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LONG-TERM STORAGE OF SEEDS FROM PLANT GENE POOL SAMPLES IN THE NATIONAL DEPOSITORY

Here, we have explored the viability of plant gene pool samples deposited for long-term storage at the National Depository for Plant Genetic Resources, where 50,743 seed samples from 741 species of 308 crops, originating from 123 countries, are stored at temperatures ranging from $-18... -20$ °C in sealed foil bags. The intake of samples for long-term storage over the past 24 years has been analyzed by crop group and origin. The dynamics of seed germination rates for various crops was assessed over a 20-year storage period. Among 13 crop groups, namely cereals (28.97%), corn (17.23%), grains (8.87%), legumes (15.53%), oilseeds (5.74%), industrial crops (7.37%), medicinal and essential oil crops (2.00%), forage crops (6.40%), vegetable and melon crops (7.36%), potatoes (0.01%), ornamental flower and herb crops (0.01%), fruit (0.01%), forest and woody ornamental (0.50%) crops, there have been identified the most cold-tolerant species capable of maintaining high germination rates (90–98%) for 15–20 years, as well as the samples with low seed longevity. The research findings are important for extending seed viability during long-term storage in gene banks and for further use in breeding programs.

Key words: low temperature storage, depository, sample, genetic diversity, similarity, culture.

In recent years, human activities have significantly impacted the Earth's surface and accelerated the biodiversity loss [1, 5]. In particular, when compared to the period before the dawn of human civilization, plant biomass has declined by approximately 50% [2], and nearly 40% of plant species are now endangered [8]. As a result, the need to preserve plant genetic resources has arisen. Global experience shows that the most reliable method for long-term storage of genetic material is *ex situ* preservation, *i. e.*, in banks for plant genetic resources (gene banks). Gene banks are also the most efficient way to enhance and utilize plant genetic resources. They store the samples of genera, species, and varieties of crop plants, as well as their wild

relatives, which are the genetic basis for useful traits and properties. Gene banks provide rapid access to gene pool samples for use in breeding, scientific, educational, and other programs [4, 16].

According to the FAO, in 2022, 116 countries worldwide operate plant genetic resource banks, including 19 regional and international ones, which store more than 5.9 million samples of seeds, tissues, and other plant materials from crop plants and their wild relatives [17].

In this way, there has been solving an important task of halting biodiversity loss and enhancing the productivity and stability of agricultural production, aimed to meet the food needs of global population projected to exceed 9 billion by 2050 [3].

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Currently, there are eleven major gene banks in the world: Agricultural Research Service (ARS) of the U.S. Department of Agriculture, Beltsville, USA; International Rice Research Institute (IRRI), Los Baños, Philippines; International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India; International Maize and Wheat Improvement Center (CIMMYT), El Batán, Mexico City, Mexico; Dutch-German Potato Gene Bank, Braunschweig, Germany; International Potato Center (IPC), Lima, Peru; International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; Northern Gene Bank, Lund, Sweden; Asian Vegetable Research and Development Center (AVRDC), Taiwan; Aegean Regional Agricultural Research Institute (ARARI), Izmir, Turkey; International Center for Agricultural Research in Dry Areas (ICARDA), Syria. Large plant collections are housed at the national centers: Royal Botanic Gardens, London, UK; Institute of Plant Breeding, Cambridge, UK; National Institute for Agricultural Research (INRA), Versailles, France; Central Institute of Genetics and Crop Research, Gatersleben, Germany; Institute of Crop Breeding, Wageningen, the Netherlands; and National Institute of Agricultural Sciences at Kyoto University, Japan [15].

