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Changes in Germination of Some Seasonal Flower Seeds Over 16 Years in Cold Storage

Bazı Mevsimsel Çiçek Tohumlarında 16 Yıllık Soğuk Depolama Sonrası Çimlenmedeki Değişimler

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Abstract

This work was carried out to test the changes in germination percentages of 11 seasonal flower seed species stored in cold storage (5 °C, 6.7-7.6 % seed moisture) hermetically sealed over 16 years. *Pelargoniums, tagetes, zinnias, petunias* and *gazanias* were found to be the most resilient species, in which seed germination was reduced between 0 and 3% following storage compared to initial germination values. *Dahlias, salvia* and *verbena* were medium tolerant species, as they lost germination at between 22 and 30%. The most sensitive species were *antirrhinum, viola* and *impatiens*, which had a germination loss as high as 94%. As vigour indication, 7th day radicle counts showed that the more resilient species had higher earlier germination values. Comparison of the storage of hybrid and open-pollinated cultivars made in two species, *Pelargonium* and *Viola*, indicated that germination was not lost in *pelargonium* hybrids, but germination loss was higher than 77% in both open pollinated and hybrid seed types of *Viola*. The results indicated that seed storage potential in flowers was greatly affected by species differences and should be taken into account for plant production practices.

Keywords: Flower seeds, Germination, Storage,

Özet

Bu çalışma 11 adet farklı mevsimlik çiçek türü tohumunun 5°C'de %6.7 ile 7.6 tohum neminde 16 yıl boyunca hermetik şekilde depolanması sonucu çimlenme oranlarındaki değişimi test etmek için yürütülmüştür. Sardunya, kadife, Kirli hanım, petunia ve Koyun gözü türleri depolamaya en dirençli türler olarak ve bu türlerde çimlenme başlangıç yüzdelerine göre sadece %0-3 arasında değişmiştir. Yıldız çiçeği, ateş çiçeği ve mine çiçeği tohumları orta düzeyde dirençli türler olarak saptanmış ve çimlenmedeki canlılık kaybı %22 ile 30 arasında değişmiştir. En hassas türler olarak aslanağzı, mor menekşe, camgüzeli ve menekşe saptanmış olup bu türlerde çimlenme kaybı başlangıç canlılığına göre %94'e kadar ulaşmıştır. Tohum gücündeki değişim olarak 7th gün çimlenme değerleri saptanmış ve depolamaya dirençli olan türler bu sayımda da yüksek değerler vermiştir. Hibrit ve açık tozlanan çeşitler menekşe ve sardunyada karşılaştırılmıştır. Sardunyada hibrit tohumlar depolamada canlılıklarını hiç kaybetmezken menekşede hem hibrit hem de açık tozlananlarda canlılık kaybı %77'ye kadar çıkmıştır. Sonuçlar çiçek tohumlarının türler bazında depolamaya dirençliliklerinin farklı olduğunu ve bunun bitki üretiminde dikkate alınması gerektiği sonucunu göstermektedir.

Anahtar Kelimeler: Çiçek tohumları, Çimlenme, Depolama

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1. Introduction

The longevity of the seeds during storage depends on pre and post-harvest factors. Harvest maturity, processing, seed drying etc. can be considered as some of pre-storage factors. Storage temperature, relative humidity/seed moisture and oxygen are post-storage factors (Ellis, 2022; Nadarajan et al., 2023; Corbineau, 2024). The genetic structure (species differences) of seeds also plays an eminent role in storability. There are differences among the flower species regarding storability. Certain flower species store better than others (McDonald, 2005, Demir et al., 2020a). If a seed species has a short storage life, then it is necessity to purchase new seed on an annual basis. In all cases, understanding the species-based seed storage behaviour of flower seeds is important for seed companies since extensive inventories of seed are maintained and to ensure that the value of the seed is not lost during storage. Seed lost during storage is important issue since high value/hybrid seed use are common in flowers. It is estimated that 25% of seeds are loss viability over the storage in flowers (Schmidt, 2002). Moreover, conserving high seed germination during storage is a necessity for obtaining a high percentage of seedling emergence in modules for quality/timely plant production. It is well-known that even though seed germination is not lost seed vigour i.e. emergence under stressful environment, decrease as the storage time extends (Demir, 2011). Studies about storage behaviour of flower seeds (Demir et al., 2020a), are scarce. It was suggested that 15-25°C storage may be adequate for seeds of annual flowering plants for 1 year, but for longer storage periods temperatures lower than 15°C are needed. (Carpenter et al., 1995a) Germination after storage at 5°C was found to be higher than that after storage at 15°C for Delphinium (Carpenter and Boucher, 1992) and Gerbera (Carpenter et al., 1995b) seeds. Carpenter et al., (1995b) suggested that storage conditions can affect uniformity of germination (more aged seeds take longer time to germinate) that can be an important asset for high quality transplant production. Similarly, seed vigour differences which were reflected on seedling emergence occurred due to the extended storage conditions were observed in various flower seed species as viola (Demir et al., 2011), geranium, salvia, gazania, impatiens (Guloksuz et al., 2012) and petunia (Demir et al., 2020b).

