky bluegrass (*Poa pratensis*) and one cultivar of meadow fescue (*Festuca pratensis*) conserved in permafrost over 30 years.

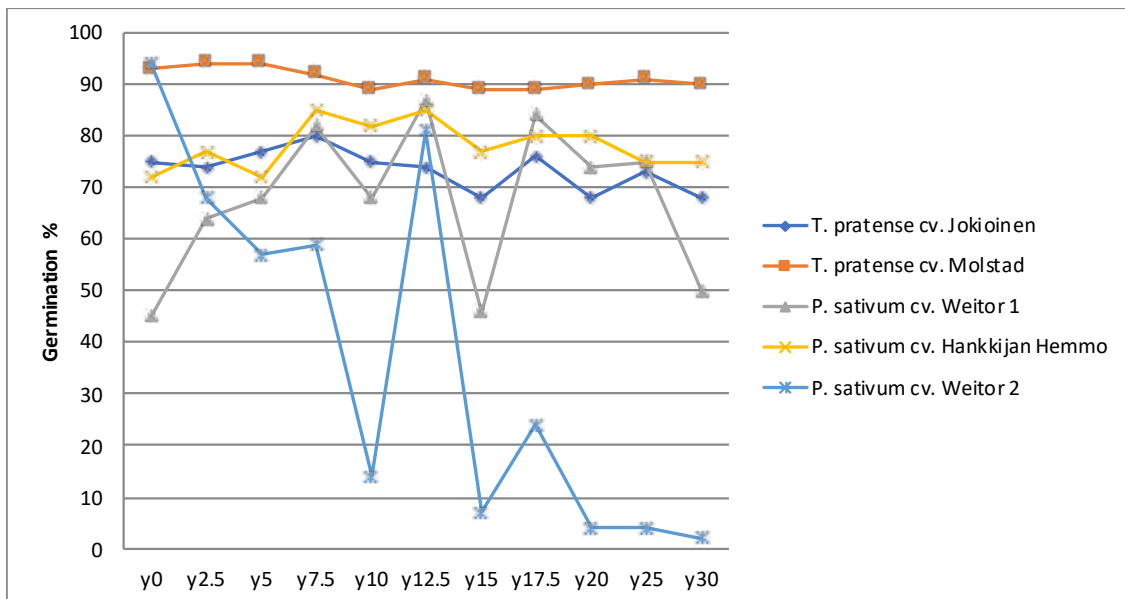


Figure 3. The germination percentages for legume species (samples from Series A); two cultivars of red clover (*Trifolium pratense*) and three cultivars of peas (*Pisum sativum*) conserved in permafrost through 30 years. Dormant and healthy hard seeds have been included in the germinated seeds.

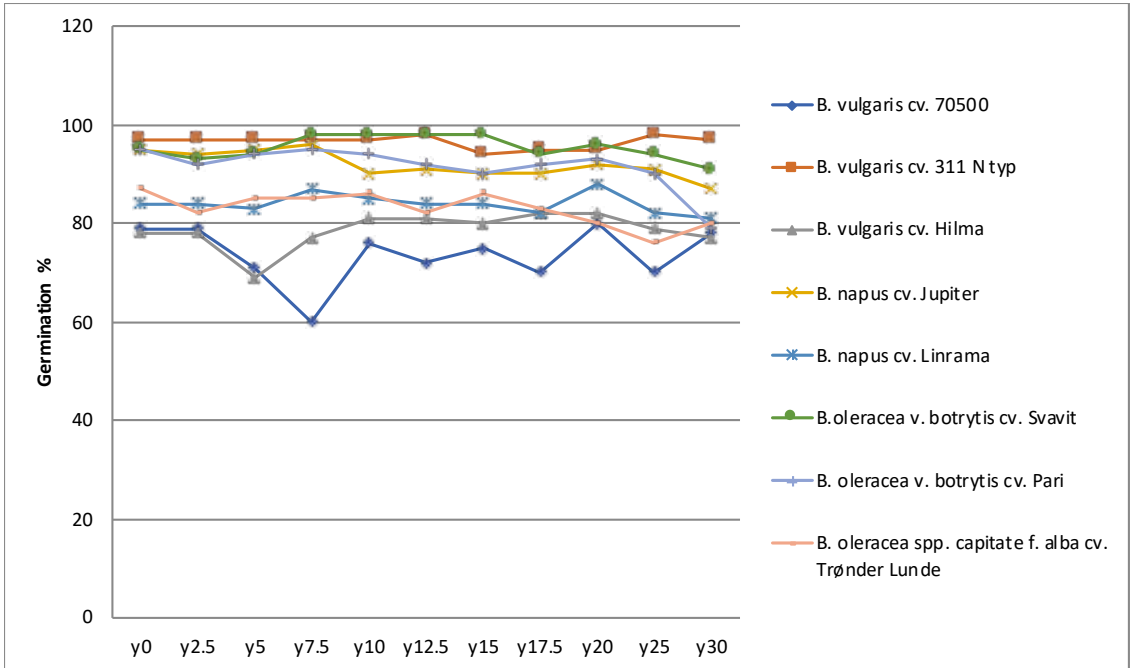


Figure 4. The germination percentages for three cultivars of beets (*Beta vulgaris*), two cultivars of oilseed rape (*Brassica napus*), two cultivars of cauliflower (*Brassica oleracea* v. *botrytis*) and one cultivar of white cabbage (*Brassica oleracea* ssp. *capitata* f. *alba*) conserved in permafrost through 30 years (samples from Series A and Series B).

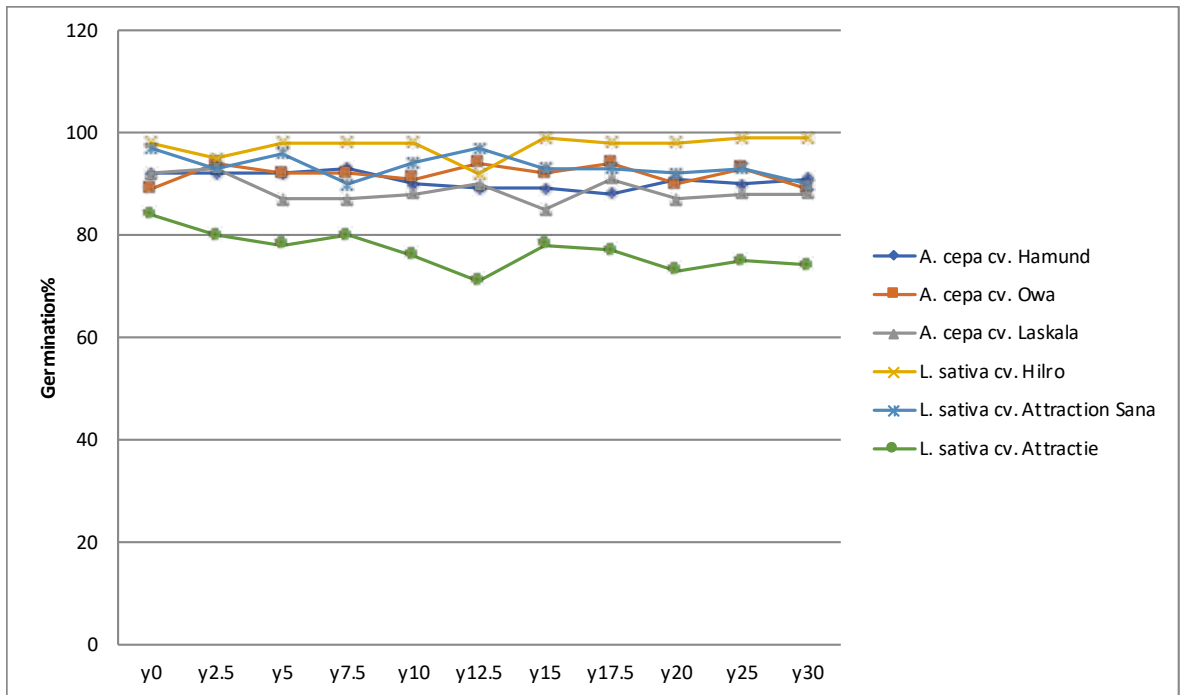


Figure 5. The germination percentages for three cultivars of bulb onion (*Allium cepa*) and three cultivars of lettuce (*Lactuca sativa*) conserved in permafrost through 30 years (samples from Series A and Series B).