In 2008, the world's largest seed vault (the Doomsday Vault) was established in the mountains of Spitsbergen. The seed storage area is located more than 100 m deep inside the mountain and beneath layers of rock 40 to 60 m thick. The vault has an additional cooling system that allows the temperature, which is normally a stable $-3...4^{\circ}\text{C}$, to be lowered to the required -18°C . The largest number of samples stored in the seed vault are the rice, wheat, and barley varieties: 150,000 samples of wheat and rice, and nearly 80,000 samples of barley. Other well-represented crops include sorghum (*Sorghum*) (>50,000 samples), bean species (*Phaseolus*) (>40,000), corn (*Zea*) (>35,000), cowpeas (*Vigna*) (>30,000), soybeans (*Glycine*) (>25,000), Kikuyu grass (*Cenchrus clandestinus*) and chickpea (*Cicer*), both with over 20,000 seed samples. Crops such as potatoe (*Solanum*), peanut (*Arachis*), cowpea (*Cajanus*), oat (*Avena*), rye (*Secale*), alfalfa (*Medicago*), the hybrid grain *Triticosecale* and *Brassica* are represented by collections ranging from 10,000 to 20,000 seed samples [14]. In 2004 and 2008, the National Center for Plant Genetic Resources transferred 2,648 samples of cereal and legume crops to the Seed Vault.

Based on seeds' ability to remain viable, plant species were divided into macrobiotics, mesobiotics, and microbiotics over a century ago. Macrobiotic seeds remain viable for over 15 years (beans, vetch, vigna, pisum, flax, soft and hard wheat, oat, millet, beet, aegilops, barley). Mesobiotic seeds remain viable for 3 to 15 years (sinapis, corn, sesame, sunflower, rye, rare wheat varieties, triticale, bean, lentil). Seeds of microbiotics remain viable for no longer than 3 years (buckwheat, zucchini, onion, rice, soybean, chickpea) [6].

Based on the duration of storage, it is divided into three main types: long-term — at a temperature of $-20 \pm 2^{\circ}\text{C}$ and a relative humidity of $15 \pm 3\%$; medium-term — when the storage period does not exceed 5 years, at temperatures ranging from 0 to 10°C and relative humidity of 20—30%; short-term — from one year to 18 months. For the latter, the temperature is $20 \pm 2^{\circ}\text{C}$, and the relative humidity — 45—50% [7, 12].

The National Center for Plant Genetic Resources of Ukraine (NCPGRU) was founded in 1993. The Center is a scientific and methodological institution responsible for establishing and managing the National Bank of Plant Genetic Resources of Ukraine, which currently comprises 156,300 samples representing 544 crops and 2,020 plant species. Among them, more than 115,000 samples are propagated by seed. For over 30 years, the NCPGRU has been storing and expanding the seed collections [9].

To ensure the long-term preservation of seed-propagated plant species in a state of high viability and genetic stability, the NCPGRU has established the National Seed Depository of Plant Gene Pool Specimens (National Depository) [13]. The National Depository ensures the viable storage of 71,800 gene pool specimens belonging to 308 crops of 741 species of cultivated plants and wild relatives. Specifically, 50,700 samples are stored in a freezer (-20°C), 15,000 samples — in a refrigerator (4°C), and 6,100 samples — at uncontrolled temperatures in hermetically sealed containers.

The National Depository has been operating since 1995 and is designed to store 100,000 seed samples. The technological level of storage at the depository enables preserving gene pool seed samples in a viable state, depending on the crop and plant species, for 15—100 years or more. This is possible only if healthy, viable seeds with an intact genetic base are grown, properly prepared, and deposited in the National Depository.

The research aim herein was to analyze the longevity of seed samples from Ukraine's plant gene pool that have been deposited in the National Depository for long-term storage over the past 20 years.

MATERIALS AND METHODS

The study material consisted of seeds from 50,743 samples of Ukraine's plant gene pool, deposited for long-term storage (LTS) at the National Depository at $-18...-20$ °C in airtight foil bags. Only clean, healthy seeds with a germination rate no lower than that specified for each crop in the methodological guidelines of the NCPGRU [18] are deposited for storage. Each batch of seeds transferred to storage is accompanied by a protocol and a list in the prescribed form.

Before storage, seeds are dried using the "MD 600" air dryer (MUNTERS, Sweden) at a temperature not exceeding 25 °C and a relative humidity of 20–30% to the moisture content optimal for each type and crop: cereals and grains — 6–7%; legumes, corn — 7–8%; oilseeds — 3–4%; forage and industrial crops — 5–7%; medicinal, essential oil, vegetable, and melon crops — 4–6%.

