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GERMINATION OF SEEDS OF *DIANTHUS PLUMARIUS* L. (CARYOPHYLLACEAE JUSS.) AFTER STORAGE PERIOD OF 43 AND 44 YEARS

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Abstract

The seed germination tests of long-term (1978- and 1979-year harvest) storage seeds of *Dianthus plumarius* were carried out. The seeds were stored in a cold chamber (0-5 °C) for 43- and 44-year period, respectively, in the hermetically sealed glass containers. The experiment was conducted in laboratory conditions, on filter-paper, in three consequent stages: initial screening (Sept/Oct. 2022, sample 1978); 6 seed samples of 100 seeds each: 3 samples from 1978 and 3 samples from 1979 year (Oct/Nov. 2022) and 12 x 100 seeds with the highest germination taken from the previous experimental batch (Nov/Dec.2022). The temperature and air humidity were monitored daily by an Electronic Temperature station. The results from trials Oct./Nov.2022 showed that the highest germination energy (GE) was found in the seed sample from 1978 (65%) while the GE varied between 42 and 46% in the samples of the 1979' harvest. The maximum percentage of germination, in almost all experimental samples, was achieved on the 14th day and until the end of the experiment remained unchanged. The highest percentage of germination was found in sample from 1978 year - 82%. A statistically significant difference was observed between samples from 1978 and the two samples from 1979 ($p \leq 0.05$, ANOVA). The germination process took place at relatively low temperatures and at a relatively small day-night amplitude. The two consecutive experiments confirmed the fact that the seed samples from 1978 year showed a higher germination energy, which could be explained by a higher water content in the seeds which were initially placed in cold storage and were preserved during the storage conditions for the specified long-term period. The high germination rates observed in the present experiment encourages further investigations.

Keywords: *Dianthus plumarius*, Caryophyllaceae, long-term stored seeds, germination.

INTRODUCTION

The successful seed germination is considered as an important stage in the plant life cycle and is a critical factor both in production and in survival of plants in natural ecosystems (Waterworth et al., 2019). In the literature, the problem of long-term seed storage has a long history with reported records of established germination of long-term stored seeds (Roberts, 1988, Solberg et al., 2020). A revolutionary turning point in this field of research was marked by the publications of Elis & Roberts (1980, 1981) and their mathematical model for calculating/prediction of germination after a storage period, knowing the exact conditions under which seeds of particular crops had been stored.

It the recent years, new studies have addressed the problem to eco-biological, physiological and genetic aspects (Black & Bewley 2000, Baskin & Baskin, 2001; Solberg et al., 2020, Zinsmeister et al., 2020, Bradford 1990, Leila et al. 1992, McDonald 2000).

Solberg et al. (2020) have shown that when comparing mathematical models for predicting seed germination with real trials, the models have significantly higher (high and very

high) germination predictions compared to the actual values obtained experimentally. The same authors indicated that the germination (half-life P0.5) under uncontrolled conditions (at room temperature conditions) was preserved for short period (5 to 10 years), while at proper storage (orthodox seeds - at low humidity and temperature), the P0.5 (the half-life of preservation of germination) was 40−60 years with significant interspecies differences observed.

In this context, an experimental work with long-term stored seeds has given an information of the ability of certain seeds to preserve their germination energy for an extended period of time and to evaluate the practical benefit of this (Yancheva 2007, Kouneva et al., 2009). In Bulgaria, the studies on long-term seed storage have been conducted in the field of ornamental plants (Institute of ornamental and medicinal plants, Sofia) as well as in agronomy (IRGR "Konstantin Malkov"-Sadovo (Institute of plant and genetic resources). Prof. Elena Shtiliyanova has organized the research work in the University of Forestry about the long-term seed storage back in 1998, with the available seed bank at the UF from 1973 to 1981 year. This seed collection has been a source for experimental laboratory studies on preservation of germination in *Dianthus plumarius* L. as follows: Shtiliyanova E. Zl. Kabatliyska (1992, published 1998), Kabatliyska, M. Dimitrova, M. Petrova (2005), Kabatliyska, Stoenchev (2006).

