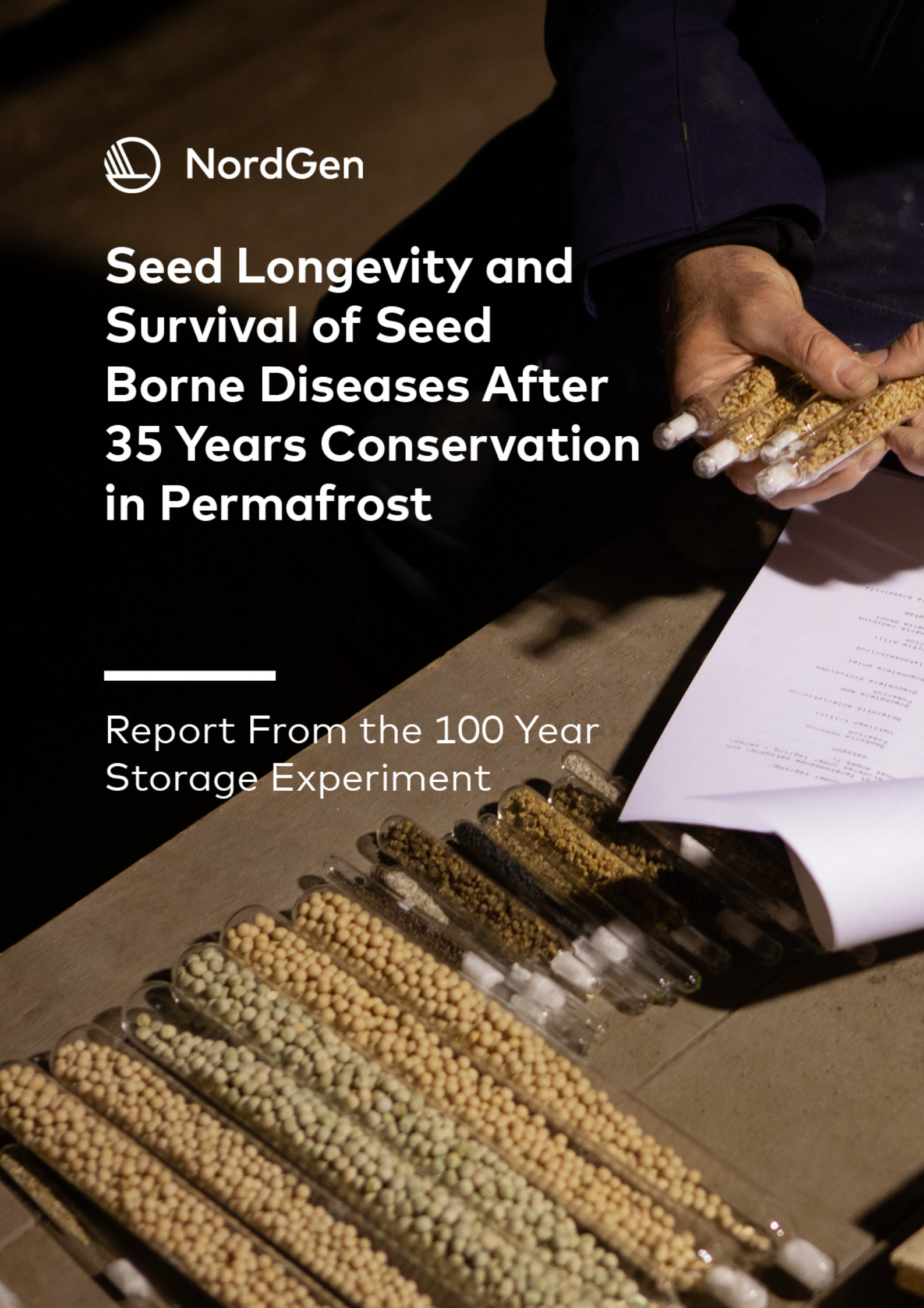

NordGen

Seed Longevity and Survival of Seed Borne Diseases After 35 Years Conservation in Permafrost

Report From the 100 Year Storage Experiment

Table of Content

Preface

The Nordic Gene Bank[1] established the 100 year seed storage experiment in Coal mine no. 3 outside Longyearbyen in 1986. The experiment was established with the aim to monitor the longevity of seeds in this Nordic back-up seed collection that were deposited in the coal mine from 1984 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.

Seed samples have regularly been withdrawn for analysis according to a fixed withdrawal and analyze plan, that will continue until the last samples are analyzed in 2086.

/Åsmund Asdal, NordGen, 6th of February 2024.

1 Predecessor to today's plant section of The Nordic Genetic Resource Center (NordGen).

In this video about the 100 year experiment, you can follow the seeds from the coal mine to NordGen's seed laboratory: ***https://vimeo.com/manage/videos/915872471***

The seeds in the experiment are stored inside a steel container in the coal mine.

Project Setup

The experimental set up included in total 41 seed lots of 17 agricultural and horticultural crop species commonly grown in the Nordic countries. The study was divided into two series. Series A studies the development of germination capacity in 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).