After drying, the seeds are packed into multi-layer aluminum foil bags, which ensure a secure seal, compact storage, and preservation of seed viability under controlled storage conditions.

Each sample is accompanied by a label indicating the crop code, National Catalog number, institution registration number, and storage location. Seed moisture content, germination energy, and germination rate are determined in accordance with DSTU 4138-2002. The seed quality is monitored before deposition and during storage using reference samples.

The primary method for testing seed viability is determining the germination rate. The initial assessment of seed viability is conducted after drying the samples prior to storage. The initial germination rate should be at least 85% for seeds of most agricultural crop species. For samples of wild and forest species, local populations, and genetic lines, which typically do not have a high germination rate, a rate of no less than 30–40% is acceptable.

The interval between subsequent seed viability tests during long-term storage is 10 years for species with high seed longevity and 5 years or less for species with low seed longevity.

The number of seeds in a sample for viability testing is 100. If the sample size is small, particu-

larly for genetic lines or wild species, the number of seeds in the sample may be reduced to 50.

If seed germination falls below the acceptable level, the sample is sent for regeneration, *i. e.*, restoration of germination or, if possible, re-collecting.

All data and information obtained during seed viability testing are recorded in the National Depository's database.

The seed from the plant gene pool samples were cultivated, evaluated, and propagated for inclusion into the National Plant Gene Bank of Ukraine, primarily at the institutions of the National Academy of Agrarian Sciences (NAAS), located in various natural and climatic zones and geographical regions of the country: from the former Luhansk Research Station in the east to the Institute of Agricultural Resources and Regional Development (Transcarpathian region) and the Institute of Agriculture of the Carpathian region of the NAAS (Lviv) in the west; from the NAAS Institute of Agricultural Microbiology and Agroindustrial Production in the north (Chernihiv region) to the Nikitsky Botanical Garden (Yalta) in the south. The institutions of the PGRU System are located in all natural and climatic zones of Ukraine: Polissya, the Forest-Steppe, and the Steppe, including Transcarpathia and the southern coast of the currently annexed Crimea [11].

The findings were processed mathematically and statistically using the Excel software (Microsoft, USA).

RESULTS AND DISCUSSION

A total of 50,743 seed samples are stored in the freezers of the National Depository at -20 °C (Fig. 1). They are represented by 13 crop groups, namely: cereals (28.97%), maize (17.23%), grains (8.87%), legumes (15.53%), oilseeds (5.74%), industrial crops (7.37%), medicinal and essential oil crops (2.00%), forage crops (6.40%), vegetables and melons (7.36%), potatoes (0.01%), ornamental flowers and herbs (0.01%), fruit crops (0.01%), and ornamental forest and woody plants (0.50%).

Seeds from 308 crops, representing 741 species, are currently in storage, including: cereals (9 crops, 55 species), maize (1 crop, 4 species), cereals (7 crops, 28 species), legumes (12 crops, 95 species), oilseeds (17 crops, 41 species), industrial crops (11 crops, 37 species), medicinal and essential oil plants (118 crops, 188 species), forage crops (53 crops, 155 species), vegetables and melons (49 crops, 81 spe-

cies), potatoes (1 crop, 15 species), ornamental flowers and herbs (28 crops, 31 species), fruit plants (4 crops, 6 species), and ornamental forest and woody ones (6 crops, 5 species).