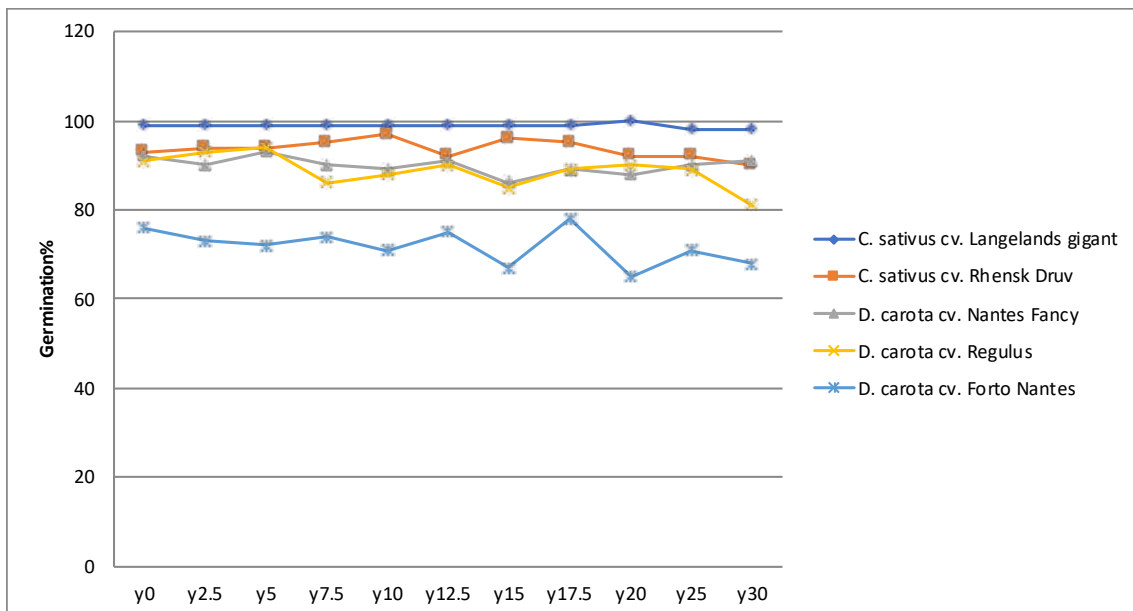


Figure 6. The germination percentages for two cultivars of cucumber (*Cucumis sativus*) and three cultivars of carrots (*Daucus carota*) conserved in permafrost through 30 years (samples from Series A and Series B).

Observed changes in germination percentage over 30 years for all 41 cultivars of 17 crops are displayed in Figure 7. Species showing the highest loss of germination percentage are grouped from the top of the figure. Remaining germination percentage after 30 years storage as average of the cultivars included is shown in Figure 8. Remaining germination ability as percentage of initial germination is showed in Figure 9.

Six samples show no loss in germination over 30 years (Figure 9). Among these, we find two of the pea samples, which is very contradictory to the third pea sample, which has lost most of its germination ability. Another large group of 28 samples has retained between 70 and 100 % of its initial germination ability. Six samples has retained between 38 and 60 percent of its initial germination percentage.

Samples showing the most significant loss in germination are both samples/cultivars of the crops rye (*S. cereale*) and rye grass (*L. perenne*), two of four samples of wheat (*T. aestivum*), and the only sample of meadow fescue (*F. pratensis*). Results for the three samples of timothy (*P. pratensis*) differ significantly as the cultivar 'Tammisto' has lost 42.5 % of its initial germination while the cultivars 'Bodin' lost 13.3% and 'Forus' lost only 2.1% (Figure 9).

Species not commented in particular above show quite similar results for the two or three cultivars involved in the experiment.

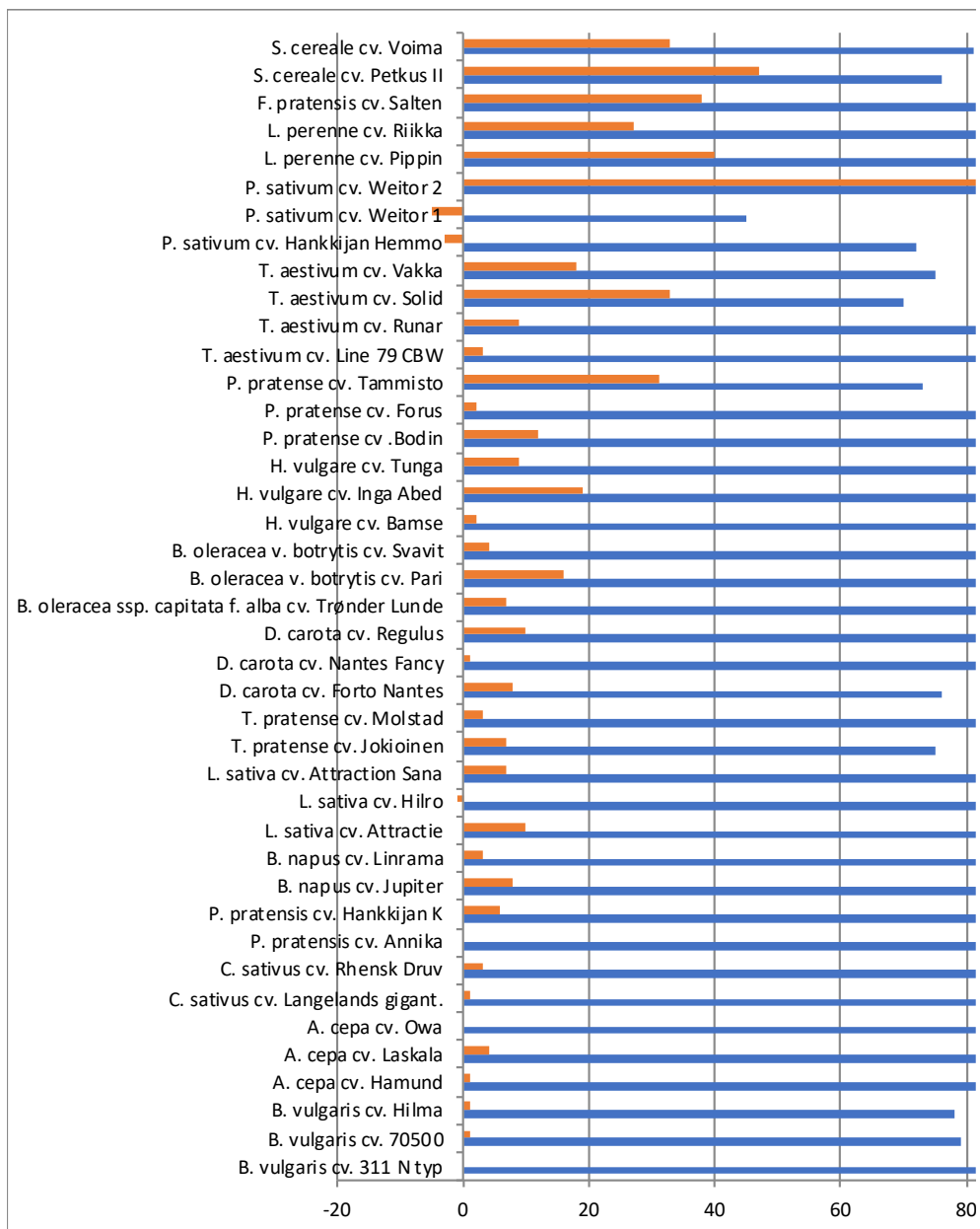


Figure 7. Loss in germination percentage (red bars) in seeds of 41 cultivars of 17 crop species over 30 year, ranged species wise. Species showing the average largest loss in germination grouped from the top of the graph. Blue bars indicate germination percentage at y0 (negative bars indicate higher germination percentage at y30 than at y0).