***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, three cultivars for pea (Pisum sativum). [1] Country codes according to ISO 3166-1 alpha-3 (Wikipedia 2019). [2] Material from Nordic sugar beet breeding programmes.*

Sample identity	Crop / species	Cultivars (country of origin)[1]
A-1-1/A-1-2	Barley (*Hordeum vulgare*)	Inga Abed (DNK), Tunga (NOR)
A-2-1/A-2-2	Wheat (*Triticum aestivum*)	Vakka (FIN), Solid (SWE)
A-3-1/A-3-2	Rye (*Secale cereale*)	Petkus II (DNK), Voima (DNK)
A-4-1/A-4-2	English ryegrass (*Lolium perenne*)	Pippin (DNK), Riikka (FIN)
A-5-1/A-5-2	Timothy (*Phleum pratense*)	Tammisto (FIN), Bodin (NOR)
A-6-1/A-6-2	Kentucky bluegrass (*Poa pratensis*)	Hankkijan Kyösti (FIN), Annika (DNK)
A-7-1/A-7-2	Red clover (*Trifolium pratense*)	Jokioinen (FIN), Molstad (NOR)
A-8-1/A-8-2/A-8-3	Pea (*Pisum sativum*)	Weitor (SWE), Hankkijan Hemmo (FIN), Weitor pt. 10468 (SWE)
A-9-1/A-9-2	Beet (*Beta vulgaris*)	311 N typ (SWE), 70500 (DNK)[2]
A-10-1/A-10-2	Oilseed rape (*Brassica napus*)	Jupiter (SWE), Linrama (DNK)
A-11-1/A-11-2	Bulb onion (*Allium cepa*)	Hamund (SWE), Owa (DNK)
A-12-1/A-12-2	Lettuce (*Lactuca sativa*)	Attraktion Sana (DNK), Hilro (SWE)
A-13-1/A-13-2	Cucumber (*Cucumis sativus*)	Langelands gigant (DNK), Rhensk Druv (SWE)
A-14-1/A-14-2	Carrot (*Daucus carota*)	Nantes Fancy (DNK), Regulus (SWE)
A-15-1/A-15-2	Cauliflower (*Brassica oleracea v. botrytis*)	Svavit (SWE), Pari (DNK)

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). Naturally infected seeds have been used, and in addition, one sample of fungal survival structures (sclerotia) was included in series B.

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	Cultivars (country of origin)[1]
B-1	*Septoria nodorum*, *Fusarium* spp.	Wheat (*Triticum aestivum*)	Runar (NOR)
B-2	*Ustilago nuda* f.sp. *tritici*	Wheat (*Triticum aestivum*)	Line 79 CBW "A" No 72 (CAN)
B-3	*Sclerotinia sclerotiorum*	Sclerotia from *Brassica*/Cabbage[2]	(NOR)
B-4	*Drechslera* spp. *Fusarium* spp.	Barley (*Hordeum vulgare*)	Bamse (NOR)
B-5	*Drechslera dictyoides*	Meadow fescue (*Festuca pratensis*)[3]	Salten (NOR)
B-6	*Drechslera phlei*	Timothy (*Phleum pratense*)	Forus (NOR)
B-7	Lettuce mosaic virus	Lettuce (*Lactuca sativa*)	Attractie (NLD)
B-8	*Botrytis allii* *Fusarium* spp.	Bulb onion (*Allium cepa*)	Laskala (NOR)
B-9	*Alternaria radicina* *Alternaria dauci*	Carrot (*Daucus carota*)	Forto Nantes (NLD)
B-10	*Phoma betae*	Beet (*Beta vulgaris*)	Hilma (GBR)
B-11	*Alternaria brassicicola*	Cabbage (*Brassica oleracea* ssp. *capitata* f. alba)	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. 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) labelled with the date for withdrawal. Altogether, 25 boxes were prepared, transported to Longyearbyen, and placed in the steel container in the abandoned gallery of Cole Mine no 3, where the Nordic Gene Bank already had its safety collection of Nordic seeds.

At the start of the seed deposit project in the coal mine in 1984, the temperature inside was measured to minus 3,7°C, and it was reported to be stable throughout the year. The temperature in the seed storage part of the coal mine has not been measured by NordGen, but as climate change effects are noticeable and significant in Svalbard, it is likely that the temperature has increased over the time span of the project, from minus 3,7°C at the start towards an estimated minus 1-2 °C in 2021. NordGen will, for the rest of the project, monitor the changes in temperatures regularly.

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 glass ampoules. Following the same procedures and test methods through the whole experiment is crucial for the reliability and the scientific value of the results.

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 (year 0 = y0). The last box will be taken out for analyses in December 2086 (y100).

NordGen's team at the wooden craters inside the steel container, 2017.

Seeds have been tested according to methods established by the International Seed Testing Association (ISTA 1985, 2017). Test methods are further described in the NordGen report *Seed Longevity and Survival of Seed Borne Diseases after 30 years Conservation in Permafrost. Report from the 100 year storage experiment* (2019).

Seeds have, for the first 30 years of the experiment, been tested by Kimen Seed Laboratory located at Ås in Norway, applying ISTA protocols published in *ISTA (1985) International Seed Testing Association. Rules, 1985. Seed Science and Technology, 13: 299–355.* NIBIO (Norwegian Institute of Bioeconomy Research has assisted in tests detecting virus contamination in lettuce.

From 2021, germination tests belonging to series A have been carried out by the NordGen seed lab in Alnarp, using the same ISTA protocol, while Kimen Seed Laboratory has continued to carry out the tests belonging to Series B.

Results – Germination

Germination results at the start and after 35 years of storage are listed in Annex 1. Graphs showing development and changes in germination over the years are shown crop group wise in figures 1-6 below. Results include both Series A and Series B. Remaining germination ability of initial germination percentage after 35 years is shown in figure 7. Results grouped according to the two analysing seed labs are shown lin figure 8.

After 35 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 35 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 35 years. Wheat and rye have declined significantly in viability, in particular during the last 15 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.