The samples stored for long-term preservation also include wild relatives of cultivated plants, specifically: 69 samples of cereal crops (wheat *Triticum araraticum*, *T. dicocoides*, *T. boeoticum*, *T. urartu*, *Aegilops bicornis*, *Aeg. columnaris*, *Aeg. crassa*, *Aeg. cylindrica*, *Aeg. geniculata*, *Aeg. kotschyi*, *Aeg. longissima*, *Aeg. lorentii*, *Aeg. markgrafii*, *Aeg. neglecta*, *Aeg. peregrina*, *Aeg. speltooides*, *Aeg. tauschii*, *Aeg. triuncialis*, *Aeg. umbellulata*, *Aeg. vavilovii*, *Aeg. ventricosa*, barley *Hordeum agrocrithon*, *H. bulbosum*, *H. murinum*, *H. spontaneum*, *H. vulgare*, maize *Zea mays*), 251 samples of legumes (soybean *Glycine canescens*, *G. latifolia*, *G. latrobeana*, *G. pescadrensis*, *G. rubiginosa*, *G. soja*, *G. stenophita*, *G. tabacina*, *G. tomentella*, bean *Phaseolus acutifolius*, the genus *Lathyrus* *L. annuus*, *L. aphaca*, *L. articulatus*, *L. chloranthus*, *L. chrysantikus*, *L. cicera*, *L. clymenum*, *L. gorgoni*, *L. hirsutus*, *L. inconspicuus*, *L. nissolia*, *L. ochrus*, *L. odoratus*, *L. pseudocicera*, *L. sativus*, *L. sphaericus*, *L. sylvestris*, *L. tingitanus*, *L. tuberosus*, *L. vinealis*; chickpea *Cicer arietinum*, *C. bijugum*, *C. chorassanicum*, *C. judaicum*, *C. pinnatifidum*, *C. reticulatum*, *C. yamashitae*; lentil *Lens orientalis*, *L. odemensis*, *L. tomentosus*; beans *Faba bona*, lupine *Lupinus angustifolius*, *L. luteus*, *L. polyphyllus*; wetch *Vicia angustifolia*, *V. articulata*, *V. benghalensis*, *V. bithynica*, *V. cordata*, *V. eriocarpa*, *V. ervilia*, *V. galilaea*, *V. grandiflora*, *V. hajastana*, *V. hirsuta*, *V. hybrida*, *V. hircanica*, *V. lathyroides*, *V. lutea*, *V. michauxii*, *V. nana*, *V. narbonensis*, *V. neglecta*, *V. pannonica*, *V. peregrina*, *V. sativa*, *V. sepium*, *V. varia*, *V. villosa*), 58 samples of oilseeds (sunflower *Helianthus angustifolius*, *H. annuus*, *H. californicus*, *H. debilis*, *H. divaricatus*, *H. floridanus*, *H. giganteus*, *H. hirsutus*, *H. microcephalus*, *H. mollis*, *H. niveus*, *H. nuttallii*, *H. petiolaris*, *H. praecox*, *H. strumosus*; castor bean *Ricinus communis*; mustard *Brassica nigra*, lallemantia *Lallemantia iberica*, safflower *Carthamus lanatus*, *C. tinctorius*, poppy *Papaver orientale*, *P. rhoeas*, *P. somniferum*, *P. argemone*; roquette *Eruca sativa*), 53 samples of industrial crops (flax *Linum alpinum*, *L. angustifolium*, *L. austriacum*, *L. campanulatum*, *L. flavum*, *L. grandiflorum*, *L. perenne*, *L. squamulosum*, *L. usitatissimum*, hemp *Cannabis sativa*, tobacco *Nicotiana glutinosa*, *N. to-*