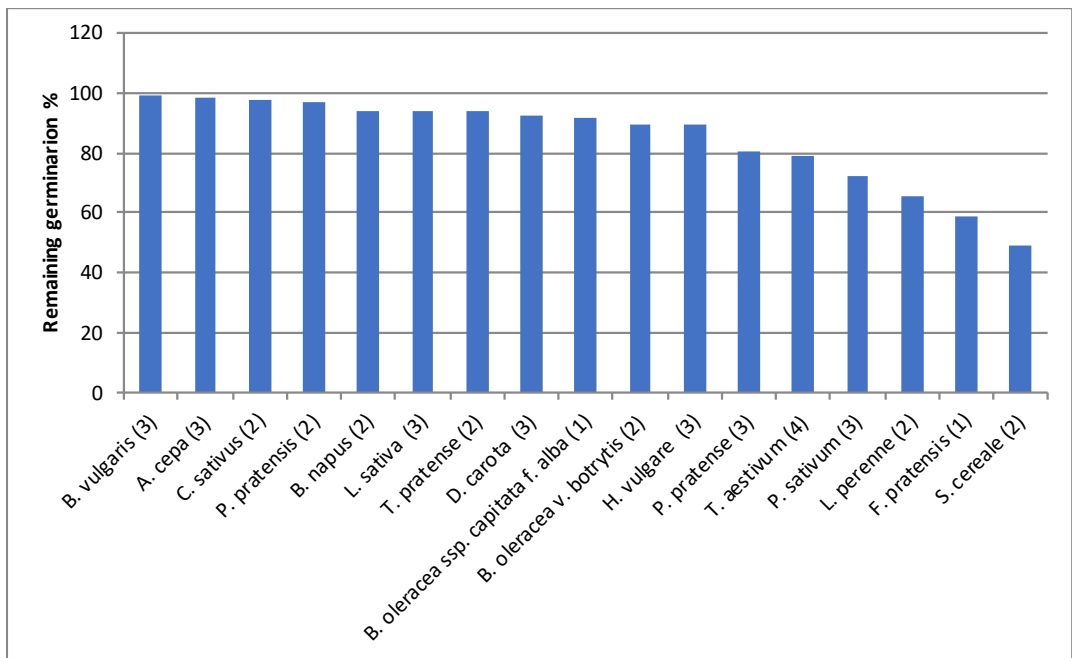


Figure 8. Remaining germination percentage of 17 crop species after 30 years storage as average of the cultivars included (number of cultivars in brackets). The results for pea (*Pisum sativum*) show the average for two cultivars with very high germination and one cultivar with close to zero germination. (See Figure 9 for details).

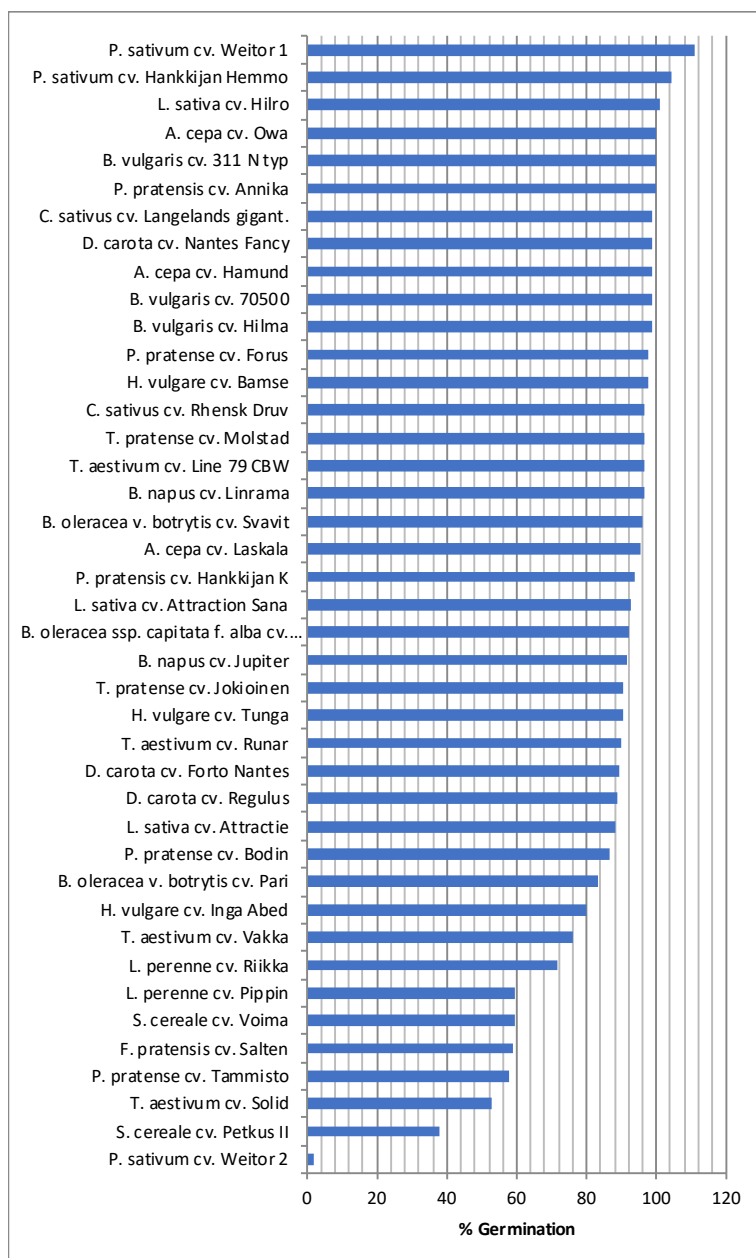


Figure 9. Remaining germination ability after 30 years of storage in permafrost, shown as percentage of initial germination percentage upon the establishment of the experiment in 1986. Species from the top show the lowest loss in germination over 30 years. Three samples show an increase in germination after 30 years in these tests.

Compiling data for all test samples (species and varieties) indicates the overall change in germination ability over 30 years. Means and standard deviation of 40 samples are displayed in Figure 10. The sample of *P. sativum*, cultivar Weitor 2 has been removed from the data set as this sample shows inexplicable results and variations, differing considerably from the other samples.

Average germination percentage for 40 test samples have decreased from 87.2 % to 76.9 % over the 30 years. A major part of the reduction has occurred during the last ten years as the average after 20 years still was 83.1 %.