When carrying over seeds (those that are left over from an earlier production year) are to be used in next production season, or seed gene bank samples for long term use, seed lots must be stored in ideal conditions. Commercial seed storage on company base is conducted ideally at 12-17°C temperatures with 40-60% relative humidity/5-7% seed moisture for medium-term storage (12-36 months) for diverse species (Walters, 2015). In seed gene bank conditions seed samples are stored at 5°C at 5-7% seed moisture for active seed collection. They normally require to re-produce every 5 years. Un-optimal storage environment is basic reason for inducing seed ageing/longevity. Our research objectives were to compare the germination percentages after storing the seeds of eleven flower species with 6.7-7.6% seed moisture content stored hermetically at 5°C for 16 years and show how the seed quality varies among the species.

2. Material and Methods

Eleven different flower seed species were used in the work. Geraniu/Pelargonium (*Pelargonium hortorum*.), Marigold/tagetes (*Tagetes erecta*), Zinnia (*Zinnia* elegans), Petunia (*Petunia grandifolia*.), Gazania (*Gazania rigens*.), Dahlia (*Dahlia variabilis*), Salvia (*Salvia splendes*), Antirrhinum (*Anthirrhinum* spp), Verbena (*Verbena* spp.), Pansy/Viola (*Viola wittrockiana*) and Impatiens (*Impatiens* spp.) (Table 1) were obtained from commercial seed companies. Seeds of gazania, salvia, antirrhinum and verbena seeds are open-pollinated but the rest was hybrid cultivars. Initial seed moisture content was determined using the low temperature oven method (ISTA, 2020) and seed moisture percentages were changed in between 6.7 and 7.6% being the lowest in geranium and the highest in salvia, pans and ımpatiens as 7.6%. among species. Four replicates of 25 seeds were placed on double layered filter paper (Filtrak, Germany) which wetted with 5 ml of KNO_3 in a petri dish (9 cm). Tests were done in April in 2008 (before storage, initial germination) and after storage in April 2024 (about 16 years after). Seeds were stored at closed glass jars with sealed caps at 5°C in the dark. In the germination tests the Petri dishes were watered with 0.2% KNO_3 and placed at 5°C, over 7 days to make the dormancy release (Baskin and Baskin 2014) initially and then transferred to 20°C in dark, subsequently. Petri dishes were placed in sealed polyethylene bags to prevent evaporation. Total germination percentages (2 mm radicle emergence) were evaluated after the 7th day of the test and final germination period after 21 days. In order to see the differences in seed survival in open-pollinated and hybrid seeds open-pollinated and hybrid cultivars in Geranium and Pansy seeds were compared. In both species one of open-pollinated and two hybrid cultivars separately germinated as described above and the effect of origin of cultivar on germination after storage was tested.

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS, Chicago, IL) by using analyses of variance. Separation between initial germination and germination after storage was made at the 5% level by the Duncan Multiple Range Test.

3. Results and Discussion

Pre-storage (initial germination) percentages in 2008 of all species ranged between 80 and 100%. Geranium marigold, zinnia, pansy seed lots had 100% germination before storage (Table 1). The initial values in petunia, dahlia, gazania, and impatiens were reported as 95, 98, 93 and 98%, respectively. The lowest initial seed germination percentages were observed in salvia, antirrhinum and verbena as 80-85% (Table 1).

Table 1. Changes in germination percentages of flower species stored at 5 °C hermetically over 16 years.