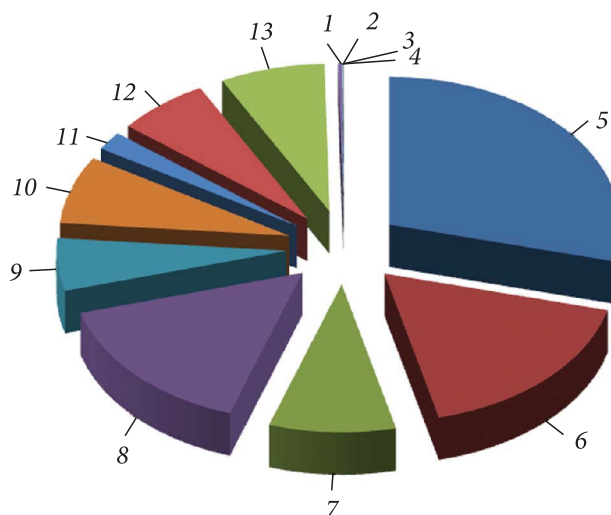


Fig. 1. Plant seeds deposited for long-term storage; number of samples: 1 — flowers and herbaceous ornamental, 140; 2 — potatoes, 26; 3 — fruits, 6; 4 — forest and woody ornamental, 11; 5 — cereals, 14832; 6 — maize, 8824; 7 — groat, 4546; 8 — grain legumes, 7954; 9 — oil bearing, 2942; 10 — technical, 3785; 11 — medicinal and essential oil-bearing plants, 1027; 12 — Forages, 3329; 13 — Vegetable and melon crops, 3767

mentosa, *N. longiflora*, *N. paniculata*, beet *Beta corolliflora*, *B. intermedia*, *B. lomatozona*, *B. maritima*, *B. patellaris*, *B. vulgaris*, chicory *Cichorium intybus*), 47 vegetable samples (parsnip *Pastinaca sativa*, carrot *Daucus carota*, lettuce *Lactuca sativa*, cress *Lepidium sativum*, dill *Anethum graveolens*, sorrel *Rumex acetosa*, *R. alpinus*, *R. confertus*, *R. crispus*, *R. tianschanicus*, rhubarb *Rheum rhaponticum*, *R. officinale*, onion *Allium fistulosum*, *A. nutans*, *A. odorum*, *A. schoenoprasum*, gourd *Lagenaria siceraria*, fennel *Foeniculum vulgare*, savory *Satureja coerulea*, *S. hortensis*, eggplant *Solanum aethiopicum*), 20 samples of flowering plants and herbs (peony *Paeonia officinalis*, *P. tenuifolia*, chrysanthemum *Chrysanthemum coronarium*, martagon lily *Lilium martagon*, stonecrop *Sedum aizoon*, melic grass *Melica altissima*, California poppy *Eschscholzia californica*, balsam *Impatiens balsamina*, stock *Matthiola incana*, nicandra *Nicandra physalodes*, pokeweed *Phytolacca americana*, desert lily *Eremurus spectabilis*, nepeta *Lophanthus anisatus*, white swallow-wort *Vincetoxicum hircundinaria*, watercress *Nasturtium officinale*), 4 fruit samples (rowan *Sorbus aucuparia*, danewort *Sambucus. ebulus*, *S. nigra* ra guilder-rose *Viburnum opulus*). The collection of medicinal and essential oil crops consists of 31% wild species. Those of potatoe, forest and ornamental crops are entirely represented by wild species.

Every year, seeds of new specimens are deposited for storage in freezers; the largest number was stored in 2010 — 4,975 (Table). The most cereal crops were stored in 2019 — 1,613 samples; corn, oilseed, ornamental, and tree crops — in 2009 (1,172, 446, 88, and 8 samples, respectively); grain crops — in 2007 (499 samples); legumes — in 2006 (801 samples), industrial, medicinal, and vegetable crops — in 2010 (594, 126, and 529 samples, respectively); the largest number of forage crop samples was deposited in 2012 — 380. Most of the potato samples (20) were placed to the depository in 2014.

The samples deposited for long-term storage come from 123 countries. The largest number of samples are from Ukraine — 23,973, Russia — 6,467, the United States — 2,309, Germany — 1,339, Mexico — 1,230, Canada — 897, Syria —

893, Kazakhstan — 800, France — 772, Belarus — 733, Hungary — 701, Poland — 699, the Czech Republic — 621, Bulgaria — 591, China — 538, Moldova — 431, Romania — 387, India — 378, the Netherlands — 360, Turkey — 360, the United Kingdom — 336, and Sweden — 318.

It has been shown that the viability of cereals, grains, legumes, and maize remained unchanged and at a high level after 20 years of storage in a freezer (Fig. 2).

The germination rates of oilseed and vegetable crops remained high (with minimum acceptable levels being 75 and 62%, respectively) for 15 years, after which it began to decline. Some ornamental flower and herb crops began to show reduced germination rate 10 years after being deposited for storage; after 15 years, this index decreased by 15%.