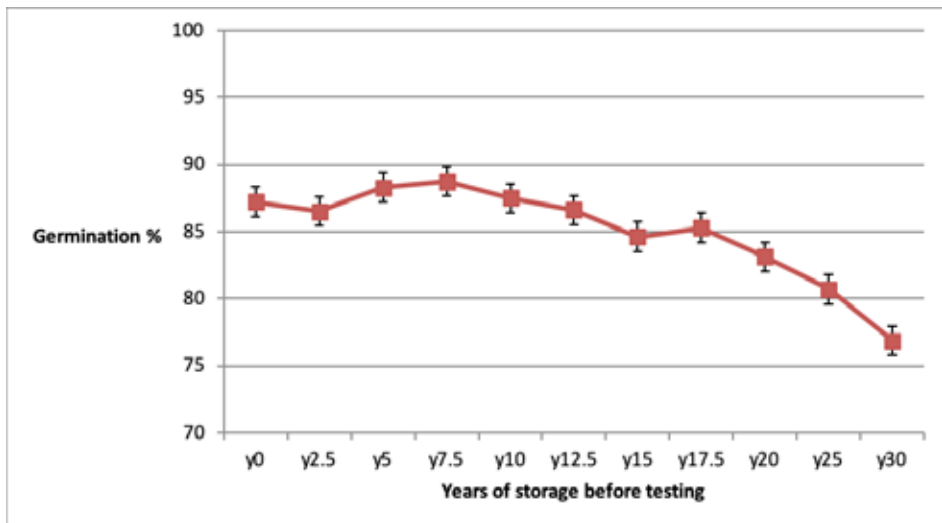


Figure 10. Mean germination of all accessions (except *P. sativum* cv. Weitor 2) at the different test occasions from y0 to y30. The square dotted red line indicates the mean values. The standard deviation and the confidence values are shown above and below.

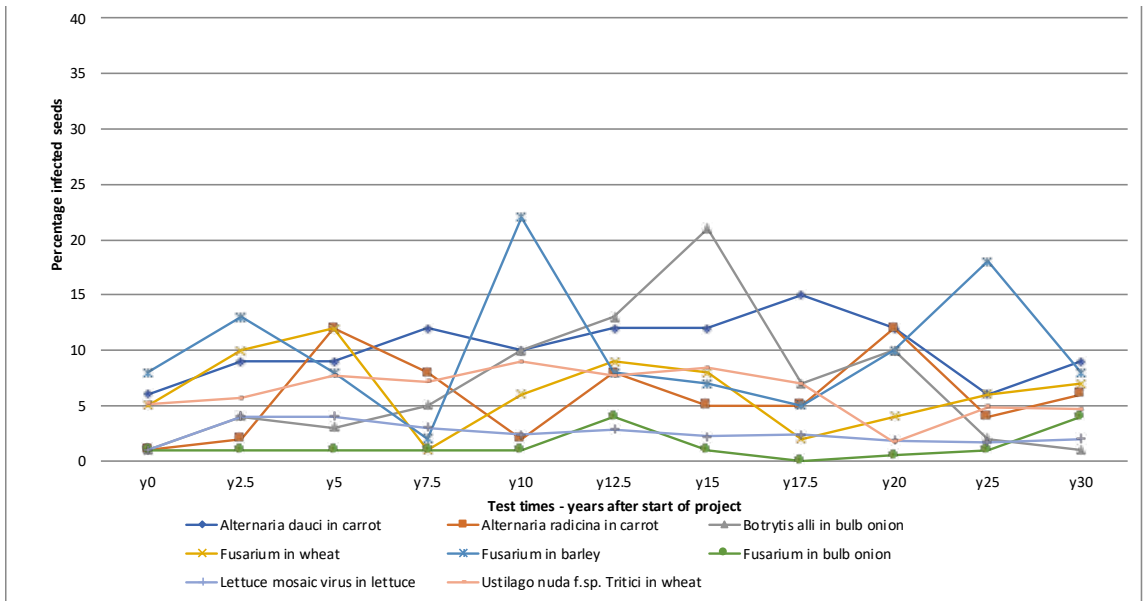


Figure 11. Fluctuations in seed infection by eight seed borne pathogens over 30 years. *Fusarium* spp. have been analysed in seed samples from three host plants.

3.2. Seed borne diseases

The initial infection level of the seed borne pathogens varied considerably among the different crop species included in the experiment (Figures 11 and 12). However, after 30 years of storage in permafrost the infection levels were in general only slightly changed in most of the species, although some variation in the levels were observed over the years.

All investigated pathogens were still detected after 30 years of storage also when the initial infection level was low, as for *B. allii* and *Fusarium* spp. in onion, *A. dauci* and *A. radicina* in carrot, *Fusarium* spp. in barley and wheat, *U. nuda f. sp. tritici* in wheat, and LMV in lettuce (Figure 11). For some of the pathogens with higher infection levels, like *D. dictyoides* in meadow fescue and *P. nodorum* in wheat, a trend towards reduced level was observed, whereas for *Phoma betae* in beet a trend towards increased infection was observed, compared to the level at the start of the experiment (Figure 12).

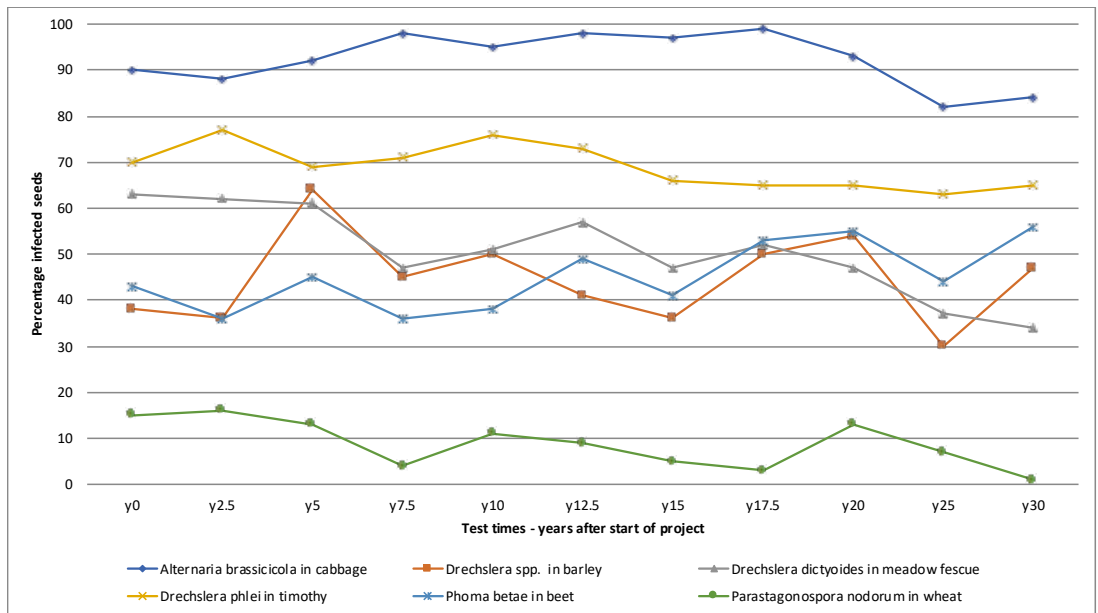


Figure 12. Fluctuations in seed infection by six pathogens over 30 years.