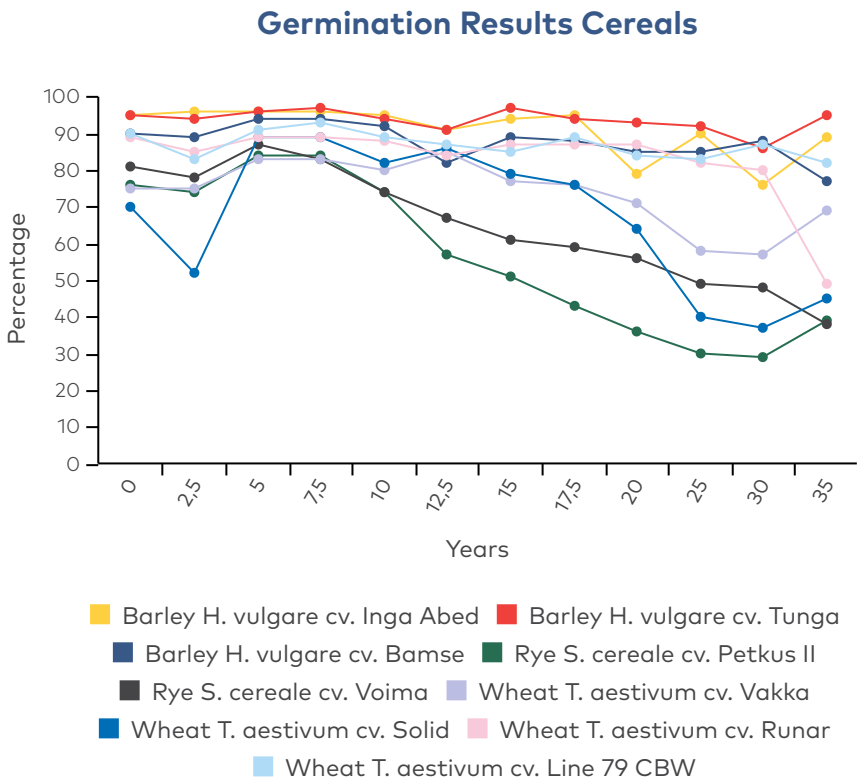

***Figure 1**. Germinations results of cereal crops, barley (2 varieties), rye (2 varieties) and wheat (4 varieties).*

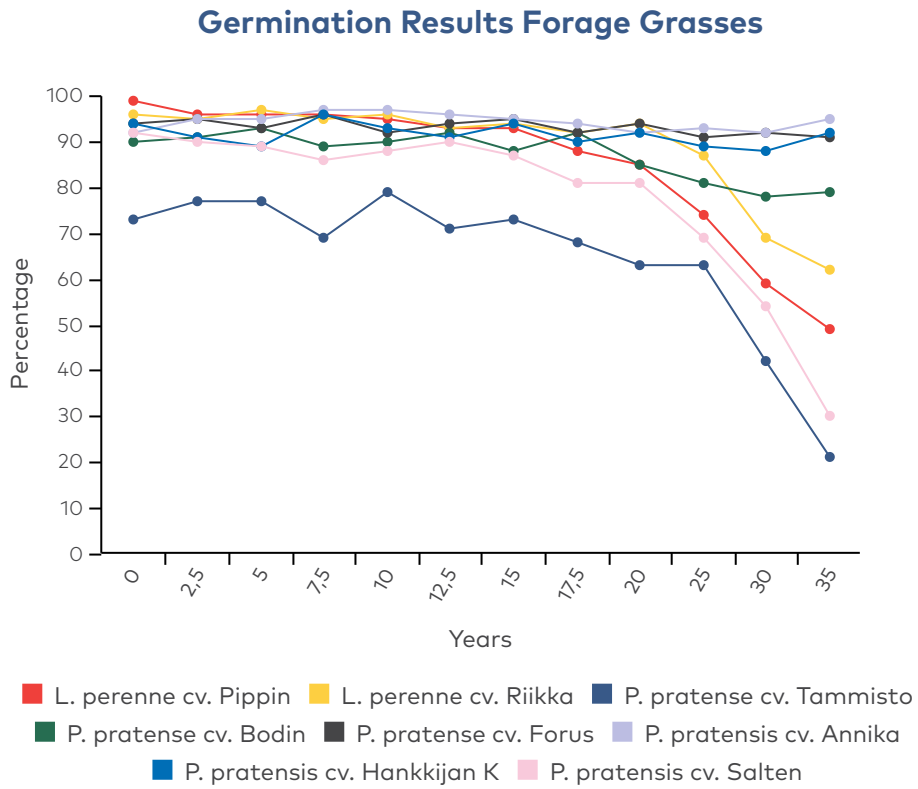

Figure 2. *Germinations results of forage grasses, perennial raigrass (2 varieties), timothy (2 varieties), meadow fescue (1 variety) and Kentucky bluegrass (2 varieties).*