Seeds of various crop groups accepted for long-term storage; number of sample

Year	Cereal crops	Maize	Grain crops	Legumes	Oilseed crops	Industrial crops	Medicinal and essential oil crops	Forage crops	Vegetables and melons	Potato	Ornamental flowers and herbs	Fruit crops	Ornamental forest and woody crops
2001	—	—	—	—	—	14	—	—	—	—	—	—	—
2002	—	—	—	—	20	27	24	29	36	—	6	—	—
2003	—	—	—	—	69	16	9	19	35	—	4	—	—
2004	—	—	—	—	30	33	41	56	45	—	8	—	—
2005	7	—	—	—	142	80	93	145	62	—	—	—	—
2006	925	484	410	801	226	335	101	188	120	—	3	2	—
2007	1025	514	499	773	325	485	15	168	167	—	—	—	—
2008	415	700	389	555	430	530	38	149	341	—	1	2	—
2009	332	1172	324	619	446	570	47	118	375	—	88	—	8
2010	1249	972	416	769	238	594	126	78	529	—	4	—	—
2011	1468	1037	361	224	211	208	48	86	248	4	2	—	—
2012	1130	694	257	350	161	118	46	380	170	2	2	—	—
2013	1034	656	419	619	104	45	98	58	108	—	3	1	—
2014	1047	477	323	311	70	395	54	120	75	20	5	—	—
2015	1303	460	461	392	59	109	35	60	54	—	—	—	—
2016	868	409	126	744	184	73	66	189	182	—	6	—	—
2017	1043	245	239	430	56	29	30	195	35	—	—	—	—
2018	835	209	56	654	50	16	64	196	223	—	2	1	—
2019	1613	107	143	402	80	42	5	178	96	—	—	—	—
2020	21	77	16	14	8	7	13	34	69	—	2	—	1
2021	30	67	18	10	—	9	13	278	113	—	2	—	1
2022	6	28	4	—	3	21	7	183	97	—	1	—	—
2023	80	159	11	164	12	7	48	243	440	—	—	—	—
2024	401	357	74	123	18	22	6	179	147	—	1	—	1
Avg/yr	618	367.7	189.4	331.4	122.9	157.7	42.8	138.7	156.9	1.1	5.8	0.3	0.5