3.3. Survival of sclerotia

Along with analysis of pathogens present in seed samples, sclerotia of the species *Sclerotinia sclerotiorum* derived from a *Brassica* seed lot have been stored under the same conditions as the seeds. Samples consisting of 20 sclerotia have been tested for viability as described above. The results show considerable fluctuations over the years (Table 5). It can, however be concluded that a significant number of sclerotia can survive for 30 years in permafrost conditions. In some samples, the sclerotia were totally or partly damaged by saprophytes.

Table 5. Survival of sclerotia (*Sclerotinia sclerotiorum*) from Brassica during 30 years of storage in permafrost.

Test year	Number sclerotia analysed	Number germinating sclerotia	% germination
y0	11	10	91
y2.5	20	20	100
y5	20	20	100
y7.5	All sclerotia damaged by saprophytes		
y10	All sclerotia damaged by saprophytes		
y12.5	20	13	65
y15	17	12	71
y17.5	20	12	60
y20	20	0	0
y25	20	8	40
y30	20	12	60

3.4. Moisture content in the seeds

The seed samples in Series A of barley (*Hordeum*), wheat (*Triticum*), rye (*Secale*), pea (*Pisum*), beet (*Beta*) and cucumber (*Cucumis*) were analysed for moisture contents during the first ten years of the experiment (Table 6).

Table 6. Moisture content of analysed seed samples during the first ten years of the experiment. Two varieties of barley (*Hordeum*), wheat (*Triticum*), rye (*Secale*), beet (*Beta*), cucumber (*Cucumis*) and three varieties of pea (*Pisum*) were included.

Crop	Cultivar	y0	y2.5	y5.0	y7.5	y10	Max/Min
<i>Hordeum</i>	Inga Abed	5.0	4.7	4.6	4.7	4.8	5.0 / 4.6
	Tunga	4.6	4.6	4.3	4.3	4.7	4.7 / 4.3
<i>Triticum</i>	Vakka	4.0	4.3	4.3	4.3	4.3	4.3 / 4.0
	Solid	6.3	5.8	5.5	5.8	5.8	6.3 / 5.5
<i>Secale</i>	Petkus II	5.3	5.1	4.9	5.1	5.2	5.3 / 4.9
	Voima	4.9	4.7	4.4	4.7	4.8	4.9 / 4.4
<i>Pisum</i>	Weitor 1	4.7	4.6	4.4	4.7	4.5	4.7 / 4.4
	Hankkijan Hemmo	4.9	4.8	4.7	4.8	5.1	5.1 / 4.7
	Weitor 2	5.6	5.0	4.9	5.0	5.0	5.6 / 4.9
<i>Beta</i>	70500	3.7	4.3	4.3	3.7	4.1	4.3 / 3.7
	311 N typ	3.5	3.7	3.6	3.9	3.5	3.9 / 3.5
<i>Cucumis</i>	Langelands gigant	3.0	3.5	3.6	2.8	2.9	3.6 / 2.8
	Rhensk Druv	2.8	3.3	2.7	2.8	2.7	3.3 / 2.7

According to standard procedure for preparing seeds for long term conservation at the Nordic Gene Bank, the drying of the seed material for the experiment was aiming at bringing all seed samples moisture contents down to 3–5 %. Tests of the actual moisture content of some of the samples in Series A were carried out along with seed germination tests in year0.

The results show that three samples had higher moisture content than recommended at the beginning of the experiment, while ten of the thirteen samples had the recommended low moisture content. For these ten varieties, the moisture content in the seeds stayed below 5% in all tested samples during the first ten years.

For all varieties and samples the variation in moisture content of seed

samples of the same variety tested within the first ten years was between 0.8% and 0.3%.

The three varieties having seed sample moisture contents above 5% were the wheat variety 'Solid' (6.3%), the rye variety 'Petkus II' (5.3%) and the pea variety 'Weitor 2' (5.6%). For all these three varieties the highest level of moisture content was measured at year0, with a difference varying from 0.4% to 0.8% during the ten years.

It is worth noting that these three varieties are those three showing the largest loss of initial germination ability after 30 years of storage in permafrost (Figure 9).

4. Discussion

The 100 year experiment has revealed valuable results concerning longevity of seeds and survival of seed borne plant diseases conserved in permafrost. First, the results are valuable and directly related to the longevity of the seeds in the Nordic backup collection that up to 2008 was conserved in the coal mine. Secondly, the observations give general knowledge when comparing with results published from other seed storage experiments.

4.1. Seed germination

The storage conditions are an important characteristic for comparing results with other experiments. Our experiment was conducted in permafrost with a stable sub-zero temperature. Most other experiments have been carried out either under ambient room conditions or under -18°C conditions. Following general recommendations and scientific evidence, storage under sub-zero temperatures should be better than storage under ambient temperatures (see e.g. Cromarty et al. 1982), but how much better -18°C storage is compared to -3.6°C (Svalbard permafrost temperature) is not known.

When discussing the results from this long-term experiment, it is important to notice that the experiment was designed without reference material kept under ambient or -18°C conditions. The value of such comparisons is then limited. The applied test method and the experiment set up did not allow for statistical analysis of significance of the results within each species. Despite the weaknesses, the observations add knowledge to the longevity of seeds and seed borne pathogens in permafrost.

Seeds of all tested species remained viable after 30 years in permafrost, when dried to 3–5 % moisture content and stored in sealed glass ampoules. Differences between crop groups but also between species and cultivars within the same species were observed. This is in line with other long-term experiments (e.g. Rincker, 1983) and can be explained by different pre- and post-harvest conditions.

In general, immature seeds lose viability faster than mature seeds (Austin, 1972; Justice and Bass, 1978). For instance, potential seed longevity in barley is highest if harvested about two weeks after end of grain filling (Pieta Filho and Ellis, 1991). The same has been found in tomato (Dias et al., 2006). Weather during seed maturation, harvesting method, mechanical damage, and the presence of pests and diseases may further influence the viability of seeds during storage (Justice and Bass, 1978; Rao et al., 2017). All seed samples in this experiment were taken from newly harvested material. Still we have no detailed information related to the weather conditions before and during harvesting, or other factors that might have influenced seed quality.

The most long-lived seeds in this experiment, displayed as minimal loss of germination percentage over 30 years, have been the vegetable seeds, here represented by cucumber, lettuce, onion, beet, cauliflower and carrots. The picture is more varying within cereal, grass and legume species. Maude and Bambridge (1985) reported that beet seeds retained a high level of germination when stored 13 years at 10°C and 50% RH. The used beet cultivars have multigerm seeds, which may partly explain good germination results for beet.

In addition, cucumber and cauliflower have seeds that can maintain viability for a long time (Nagel and Börner, 2010), while onion and lettuce are more short-lived (Roos and Davidson, 1992; Nagel and Börner, 2010). Our result is somewhat striking, as we demonstrate that also onion and lettuce seeds maintained viability very well over a 30 year period. The good germination results in this study may be due to high quality of seed lots.

Among cereals, barley seeds maintained high germination levels over 30 years, while wheat and rye declined in viability. This was especially clear for the last ten years of the 30-year period. The results correspond well with the literature, showing that rye can be relatively short-lived (Nagel *et al.*, 2010). The mixed result in wheat with one cultivar showing a low decline and the other high decline in viability could be explained by different pre-harvest conditions or there could be a genetic factor.

The grasses and legumes show a varied picture. Some species and some cultivars maintained viability while other declined, and the drop in viability was considerable during the last five years.

From literature, barley, peas and clover seeds are known to be long-lived compared to most other crops (Rincker, 1983; Steiner and Ruckenbauer, 1995; Nagel *et al.*, 2009; Nagel and Börner, 2010). In this context, the variable results for peas are difficult to explain.