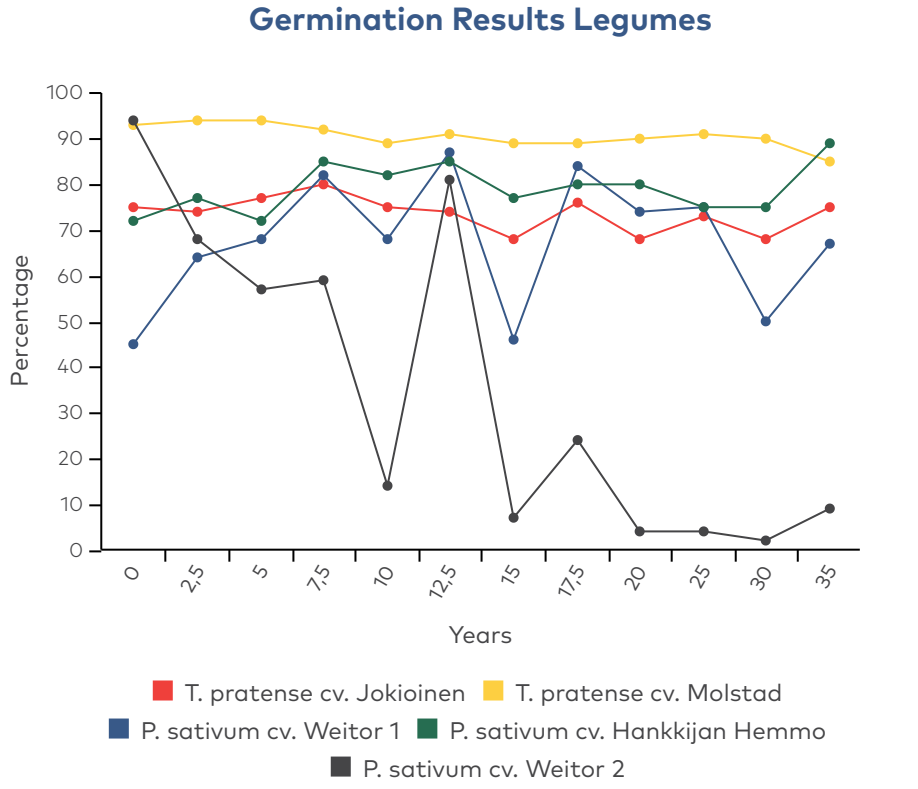

Figure 3*. Germinations results of legume crops, red clover (Trifolium pratense) (2 varieties) and pea (Pisum sativum) (3 varieties). (Results for the pea variety 'Weitor 2' is not included in further summarizing analysis).*

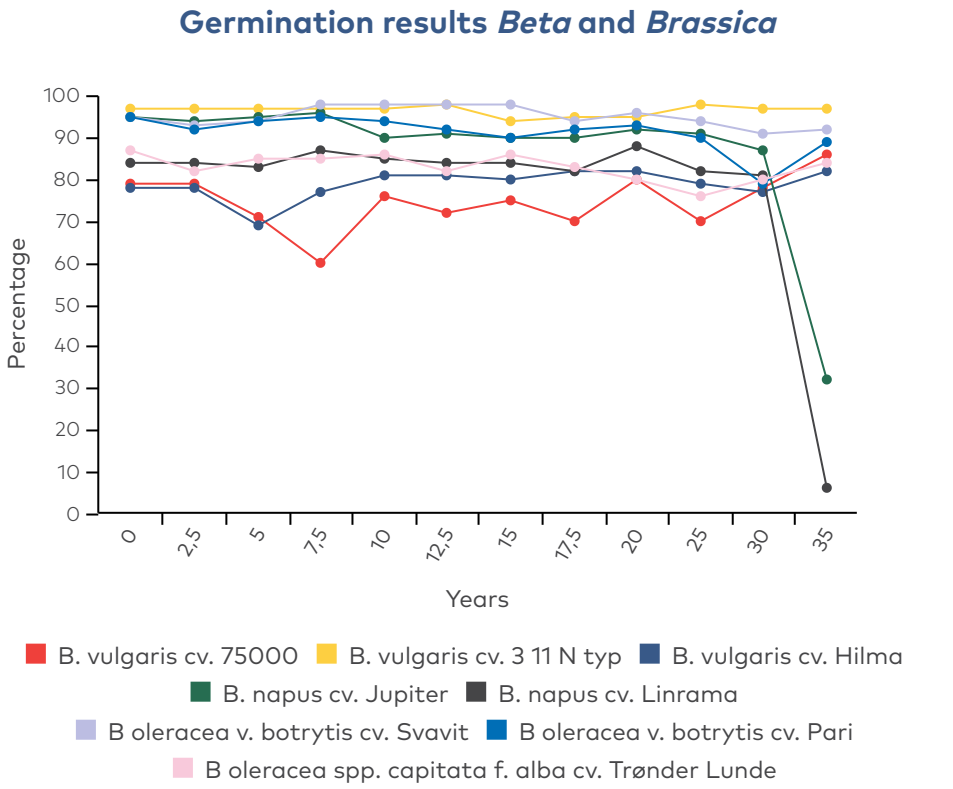

Figure 4. *Germinations results of Beta (1 variety) and Brassica species (3 species/varieties).*