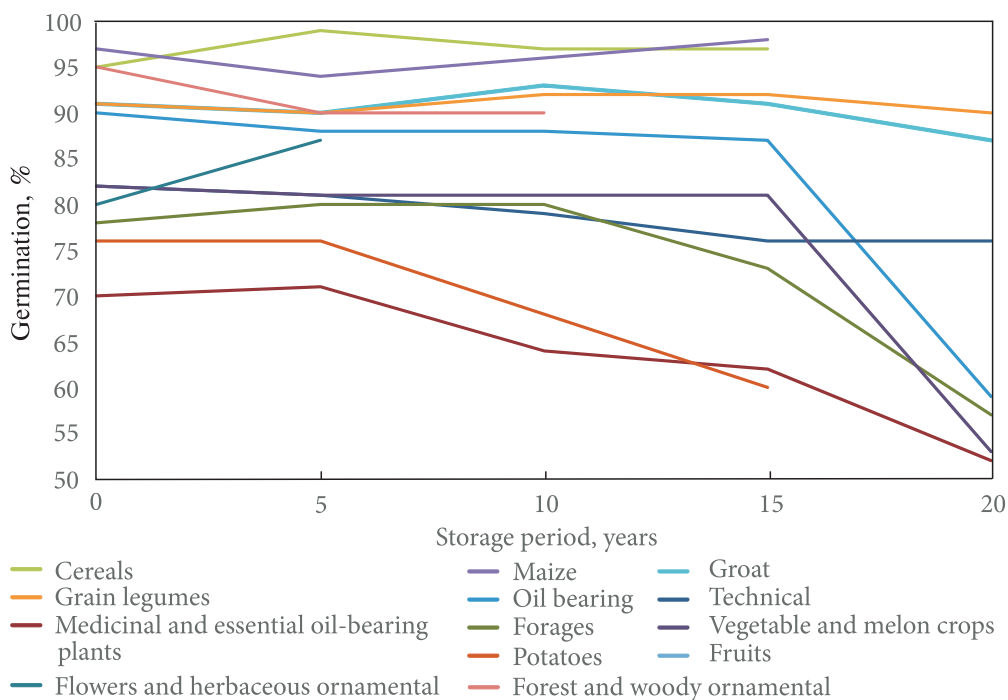


Fig. 2. Seed viability by crop groups



Fig. 3. Monitoring of seed viability using the following examples: a — cucumber (*Cucumis sativus*); b — milk thistle (*Silybum marianum*)

This proves the need for more thorough monitoring of these crops' seeds during storage.

The germination rate of industrial crop seeds remained at an acceptable level — 76% — over a 20-year storage period. For medicinal plants, germination rates decreased by 20% within 20 years and was 52%, but still remained at an acceptable level, since for some medicinal crops the maximum germination rate is 50%, and the minimum acceptable level is 30—40%. It is worth noting that we observed exceptions to the general viability results. Moni-

toring of milk thistle *Silybum marianum* sample, which has been stored in the Depository since 2005 (Fig. 3), showed its germination rate as gradually increased: from 32% in 2005 to 96% in 2025. As reported by various researchers, this increase may be related to biochemical composition of seeds [18].

CONCLUSIONS

The National Depository plays a vital role in long-term preservation of the biodiversity of the National Gene Bank and in supporting scientific, breed-

ing, and educational programs. An analysis of 50,743 samples of Ukraine's plant gene pool for longevity has revealed that seeds of cereal, grain, and legume crops are more resilient to storage at sub-zero temperatures (–18... –20 °C) which maintain high germination rates (90–98%) for 15–20 years. The oilseed, industrial, and vegetable crops remained viable for 15 years with germination rates of 76–87%. Forage, medicinal, essential oil, flower, and ornamental crops were characterized by low

seed longevity. In particular, the varying durability of seeds from the Ukrainian plant gene pool may be related to seed adaptability to adverse conditions and biochemical composition. Assessing seed longevity enables the improvement of long-term storage protocols and the assessment of seed condition in the National Gene Bank of Plants of Ukraine, which is crucial for extending seed viability during long-term storage in gene banks and for their subsequent use in breeding programs.

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ДОВГОСТРОКОВЕ ЗБЕРІГАННЯ НАСІННЯ
ЗРАЗКІВ ГЕНОФОНДУ РОСЛИН У НАЦІОНАЛЬНОМУ СХОВИЩІ

У роботі досліджено життєздатність зразків генофонду рослин, закладених на довгострокове зберігання у Національне сховище генетичних ресурсів рослин, де за температури $-18...-20$ °С у герметичних пакетах із фольги зберігається 50 743 зразків насіння 741 виду 308 культур, які походять з 123 країн світу. Проаналізовано надходження зразків на довготривале зберігання за останні 24 роки за групами культур та походженням. Оцінено динаміку схожості насіння різних культур протягом 20 років зберігання. Серед 13 груп культур, а саме: зернових (28,97 %), кукурудзи (17,23 %), круп'яних (8,87 %), зернобобових (15,53 %), олійних (5,74 %), технічних (7,37 %), лікарських та ефіроолійних (2,00 %), кормових (6,40 %), овочевих та баштанних (7,36 %), картоплі (0,01 %), квіткових та трав'яних декоративних (0,01 %), плодових (0,01 %), лісових та деревних декоративних (0,50 %), встановлено найбільш витривалі до зберігання за від'ємних температур, які підтримують схожість на високому рівні (90—98 %) протягом 15—20 років та зразки з низькою довговічністю насіння. Проведені дослідження мають важливе значення для подовження життєздатності насіння під час тривалого зберігання у банках генетичних ресурсів та для подальшого використання у селекційних програмах.

Ключові слова: низькотемпературне зберігання, сховище, зразок, генетичне різноманіття, схожість, культура.