Pea seeds require germination in sand (or between paper) as outlined by ISTA (ISTA 1985). However, the ultra-low water content commonly found in genebank accessions may not necessarily fit into established standards for water supplies. Ultra-dried peas may need a pre-test water soaking treatment before germination or perhaps the required amount of water added to the sand should be higher.

According to ISTA, peas are to be germinated between paper, in sand or on top of paper with a layer of sand. In the experiment, the sand method was chosen and has been followed for 30 years. However, the protocol of water supply added is based on empiric experience by the laboratory and not by prescribed volumes given by ISTA. Whether the ultra-low water

content commonly found in genebank accessions in some cases does not fit into established protocols for water supply is an open question. Perhaps ultra-dried peas need a pre-test water soaking treatment before germination or perhaps the required amount of water added to the sand should be higher.

4.2. Survival of seed borne diseases

Seed of most crops carry a wide variety of pathogens (Neergaard, 1977). Knowledge of the duration of survival of pathogens on seed is important for genebanks in relation to the risk of spreading disease through germplasm exchange. The infected materials included in the 100 year storage experiment give examples of survival of seed-borne diseases on some crops commonly grown in Nordic countries when dry seeds are stored at temperatures a few degrees below zero.

All pathogens involved in the 100 year experiment have survived through the 30 years of permafrost storage. The infection levels, expressed as percentage of infected seeds, were in most cases only slightly changed over the years.

There is not much recent literature on the longevity of pathogens on seeds. However, a few earlier studies have reported that some fungi may live for a number of years during storage. Hewett (1987) found around 75-80 % viability of seed-borne pathogens in a comprehensive study of 80 samples of cereals, pulses and some vegetables, after deep freeze storage (-20°C) of dry seeds for up to 14 years. His study included pathogens such as *Drechslera spp.* in barley, *P. betae* in sugar beet and *A. dauci* and *A. radicina* in carrot. Our results are largely in agreement with the results from Hewett (1987).

A survey of seed-borne fungi on genebank-stored (-1°C, 30 % RH) cruciferous seed from Japan reported that the seed lots were frequently infected by pathogenic fungi, such as *A. brassicicola* after around 10 years of storage (Nishikawa *et al.* 2014). This is in line with the high level of *A. brassici-*

cola on cabbage seed we observed during the 30 years storage in permafrost. On the other hand, Maude and Humpherson-Jones (1980) reported declined viability of superficial *A. brassicicola* on cabbage seeds after two years storage at 10°C and 50 % RH. However, internal infection persisted for up to 12 years. In contrast to the stable or slightly increased infection level of *P. betae* in our permafrost storage experiment, Maude and Bambridge (1985) reported that seed infection by *P. betae* declined from 27.5 to 4.5 % in beet seed stored for 13 years at 10°C and 50 % RH.

The high survival of pathogens in cereal seeds in our experiment is partly in contrast to a Canadian 10 years storage study carried out almost 70 years ago, where most of the infected seeds became free of fungi before the end of the storage period (Machacek and Wallace, 1952). However, in their study the seed samples were stored in an aerated box in an unheated dry shed.

A more recent evaluation of seed-borne fungi (including *Drechslera* and *Fusarium spp.*) in 36 rye accessions after 30 years of storage at -4°C in the national Spanish genebank did not reveal any strong effect of storage (Garcia *et al.*, 2016), which is generally in accordance with our results.

Machacek and Wallace (1952) reported that *S. nodorum* in wheat died out rather rapidly and the fungus was not detected after eight years. It is interesting that *S. nodorum* was one of the fungal species that seemed to lose viability in our storage experiment. In contrast, Hewett (1987) reported high survival of this fungus after 14 years storage at -20°C, and Cunfer (1981) observed that *S. nodorum* even increased by 1–25 % during storage at 25°C for two years after harvest. *Fusarium spp.* is known to decline rapidly on seeds stored at room temperature, and Hewett (1967) observed a considerable reduction of *Fusarium* on wheat seed after 4–8 months. However, in our permafrost study no reductions in *Fusarium* levels on barley, wheat and onion seed were observed, in spite of low initial infection levels.

In spite of storage under not controlled conditions, Machacek and Wallace (1952) reported considerably increased infection levels of *Drechslera* in barley during the second and third years of storage, which is similar to our

observations after 5 years. After 10 years, Machacek and Wallace (1952) reported approximately 40 % survival of *Drechslera* in barley compared to the initial infection level. We observed no decline of *Drechslera spp.* in barley after 30 years in permafrost. This indicates that this pathogen can survive a long time in barley seed stored at temperatures both below and above zero degrees.

Our wheat sample naturally infected with *U. nuda f. sp. tritici* showed no reduction in infection level during 30 years. Similarly, wheat seed harvested from plants that had been artificially inoculated with *U. tritici (=)*, showed no decline in infection level during 20 years of storage at -15°C and low relative humidity (Menziez et al., 1997).

The conclusion on the seed-borne pathogens after 30 years of storage in permafrost is that their viability is well maintained. This is in line with other studies of dry seed stored at temperatures below zero. The conditions commonly used in genebanks to maintain seed longevity therefore usually also favour survival of seed borne pathogens.

5. Recommendations

1. The 100 year experiment should be pursued according to the plan for the rest of the project period.
2. Future analyses should report results from the four parallels (4x100 seeds separately) and not only the average data, for both germination and seed health tests.
3. From year 30 the germination analysis should include counting of normal seedlings emerging at an early stage (first count). Analysis in previous years included only the final count, which is a test of germination potential under optimal conditions. Because the number of normal seedlings at first count may serve as an indicator of the seed vigour and the storage ability of the seeds, it is recommend including the first counts in the seed analysis for the rest of the experimental period.

4. It is recommended to establish a new 100 year experiment related to conservation of seeds at -18°C in the Svalbard Global Seed Vault according to the following guidelines.
 - a) Species chosen for the new experiment should include crop species that are important for food and agriculture in different regions and climates.
 - b) Major international and national gene banks should be invited to participate in the project, e.g. by suggesting species and accessions to be investigated and to provide seeds. A reference group consisting of these and other stakeholders should be established.
 - c) Detailed instructions for preparing, packing and shipment of seeds should be worked out. Details about the seed production and growing conditions should be recorded according to a standardized plan.
 - d) The experiment should include test samples stored at -18°C at the Svalbard Global Seed Vault. Recommended controls and comparisons could be storage of different harvests (years), different growing conditions and seed quality factors of the same variety.
 - e) The participating gene banks could according to own preferences store samples of the same seed yield at their primary gene bank storage conditions, for analysis at their own seed labs.
 - f) The experimental design should include four replicates of each species and variety in order to allow statistical analysis of the results. It is recommended that all samples from the Seed Vault experiment are tested at the same laboratory. In order to facilitate easy transport and to avoid customs problems using a seed lab in Norway would be advantageous.

6. Acknowledgements

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Thanks to staff at Kimen Seed Laboratory in Ås, Norway and former staff of the Norwegian State Seed Testing Station, and the Norwegian Institute of Bioeconomy Research for thorough and accurate analysis of samples, following the same procedures throughout the 30 years.

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In addition to the authors of this report, a range of Nordic Gene Bank and NordGen staff has been involved from the establishment in 1986 until today. A special thanks to all of these.

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Annex 1

Storing seeds in a coal mine in Svalbard has attracted significant attention from media and others right from the idea was launched in the early 1980ties. Despite increased tourism and easier access, Svalbard is still considered to be an exotic place. In the early 1980ties Svalbard and Longyearbyen was even more mysterious and surrounded by myths about polar bears, explorers and hunters overwintering in remote cabins and surviving on reindeer meat and seal blubber.