The genus *Dianthus* L. is the source of many ornamental species. Carnations are one of the most desired flowers because of their excellent durability and ornamental characteristics (Gonzalez-Amaya et al., 2018). Carnation is one of the most popular greenhouses cut flower crops (Lopez et al., 2010, Dyaberi et al., 2015, Castilla & Gonzales, 2014) and one of the most important crops in the world (Rabnawaz et al., 2020). The co-germination of seeds is an important alternative to reduce costs in seed propagation of some *Dianthus* cultivars (Gonzalez-Amaya et al., 2018). The species of the genus are suitable for different types of compositions. In our practice, the species of the genus *Dianthus*, including *D. plumarius* L., are recommended for flowering meadows (Kouneva et al., 2008, 2009) and *D. plumarius* L., together with two other representatives: *D. monspessulanim* var. Sferenbergii and *D. biflorus* Sm., when grown in light and porous substrate they are recommended for extensive and semi-extensive roof-landscaping (Yancheva, 2016). Besides their ornamentality, the species of the genus are extremely resistant to drought (Dimitrova et al., 2020) and aggravated soil conditions. Modern studies on the seed germination of species of the genus under deteriorated soil conditions show good adaptation qualities - *Dianthus superbus* L. in soils contaminated with copper dichloride ($CuCl_2$) (Brînză et al., 2019); *Dianthus carthusianorum* L. in soils rich in copper, lead and cadmium (Muszynska et al., 2013). The studies on the germination of seeds of *Dianthus* sp. showed a significant increase in the percentage of germinated seeds in *D. barbatus* cv. 'Purple' when combined with soaking for 6 hours and KNO_3 (concentration 250 mg/l) (González-Amaya et al., 2018). Hazar & Baktir (2012) have carried out detailed studies on the effect of temperature, growing medium and age of seeds on the germination process of several representatives of the genus *Dianthus*. The authors indicated 20°C as an optimal temperature for germination of seeds of *Dianthus calocephalus* Boiss and a significant difference between the germination percentage of fresh and stored seeds in favor of freshly harvested. The same authors (Hazar & Baktir, 2013) studied the influence of substrate, seed age and different options for pre-sowing seed preparation - treatment with different concentrations and different exposure to gibberellic acid solutions and different heat treatment of seeds, as well as the influence of different substrate on germination of *Dianthus orientalis* Adams. The authors indicated that the

storage of seeds at the temperature range of 5-10°C resulted in decreased germination. Rabnawaz et al. (2020) stated that after comparing chemical treatment (salicylic acid, glycine betaine, potassium nitrate and ascorbic acid) and hydropriming on seeds growth and quality characteristics of carnations, the best results in seed germination were obtained with hydropriming (water treatment) - 43.04%. The shortest time for the formation of the first flower of the plant was observed after hydropriming treatment. All other treatments improved the production characteristics of plants when compared to plants from untreated seeds. For a crop of fertile seeds (as a prerequisite for long-term preservation of germination), a certain relationship has been established between the time of planting of young carnation plants and the corresponding flowering time. Sharma et al. (2015) found that an early planting period of *Dianthus barbatus* resulted in the formation of the highest number of flowers, seed pods and seeds per pod. Dyaberi et al. (2015) found significant differences between seed pod setting ability and seed number and fertility among individual phenotypes.

The purpose of this paper is to present the results of germination of long-term stored seeds of *Dianthus plumarius*, yield from 1978 and 1979 year, by estimating the germination energy and total germination rate.

MATERIALS AND METHODS

The tested *Dianthus plumarius* seeds were stored in a cold chamber (0-5 °C) since their harvest (1978, 1979 year) till 2022 (43- and 44-year period, respectively) in hermetically sealed glass containers. The seed have been collected from an experimental field of the University of Forestry-Sofia (at that time named as the Higher Institute of Forestry). The age of the plants at the time of seed collection was 5 years.