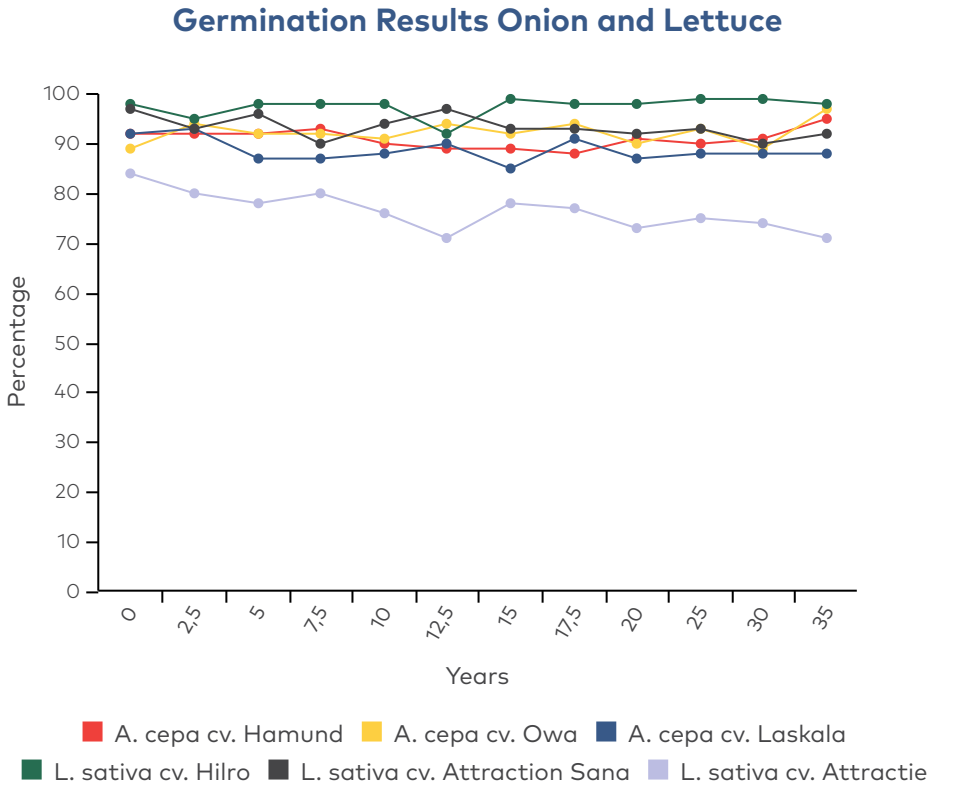

Figure 5. *Germinations results of onion (Allium cepa) (3 varieties) and lettuce (Lactuca sativa) (3 varieties).*

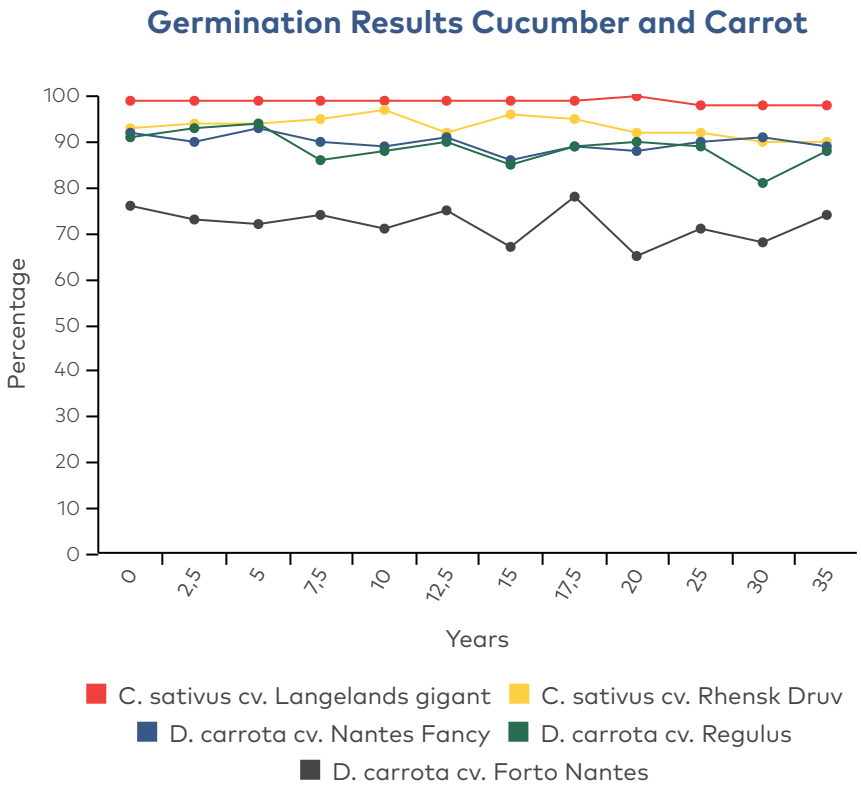

Figure 6. *Germinations results of Cucumber (Cucumis sativus) (2 varieties) and carrot (3 varieties).*