Species	Hybrid	Open pollinated	Seed moisture (%)	2008 (%)	2024 (%)	Germination difference (%)
Geranium	+		6.7	100	100	0
Marigold	+	-	7.1	100	98	2
Zinnia	+	-	7.5	100	98	2
Petunia	+	-	-	95	95	0
Gazania	-	+	7.3	93	90	3
Dahlia flower	+	-	7.5	98	75	23
Salvia	-	+	7.6	80	50	30
Antirrhinum	-	+	-	85	24	61
Verbena	-	+	7.2	56c	34	22
Pansy	+		7.6	100	6	94
Impatiens	+	-	7.6	94	0	94

Seed germination after 16 years of storage had changed variously according to the species. In geranium, marigold, zinnia petunia and gazania seeds it was just in between 0-3%. The difference in germination in between 2008 and 2024 was 23% in dahlia, 30% in salvia, 61% in antirrhinum, 46% in verbena 94% in pansy and 94% in impatiens (Figure 1).

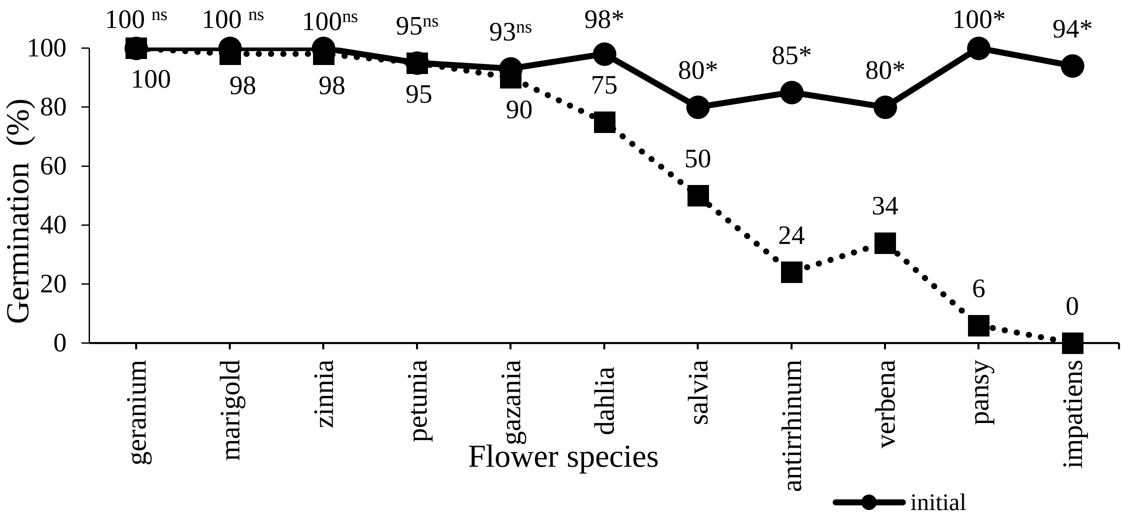


Figure 1. Differences in relation to species in germination percentages of flower species stored at 5 °C hermetically for 16 years

Seventh day germination percentages of the species as a vigour index in between 2008 and 2024 storage years showed that 7th day germination differences of geranium marigold, zinnia, petunia, gazania and dahlia seeds were smaller (Figure 2). While the differences after storage in antirrhinum, verbena, pansy and impatiens seeds were greater (Figure 2).