After some preliminary tests (Sept/Oct. 2022, sample 1978) (1) the experiments were repeated (16.10-09.11.2022 with 6 seed samples of 100 seeds each) from 6 different sources (provisionally named 1978 (1, 2, 3) and 1979 (4, 5, 6) (Fig. 1) and again between 16.11-12.12.2022 with the highest performing samples from a previous experimental batch (4x100) for each variant, labeled respectively: 78-1; 79-4 and 79-5, with indicators: a, b, c and d for each sample (Fig. 2).

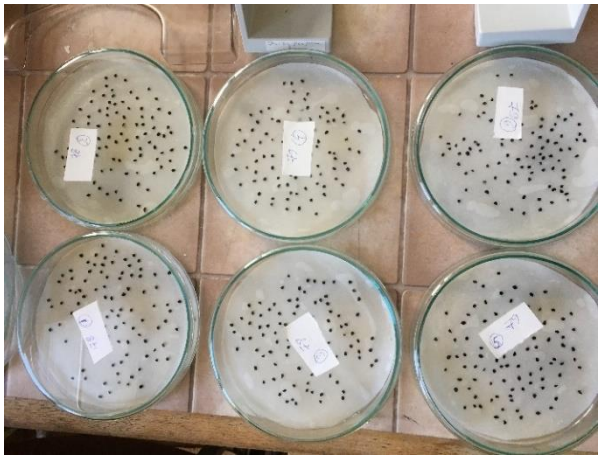


Fig. 1 Seed samples of 100 seeds each from 6 different sources (Nov/Dec 2022)

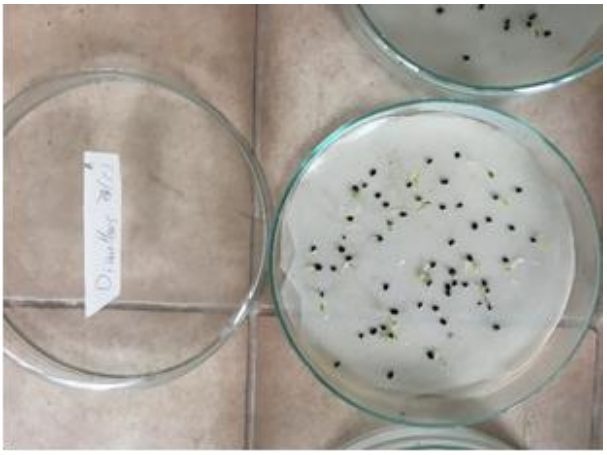


Fig. 2 Germinated seeds from a sample 79-5(b) (Nov/Dec 2022)

The seeds were placed on filter paper in laboratory conditions. For a more accurate count, the germinated seeds (at root tip and cotyledon leaves appearance stage) were removed with tweezers from the filter paper surface and placed on a soil substrate for further cultivation. The temperature and humidity of the air were monitored daily by an Electronic

Temperature station (Meyer, 2022). In the experimental period (16-09.11.2022) the minimum temperature was 18.6°C, the maximum 22.8°C; the values for air humidity were 44 and 57%, respectively. The average values of these indicators for the period were 19.3°C and 46%. In the period 16.11-12.12.2022, the values were respectively: for min 17.6°C, max 21.9°C, average 19.3°C, and the values of air humidity: min 41%, max 56% and average 48%.

For the final experiment (started on 16.11.2022), the GE% (Germination energy) record was carried out on 24.11.2022 and the final observations - on 12.12.2022, when a constant number of germinated seeds was established for the particular sample.

The data was processed with SPSS-20 (IBM, ver.20). Taking into account the experiment specifics and the number of repetitions, the values discussed are minimal, maximal and the median. Analysis of variance (ANOVA) was used to establish a statistically significant difference between the germination energy and the total number of germinated seeds between 1978 and 1979 year samples.