This Annex to the report from the first 30 years of the 100 year storage experiment contains a set of newspaper articles and photos that extends the picture of the experiment, what it looks like and how it has been perceived and featured in media.

Before the first seed were brought into the container in the mine No 3 in November 1984, several questions had to be investigated. Arne Wold and Flemming Yndgaard from the Permafrost working group made an expedition to Svalbard 4-7 July 1983 and met representatives for the mining company Store Norske Spitsbergen Kulkompani (SNSK). Two mines were visited (No 7 and No 3), and it was found that one of the transverse passages, AT₂, in mine No 3 was the most suitable for hosting a seed security storage.

Arne Wold and Flemming Yndgaard visited Svalbard a second time in November 1984 with the aim of inspecting the storage facility, and to bring the first seed samples into the steel container. The first dispatch of glass ampoules with seeds from the Nordic Genebank for the security storage arrived to Svalbard and was taken into the coal mine storage on the 14th of November 1984.

The start of the Nordic Permafrost Security Seed Storage was celebrated by SNSK giving a reception in the evening of the same day. Reports from the inauguration event hosted by SNSK say that a delicious casserole dish was served with red wine in the SNSK mess hall, and the NGB board chair Arne Wold presented a crystal vase with inscription and flowers together with deep thanks for all kind assistance from the mine company.



Figure 1. *Transport of the steel container from the airport and into the coal mine was carried out by Store Norske Spitsbergen Kulkompani. Images from Hempel Information, Number 7, August 1985. Hempel is the company that provided the long-term corrosion protection of the steel container.*

The event of bringing the first seeds into the new steel container seed store in the mine was documented by the local newspaper Svalbardposten No. 12, 1984/85 (see facsimile below).



Figure 2. Quote from Svalbardposten (to the left) translated into English:

Onsdag den 14. november 1984 kommer til å bli en merkedag både i Store Norske og Nordiska Genbankens historie. Da ble nemlig verdens første permafrostlager for kulturlanter tatt i bruk. En kasse inneholdende 20 - 30 ampuller med gennematiale eller plantefra fra like mange forskjellige kulturplanter ble plassert i en spesialoppbeiret container i en sidegang i Store Norske gruve 3. I denne sidegangen er det bygget en egen hall hvor containeren er plassert. Gruvarbeiderne har gitt hallen navnet "Frynhall", og i "Frynhall" er det at Nordiska Genbanken for all framtid skal sikre gennematiale fra alle forskjellige kulturplanter i Norden mot feilslått planteforedling og mot naturkatastrofer og atomsoolen. I lagret vil man ta vare på gennematiale og dermed sikre plantenes verdifulle egenskaper.

Gruver en sammensatt i Longyearbyen omringet av fjell undertrykket Arne Møll, fra Statens Frelstkontroll og Flemming Indgaard fra Nordiska Genbanken at det er tattet være Store Norske velvillighet og samarbeidvilje at Nordiska Genbanken nå har kunne opprette verdens første permafrostlager for gennematiale.

Det var en lett nervens forsamling som onsdag formiddag rundt de 200-metere innover i gruve 3 til frelstlaget. Både Arne Møll og Flemming Indgaard hadde felt klart for seg betydningen av det som her skjedde, men resten av forsamlingen, gruvegjef Terje Johnsen og gruvarbeiderne som har arbeidet med å gjøre i stand hallen, ble usikre av de to frelstkontrollens høytidelighet og nærmest rituelle framferd. En trekasse med sårene 40 cm x 20 cm x 20 cm var alt som ble fraktet inn og plassert i den store containeren. En liten kasse, men en stor og viktig sak.

Arne Møll og Flemming Indgaard forklarte at man

PIONERARBEID I GRUVE 3



Kassen med fra har nådd fram til permafrostlageret i gruve 3. Fra venstre Arne Møll fra Statens Frelstkontroll, Terje Johnsen, Gunnar Hansen, Flemming Indgaard fra Nordiska Genbanken, Karl Johan Eide og Gunnar Thau Johnsen. Det er disse som har hatt arbeidet med å bygge opp "Frynhall" for de to frelstkontrollene.

hadde sett på mange alternativer før man valgte ut gruve 3 ved Longyearbyen til Genbankens reservelager. En av grunnene til at det ble i Store Norske gruve var det engasjement og den samarbeidvilje som selskapet hadde vist fra de første gang ble kontaktet om saken. En annen grunn var at man i denne gruvn fant at temperaturen lå konstant på - 3,7 grader gjennom hele året. Kaldet er en forutsetning for å lagre frømateriale.

Det ble undertrykket at permafrostlageret i gruve 3 er et reservelager for Nordiska Genbankens i Lund i Sverige. Såvel her er gennematiale lagret i - 20 grader, noe som gir en bedre holdbarhet enn ved - 3,7. Problemet er bare at dette hovedlageret er avhengig av strømtilførsel for å holde temperaturen nede. Svakter strømtilførselen er lagret ødelagt, og da er det en usikkerhet å vite at man har et reservelager å ty til på Svalbard.

Ikke all utviling er av det gode, og dette er en av hovedgrunnene til at



Arne Møll og Flemming Indgaard hadde en stor dag onsdag da de kunne ta i bruk verdens første permafrostlager for gennematiale.

det er så viktig å ta vare på eldre gennematiale. Moderne planteforedling kan gjøre at man mister gode egenskaper ved kulturlanterne. Både ved

Wednesday the 14th November 1984 is going to be a memorable day in the history of Store Norske and the Nordic Genebank. This was namely the opening date of the World's first permafrost storage of crop plant seed.

A box containing 20 - 30 vials with germplasm or plant seed from as many different crop plants was placed in a specially prepared container in a gallery in the Store Norske's mine 3.

Figure 3. Shortly after the first deposit of seed samples from the NGB Nordic seed collection it was decided to establish the 100 year experiment. Pictures below show the preparation of glass tubes in wooden boxes for the experiment, probably in the autumn 1986.





Figure 4. Chairman of NGB board Even Bratberg in the steel container when the material for "The 100 year trial" was in place alongside the Nordic Safety Base Collection. The Safety collection was moved to the Seed Vault in 2008.



Figure 5. The steel container is placed on a wooden platform in the mine gallery which is strengthened by wood pillars. The photo is taken in February 2017. Only boxes containing seed glass tubes belonging to the 100 year experiment are still stored in the container.