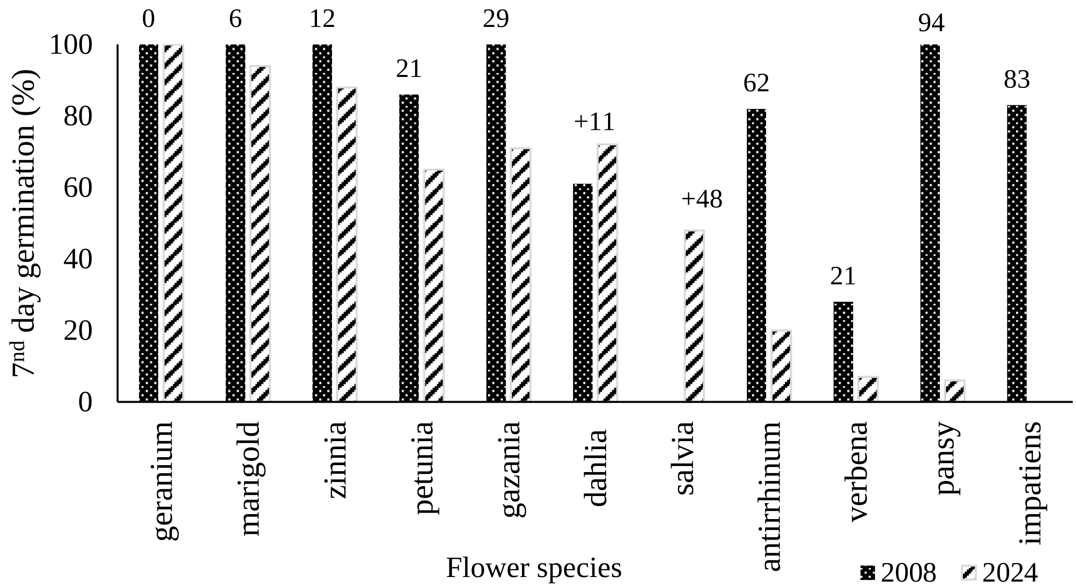
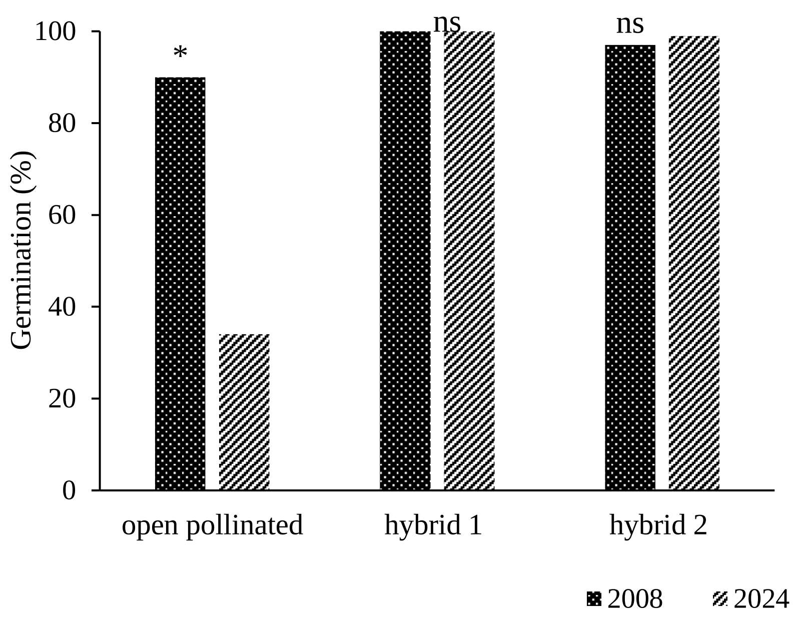


Figure 2. Germination of flower species on 7th days of germination test before (2008) and after (2024) storage. The differences were shown on top of the bars.

Figure 3 shows the difference between initial germination and germination after storage in two hybrids and one open-pollinated cultivars in geranium and pansy seeds. Results indicated that geranium seed lots lost germination less than pansy seeds regardless of origin of the cultivars.



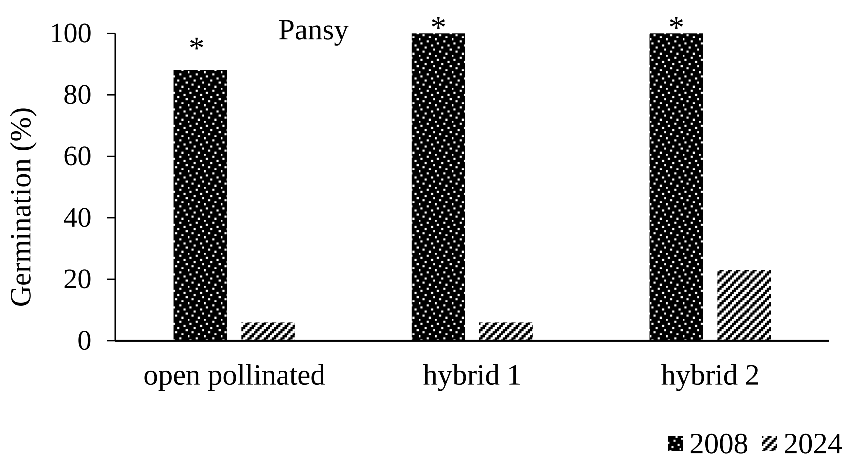


Figure 3. The difference in germination percentages after 16 years of storage in Geranium and Pansy seeds at 5°C in relation to open-pollinated and hybrid cultivars. ns:Not significant, *<0.05

Hybrids of Geranium seeds did not lost germination significantly (P>0.05) while open-pollinated one lost reasonable percentages after 16 years of storage. In pansy seeds seed germination was reduced significantly (P<0.05) with the storage. Both hybrid pansy seed lots germination reduced from 88 to 6% in open-pollinated and from 100% to 23%, in hybrid. Table 2 gives the summary of the resilience level of species to seed storage. Geranium, marigold, zinnia, petunia and gazania seeds appeared to be the most resilient species, dahlia, salvia, antirrhinum and verbena are sensitive and pansy and impatiens are very sensitive to storage conditions.