RESULTS AND DISCUSSION

The analysis of the data from Table 1 shows that the experiment with the longest-term stored seeds of *Dianthus plumarius* at the time of the study: 19 years, greenhouse/sand, with a significant temperature amplitude (20°C between the values of max and min air temperature), the obtained germination percentage was 66.7%; for seeds stored for 27 years - laboratory/ paper, with a temperature amplitude and sowing conditions similar to the present experiment (amplitude max/min 6°C and filter paper), the established germination was 65%. However, there was no evidence that the seeds used were from the same stored specimens as the seeds in the present study. For freshly harvested seeds (refrigerated for less than 1 year), the value reported by the authors was between 91.6% (1998) and 89% (2006).

In the current experiment (16.10-09.11.2022), the positive records concerned samples 78-1, 79-4 and 79-5. The obtained results are presented in Table 1, and the Germination Energy (GE) and germination dynamics are presented on Fig 3.

Table. 1. Germination of seeds of *Dianthus plumarius* L., stored for 43 and 44 years, experiment 16.10-09.11.2022. (Min/Max temp. 18.6/22.8 °C; Min/Max air humidity 44/57%)

Year of harvesting	**Period of storage/years**	**Number of samples /seeds**	**Description of the experimental plot**	**Total germination rate % (21 day)**
1978-1	**44**	**4x25**	**Filter paper/lab.**	**82**
1978-2	44	4x25	Filter paper/lab.	-
1978-3	44	4x25	Filter paper/lab.	-
1979-4	**43**	**4x25**	**Filter paper/lab.**	**75**
1979-5	**43**	**4x25**	**Filter paper/lab.**	**71**
1979-6	43	4x25	Filter paper/lab.	-

The results showed the highest germination energy of the seed sample 1978a (65%) while in the samples of the 1979 harvest it varied between 42 and 46%. In almost all experimental samples the maximum percentage of germination was achieved at 14th day and until the end of the experiment remained almost unchanged. The highest percentage of germination was found in sample 1978a - 82% (fig. 3). The following results were obtained from the Nov/Dec 2022 experiment (fig.4).

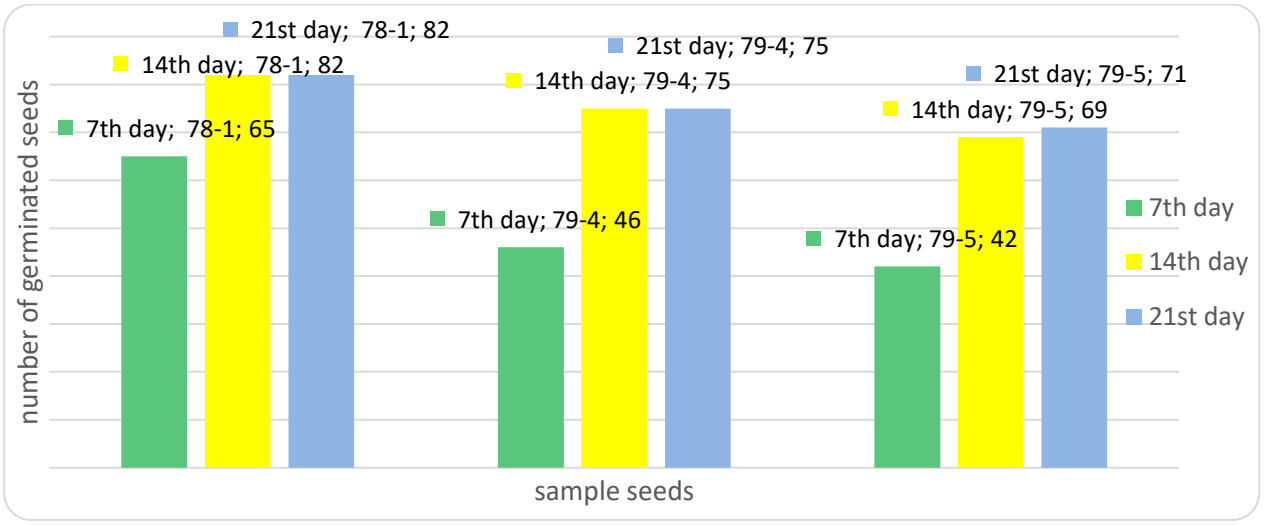


Fig. 3 The germination energy and germination dynamics at samples 78-1, 79-4 and 79-5 (16.10-09.11.2022)