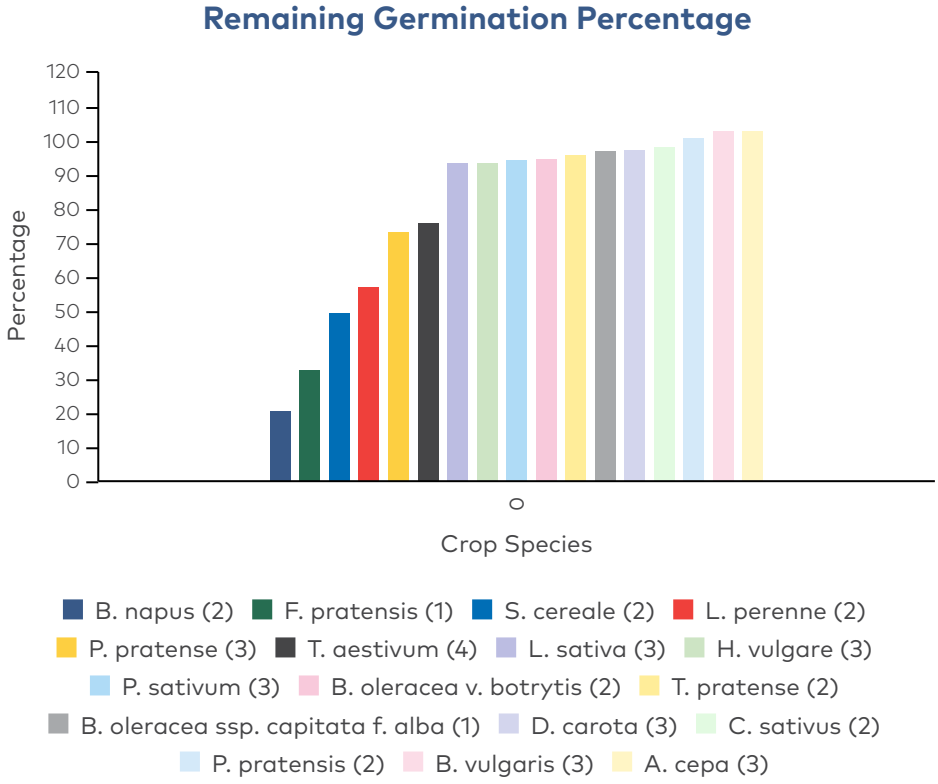

Figure 7. *Remaining germination percentage of 17 crop species after 35 years storage as average of the cultivars included (number of cultivars in brackets). The results for pea show the average for two cultivars with very high germination. One cultivar with close to zero germination is not included in the average shown here. (See Figure 3 for details).*

As explained, from 2021, the two series of experimental materials have been analysed in two different seed labs. Series A comprising 31 samples of 16 crops have been analysed at the NordGen seed lab in Alnarp, while series B comprising 10 samples with seeds of nine species have been analysed at Kimen seed lab in Ski, Norway. Analysis of seeds in series B have included test of survival of seed borne pathogens in addition to germination tests.

The total plant health status is not known for any samples/series. The only background information available is that samples in Series B are contaminated with the pathogens listed in table 2. The pathogens involved are mainly problematic when cultivating the plants in field conditions. The possible effect of the pathogens on germination is not studied.

As eight out of nine species in Series B are represented also in Series A, a comparison between germination results in the two series is shown in Figure 8. Average remaining germination percentage of crops in Series A is 83,0%, while it is 85,8% in Series B. There are no systematic trends in this comparison, and the experimental material is too scarce to draw any conclusions regarding the effect of either pathogen contamination or differences between the two seed labs.

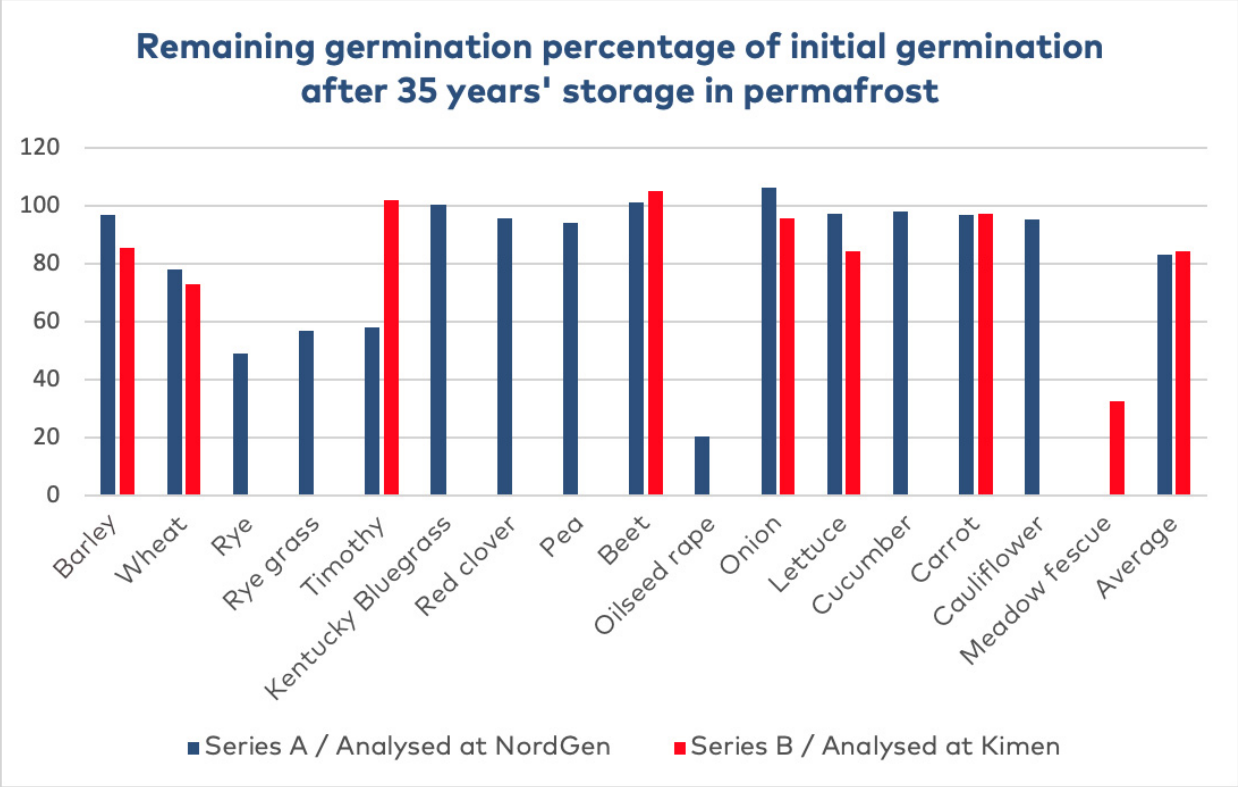

Figure 8. *Remaining germination percentage of 17 crop species after 35 years storage as average of the cultivars included (number of cultivars in brackets). This figure shows test results analysed by NordGen (blue columns) and Kimen Seed Lab, (red columns) respectively.*

Results – Seed Borne Diseases

Results showing the survival of seed borne plant diseases are shown in figure 10. The initial infection level of the seed borne pathogens varied considerably among the different crop species included in the experiment (Figure 10). However, after 35 years of storage in permafrost the infection levels were in general only slightly changed in most of the samples, although some variation in the levels were observed over the years.