Table 2. Resilience level of flower species after storage

Resilient	*	Sensitive	*	Very Sensitive	*
Geranium	0	Dahlia	23	Pansy	94
Marigold	0	Salvia	30	Impatiens	94
Zinnia	0	Antirrhinum	61		
Petunia	0	Verbena	46		
Gazania	3				

*: Germination loss after storage (%)

Results of the present work indicated that seeds of some flower species were found to be more resilient during storage than the others. This showed that genetic factors can affect longevity in seeds (Black and Bewley, 1982; Copeland and McDonald, 1995; McDonald, 1999; Demir et al., 2020b). McDonald (2005) classified flower seed species into three categories according to their storability as short (less than 1 year), medium (less than 3 years) and long (more than 3 years) storable groups. In this work we stored seeds much longer time than these periods. According to this classification, impatiens and pansy are considered as short-term, and verbena, antirrhinum dahlia, salvia was considered as medium-term storable species. Geranium, marigold, zinnia petunia and gazania considered as long storable seed species. Our results are in agreement with these findings (Figure 1). We also found similar results in our earlier study on flower seed storage at 5°C and 25°C over 24 months of storage (Demir et al., 2020a,b).

Flower seed structure is an important determinant of susceptibility to storage deterioration. In particular size/surface area ratio of the seed as well as seed coat permeability influence the rate at which water enters the seed (McDonald, 2005). The more potential for water uptake the greater the rate of seed deterioration (Ellis, 2022). Smaller seeds have greater surface area to volume ratio compared with larger seeds. Our results did not support this hypothesis. Zinnia has greater size/surface rate compared to verbena and petunia but zinnia and petunia appeared to be more resilient to storage (Figure 1, Table 2). However, we stored seeds at very similar seed moisture not let seeds uptake water from the air humidity. That could be one reason. In our study seeds remained at the similar moisture content (6.7 and 7.6%) hermetically packed throughout the storage. These seed moisture percentages are very similar levels that are used in seed gene banks for long-term storage (Walters, 2015). McDonald (2005) suggested that 5-6 % is ideal seed moisture contents for flower seeds. Storage temperature of 5°C is also used for active collections in seed gene bank for diverse plant species (Ellis, 2022). These reports indicate that the storage conditions in our work tallying with the conclusions of the earlier studies.

Seed chemical structure is another factor that related to ageing (Demir and Ozcoban, 2007; De Vitis et al., 2020). In general, seeds with high oil content such as impatiens and pansy exhibit shorter storage life due to the lipid peroxidation. Our work confirmed this assumption that impatiens seeds can be considered in the short storable seed category. Loss of germination in impatiens seeds were 94% after 16 years at 5°C (Figure 1) and seeds lost germination completely.

Differences in pre-storage maturation level among the seeds are effective on seed longevity (De Vitis et al., 2020; Corbineau, 2024). Flower crop seeds produce seeds at various maturation levels due to the inflorescence structures where mature seeds are produced on the bottom and less mature seeds on top of the inflorescence. Therefore, flower seed population represents a heterogonous population of individuals, each seed differing in its ability to store. This is also the case in various continuously flowering vegetable species too (Samit and Demir, 2001; Groot, 2022). So, a seed lot contains immature and mature seeds together after once-over harvesting. Then less mature seeds lose germination ability during storage more rapidly than mature ones. We included seed lots with very high initial germination percentages i.e.>80% before storage in all species. So, we aimed to store seed lots that had very high germination level and uniform germination before storage. So that we would see the species differences in storage.

Flowers are produced through transplants in general. The extent of seed ageing affects transplant quality i.e. size, weight (Guloksuz et al., 2011; Demir at al., 2011). Fast and rapid seedling emergence is important and valuable in high quality transplant production in bedding plants (Demir et al., 2020a). The first sign of seed ageing due to the un-optimum storage conditions is late and irregular germination in a population which delays healthy and uniform transplant production (Mavi et al., 2010; Demir et al., 2020b). Therefore, storage at optimum conditions are utmost important for efficient transplant production (De Vitis et al., 2020). This may be one reason that sensitive seeds can be renew and newly produced seed lots are used in every year rather than previous year's production.