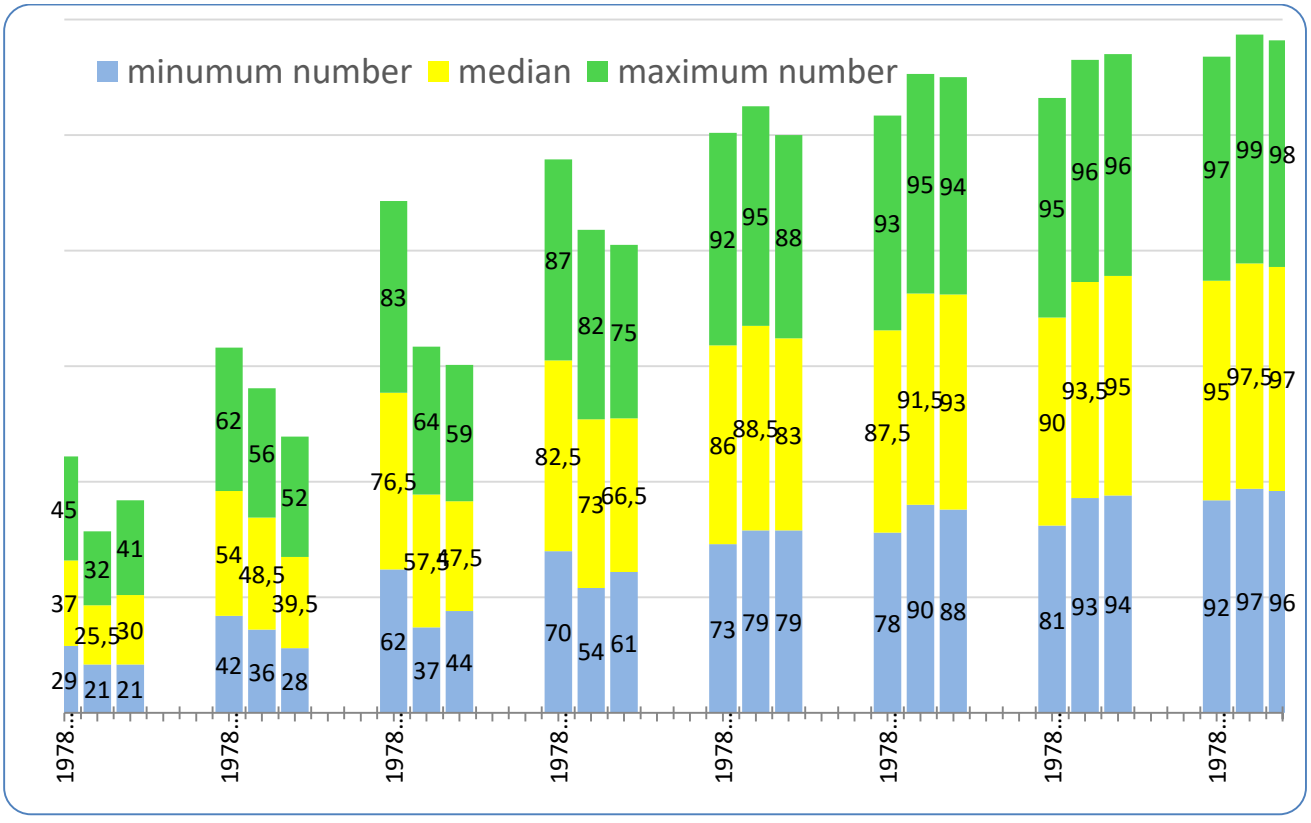


Fig. 4 Value of median, minimum and maximum number of germinated seeds on reporting dates for yields 1978-1, 1979-4, 1979-5.

Estimating the median values, in experiment '78, at all reporting dates up to 02.12 (16th day of the experiment) the number of germinated seeds exceeded those of the two seed samples from 1979 year. By the end of the experiment, the number of germinated seeds for the three batches was almost equal.