All investigated pathogens were still detected after 35 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. 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.

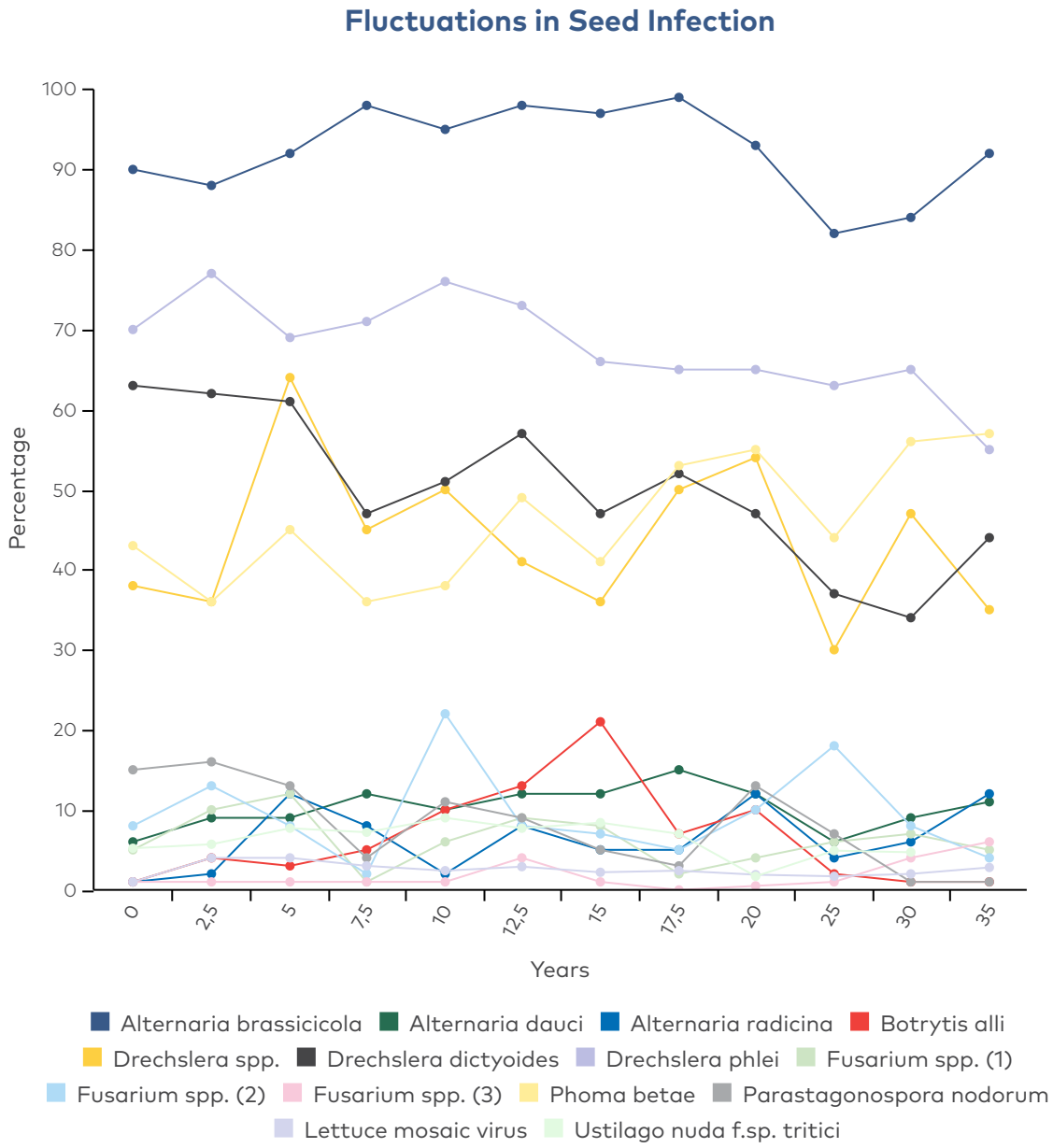

Figure 9. *Fluctuations in seed infection by 14 seed borne pathogens over 35 years.*

When considering these results, it is important to stress that the project is characterized as a monitoring project with the aim to monitor changes in germination of the backup seed samples of the Nordic seed collection that were deposited in the coal mine at -3.7°C in 1984. The number of samples and replicates do not allow for broad statistical analysis and the scientific evidence and relevance is weak.

It is also important to note that the results are extracted from seeds conserved in permafrost at minus 3.7°C and that the observations are not valid for conservation of seeds at -18°C, which is the temperature that is used for long term conservation of seeds by most genebanks and by the Svalbard Global Seed Vault.

a) The results show that 11 out of the 17 species after 35 years storage in permafrost have retained more than 90 percent of their initial germination percentage. The same figure five years ago was that 9 out of 17 species had results better than this threshold. On average, the observations then show that the germination percentages of these seeds more or less are the same as they were five years ago, with one major and three minor exceptions:
1) A major drop in germination of seeds of oil seed rape (*Brassica napus*) has been observed from 30 to 35 years of storage. This drop applies for both varieties of the species and oil seed rape is now, after 35 year storage ranged as having the poorest germination ability among the 17 crops in the project. Five years ago, seeds of oil seed rape germinated at a rate of 87%. Five years later this figure has dropped to 19%.
2) Three crops have entered the group of crops that has retained more than 90% of their initial germination ability, namely cauliflower (*B. oleracea v. botrytis*), barley (*Hordeum vulgare*) and the two varieties of peas (*Pisum sativum*). This mean that the germination results have slightly increased from 2016 to 2021, however again, no significant change, but an interesting observation though.