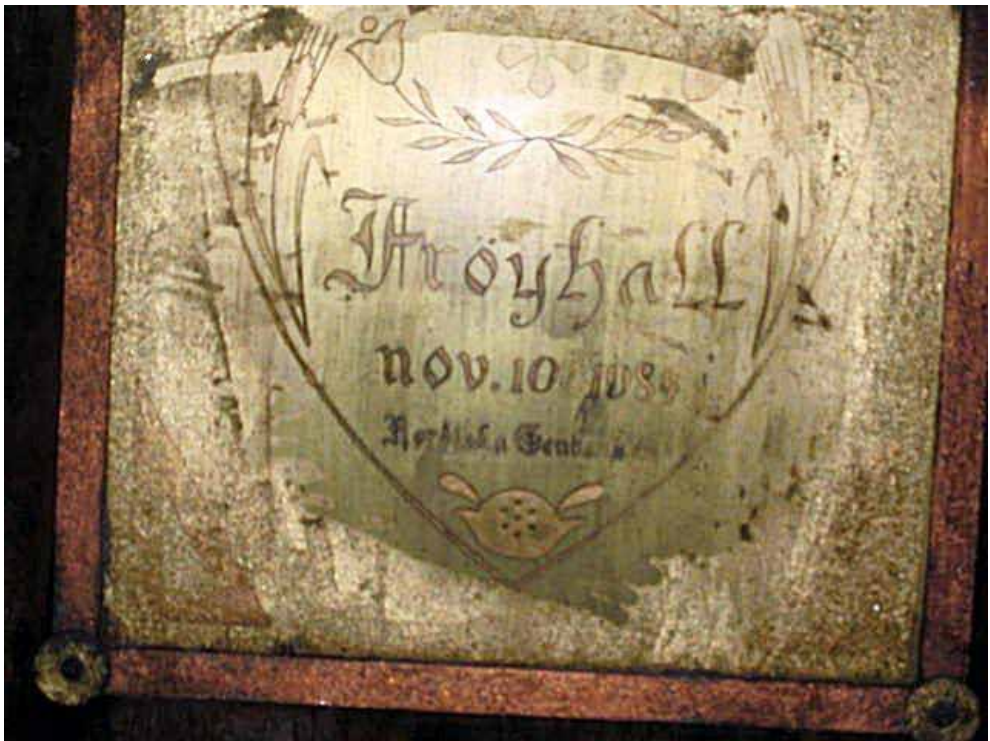


Figure 6. The wooden door leading in to "Frøyhall", with a nice plate made by a local artist in November 1984.

The opening of the first permafrost seed storage in the world was paid significant attention all over the world.

Figure 7.
Facsimile of
China Daily on
Tuesday the
23rd of July
1985.



Figure 8. Drawing
from The Messenger/
Fort Dodge Iowa, Sun-
day 14th of July 1985,
during times where
fears of nuclear apoca-
lypse were prevalent.

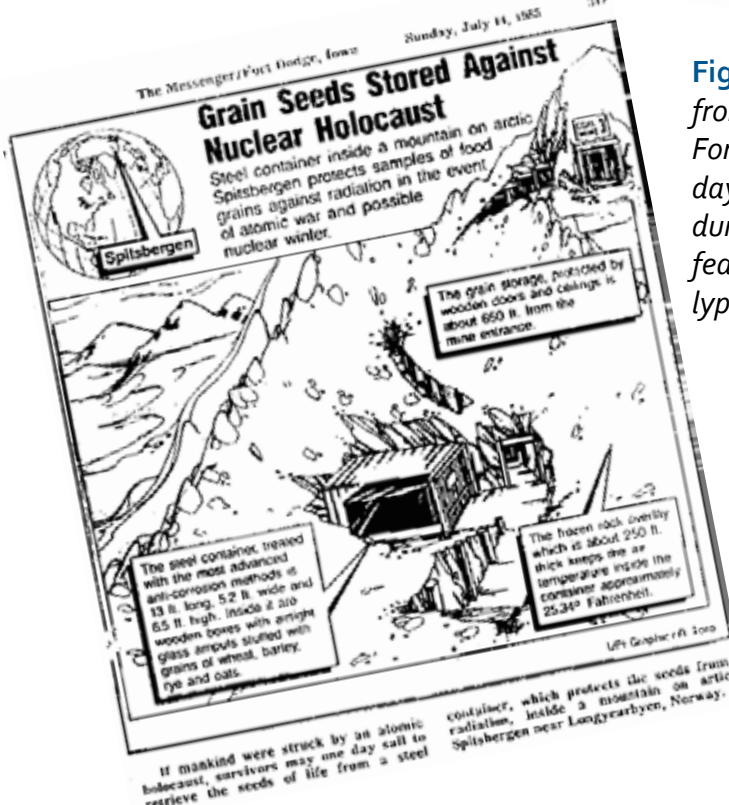




Figure 9. Birte Mattson (left) and Birgitte Lund at NGB preparing a new shipment of seeds for the Nordic safety base collection in the coal mine in 1991 or 1992. For the shipment, the wooden seeds boxes were placed in insulated polystyrene cylinders.

Withdrawal of test samples has been carried out every fifth year, in strict accordance with the project plan. A significant number of people have been involved in the process of bringing seeds out of the coal mine and shipment of seeds to the seed laboratory in Ås, Norway. Involved persons include local SNSK staff and individuals from Kimen Seed Lab., NGB / NordGen and from the Norwegian Ministry of Agriculture and Food.

Figure 10. *Withdrawal of test samples from the steel container. In 2001, Dagny Stave Larsen, Laboratory manager at Kimen Seed Lab, travelled to Svalbard and brought the actual seed box back to Ås. NordGen Seed Vault information officer, Roland von Bothmer carried out the withdrawal in 2011, when seed boxes containing seeds belonging to the ordinary Nordic seed collection had been moved to the Seed Vault in 2008.*



Figure 12. Håkon Tangerås and Dagny Stave Larsen have been in charge of the seed testing at Kimen during major parts of the project period. (Photos Even Bratberg)

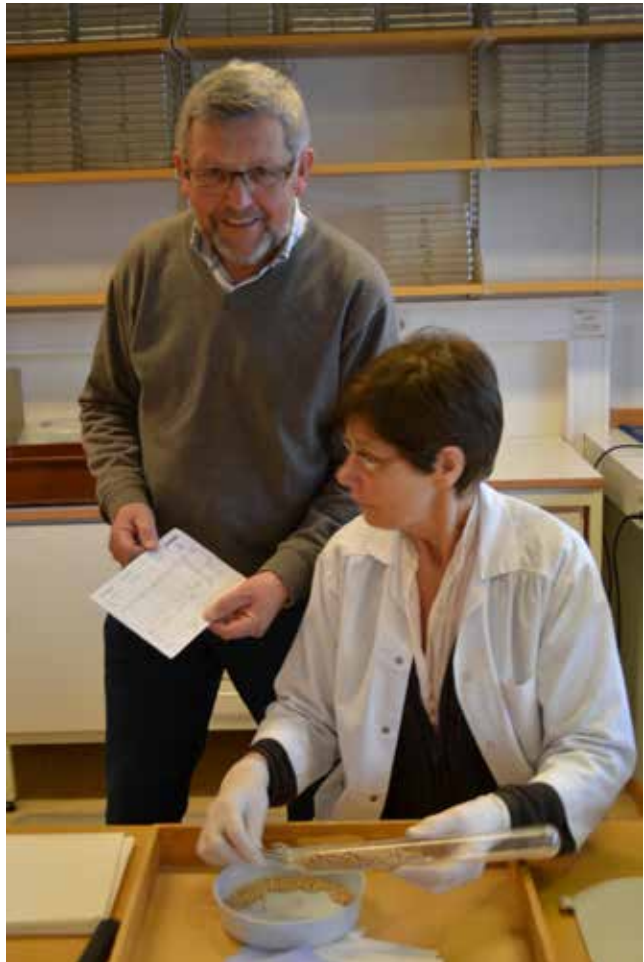


Figure 13. Germination of small seed types at Kimen



Figure 14. Testing seed for contamination by lettuce mosaic virus have been carried out by sowing the lettuce seeds and evaluate virus symptoms. The analyses have been conducted by the Norwegian Institute of Bioeconomy Research in Ås (previously Planteforsk/Bioforsk etc.) Photos from Dag-Ragnar Blystad, who has been in charge of these particular analyses through major parts of the project.



Seed longevity and survival of seed borne diseases after 30 years
conservation in permafrost. Report from the 100 year storage experiment

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