In the previous studies with seeds from the same source (the UF collection-harvest 1978) a germination rate of 68.3% (after 14

years of storage) and 65% (after 19 years of storage) was observed at March-April sowing period/ greenhouse/ sand media (Stilyanova & Kabatliyska, 1998).

The seeds from different plants of *Dianthus plumarius*, collected in 2004 and stored in fridge for 1 year showed a germination rate of 89% at April-June sowing period/ laboratory/filter paper media (Kabatliyska & Stoenchev 2006).

The results of the ANOVA test for statistically significant differences are presented in Table 2.

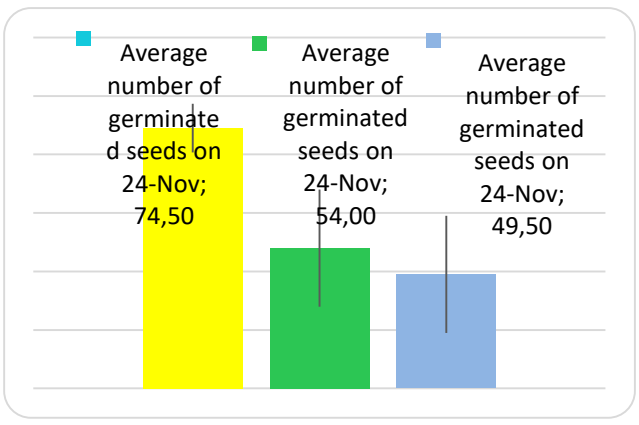
Table 2. Significance of the factor „harvest year“ on the reporting dates 24.11.2012 and 12.12.2022 estimated by analysis of variance (ANOVA).

DependentVariable / Defining factor	Average number of germinated seeds on reporting date		
	df	F	P (Sig.)
	Average number of germinated seeds on 11/24/2022.		
Harvest year	2	7,758495146	,011*
	Average number of germinated seeds on 12/12/2022.		
Harvest year	2	4,500	,044*

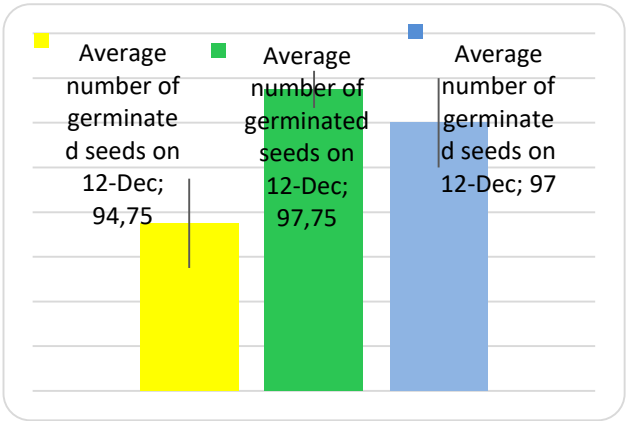
Note: * Proven influence at p≤0.05; df - degrees of freedom; F - Fisher's criterion; p (Sig.) - significance level

There was a statistically significant difference between the 1978 crop - it showed higher germination energy when reported on November 24th, but the total number of germinated seeds was lower than the 1979 crop - last reported on December 12th.

In Fig. 5 a, b the values are presented as M+/-SE, where M is the mean, SE is the standard error of the mean. Means followed by different lowercase letters are not significantly different, and those with different lowercase letters are different, at p≤0.05, as determined by one-way analysis of variance and post hoc LSD test.



a)



b)

Fig. 5 a,b. Statistically significant difference between mean number of seeds from 1978 and both samples from 1979 on the 24.11.2022 (a) and the 12.12.2022 (b)

CONCLUSION

The germination process took place at relatively low temperatures and a relatively small diurnal amplitude. The two consecutive experiments confirmed the fact that the seed samples taken in 1978 year showed a higher germination energy, which could be explained by a higher water content of the seeds when initially placed in the cold storage and

respectively preserved better during the storage conditions. The observed high germination rate of long-term stored seeds of *Dianthus plumarius* encourages future studies.

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