b) Regarding average results, there has been no major change in germination of seeds of the three cereal crops (barley, wheat and rye). However, as shown variety wise in figure 1, the cereal samples belonging to Series A that from 2021 have been analysed at NordGen, show an increased germination percentage compared to results from five years earlier (except for one of the two rye samples), while samples belonging to Series B that have been tested at Kimen seed lab shows a slight decrease in the germination percentage. The same protocol has been followed at both seed labs, and the observation is probably purely random, however, the observation should be paid further attention at the tests scheduled for 2026.

c) The crops *Beta vulgaris, Allium cepa, Cucumis sativus, Poa pratensis, Trifolium pratense, Brassica oleracea ssp. capitata f. alba and Daucus carota* still retain > 95% of the germination percentage analysed when the project was established in 1986.

d) The drop in germination of timothy and English ryegrass that started 10-15 years ago continues in the analysis carried out in 2021.

e) The 11 pathogens that are included in the project have survived over the 35 years, more or less at the same contamination levels as at the start of the project. The results are limited in number of replicates and too varying to draw any conclusions or to suggest more detailed trends or general knowledge from the pathogen survival analysis program.

NordGen's Executive Director, Lise Lykke Steffensen and Seed Vault Coordinator, Åsmund Asdal in Coal Mine 3, February 2022.

Some of the experiment's seed samples that were taken out for analysis.

Annex 1

Germination percentages of seeds of 41 varieties of 17 crops conserved in permafrost in Store norske Coal mine 3 in Svalbard analysed at the start in 1986 and in 2021.

Sample	Variety	Crop /Variety	Start 1986	2021	
Series A/B			**y0**	**y35**	**Decrease**
A-1-1	Inga Abed	H. vulgare cv. Inga Abed	95	89	6
A-1-2	Tunga	H. vulgare cv. Tunga	95	95	0
A-2-1	Vakka	T.aestivum cv. Vakka	75	69	6
A-2-2	Solid	T.aestivum cv. Solid	70	45	25
A-3-1	Petkus II	S.cereale cv. Petkus II	76	39	37
A-3-2	Voima	S.cereale cv. Voima	81	38	43
A-4-1	Pippin	L.perenne cv. Pippin	99	49	50
A-4-2	Riikka	L.perenne cv. Riikka	96	62	34
A-5-1	Tammisto	P.pratense cv. Tammisto	73	21	52
A-5-2	Bodin	P.pratense cv. Bodin	90	79	11
A-6-1	Annika	P. pratensis cv. Annika	92	95	-3
A-6-2	Hankkijan K	P. pratensis cv. Hankkijan K	94	92	2
A-7-1	Jokioinen	T.pratense cv. Jokioinen	75	75	0
A-7-2	Molstad	T.pratense cv. Molstad	93	85	8
A-8-1	Weitor 1	P.sativum cv. Weitor 1	45	67	-22

A-8-2	Hankk. Hemmo	P.sativum cv. Hankkijan Hemmo	72	89	-17
A-8-3	Weitor 2	P.sativum cv. Weitor 2	94	9	85
A-9-1	70500	B.vulgaris cv. 70500	79	86	-7
A-9-2	311 N typ	B.vulgaris cv. 311 N typ	97	91	6
A-10-1	Jupiter	B.napus cv. Jupiter	95	32	63
A-10-2	Linrama	B.napus cv. Linrama	84	6	78
A-10-1	Jupiter	B.napus cv. Jupiter	95	32	63
A-11-1	Hamund	A.cepa cv. Hamund	92	95	-3
A-11-2	Owa	A.cepa cv. Owa	89	97	-8
A-12-1	Hilro	L.sativa cv. Hilro	98	98	0
A-12-2	Attraction Sana	L.sativa cv. Attraction Sana	97	92	5
A-13-1	Langelands gig.	C.sativus cv. Langelands gigant.	99	98	1
A-13-2	Rhensk Druv	C.sativus cv. Rhensk Druv	93	90	3
A-14-1	Nantes Fancy	D.carota cv. Nantes Fancy	92	89	3
A-14-2	Regulus	D.carota cv. Regulus	91	88	3
A-15-1	Svavit	B.oleracea v. botrytis cv. Svavit	95	92	3
A-15-2	Pari	B.oleracea v. botrytis cv. Pari	95	89	6

B-1	Runar	T.aestivum cv. Runar	89	49	40
B-10	Hilma	B.vulgaris cv. Hilma	78	82	-4
B-11	Trønder Lunde	B.oleracea ssp. capitata f. alba cv.	87	84	3
B-2	Line 79 CBW	T.aestivum cv. Line 79 CBW	90	82	8
B-4	Bamse	H. vulgare cv. Bamse	90	77	13
B-5	Salten	F.pratensis cv. Salten	92	30	62
B-6	Forus	P.pratense cv. Forus	94	96	-2
B-7	Attractie	L.sativa cv. Attractie	84	71	13
B-8	Laskala	A.cepa cv. Laskala	92	88	4
B-9	Forto Nantes	D.carota cv. Forto Nantes	76	74	2

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NordGen

The Nordic Genetic Resource Centre (NordGen) is the Nordic countries' gene bank and knowledge center for genetic resources. NordGen is an organisation under the Nordic Council of Minister and works with the mission of conserving and facilitating the sustainable use of genetic resources linked to food, agriculture and forestry.

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