Storing seeds in hermetic, air and waterproof packets is important to get remained seed moisture stable during storage. Storing seeds in non-hermetic conditions, i.e. open foil packets in high relative humidity, can culminate in rapid seed deterioration in a short period. In our work we stored seeds at air proof jars with the stable seed moisture. Even though this moisture is ideal, seeds should be retested for germination when they are stored for a period of more than 1 year (McDonald, 2005). Our work shows that such retests are particularly important for some species such as antirrhinum, pansy, verbena and impatiens seeds (Figure 1).

Hybrid seed use is widespread in flowers (Carpenter et al. 1995a; McDonald, 2005). We observed that even though seed lots are hybrids seeds are more prone in some species to ageing than those of the others. In our case geranium seeds were more resilient but pansy seeds were more sensitive regardless of origin of the cultivar. This is obviously proved that species differences are very important on seed longevity rather than the origin of seed cultivar. This is in agreement with earlier findings that species differences are important (Carpenter et al., 1995a, b; McDonald, 1999; Demir et al., 2020a,b) in seed storability.

4. Conclusion

Germination percentages during storage among the flower seeds showed a variation. Pelargonium, gazania, zinnia and tagetes seed lots did not lose germination at all while antirrhinum, petunia, impatiens, viola, salvia anddahlia seeds had lower germination rates at various levels. Hermetic storage at low temperature is important for maintaining high germination in flower seeds but our results indicated that particular attention must be paid to antirrhinum, petunia, impatiens, viola, salvia and dahlia seeds even though storage conditions are very ideal (5°C, 6.7-7.6 % seed moisture).

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References

Baskin, C.C., & Baskin, J.M. (2014). *Seeds:Ecology, Biogeography and Evolution of Dormancy and Germination*. 2nd Edition Elsevier San Diego: Academic Press.

Bewley, J.D., & Black, M. (1982). *Viability, dormancy, and environmental control,* Physiology and Biochemistry of Seeds. New York: Springer-Verlag,

Carpenter, W.J., & Boucher, J.F. (1992). Temperature requirements for the storage and germination of Delphinium x cultorum seed. *HortScience*, *27*(9), 989-992.

Carpenter, W.J., Ostmark, E.R., & Cornell, J.A. (1995a). Evaluation of temperature and moisture content during storage on the germination of flowering annual seed. *HortScience, 30* (5), 1003-1006.

Carpenter, W.J., Ostmark, E.R. & Cornell, J.A. (1995b). Temperature and seed moisture govern germination and storage of gerbera seed. *HortScience*, *30*(1), 98-101.

Carpenter, W.J., & Cornell, J. A. (1990). Temperature and relative humidity recommendations for storing bedding plant seed. University of Florida Bulletin. 893

Copeland, L.O., & McDonald, M.B. (1995). *Principles of Seed Science and Technology*. New York: Chapman and Hall.

Doijode, S.D. (2001). *Seed Storage of Horticultural Crops*. Philadelphia: Haworth Press.

Corbineau, F. (2024). The effects of storage conditions on seed deterioration and ageing: How to improve seed longevity. *Seeds*, 2024, 3.

Demir, I., Celikkol, T. Sarıkamıs, G. & Eksi, C. (2011). Vigor tests to estimate seedling emergence potential and longevity in Viola seed lots. *HortScience*, *46* (3), 402-405.

Demir, I., Gökdas Z. & Eken Türer, N. İ. (2020a). Changes in Seed Germination during Storage of Flower Seeds: Species Differences. *International Journal of Agriculture and Wildlife Science*, *6* (3), 416-422

Demir, I., Erturk, N. & Gokdas, Z. (2020b). Seed vigour evaluation in petunia seed lots to predict seedling emergence and longevity. *Seed Science and Technology*, *48* (3), 391-400.

Demır, I., & Samıt, Y. (2001). Seed quality in relation to fruit maturation and seed dry weight during development in tomato *Seed Science and Technology*, *29*, 453-462

Demir, I., & Ozcoban, M. (2007). Dry and Ultra-dry storage of pepper, aubergine, winter squash, summer squash, bean, cowpea, okra, onion, leek, cabbage, radish, lettuce and melon seeds at− 20 C and 20 C over five years. *Seed Science and Technology*, *35*(1), 165-175

De Vitis, M.; Hay, F.R.; Dickie, J.B.; Trivedi, C.; Choi, J., & Fiegener, R. (2020). Seed storage: Maintaining seed viability and vigor for restoration use. *Restoration Ecology*. *28*, S249–S255.

Ellis, R.H. (2022). Seed ageing, survival and improved seed viability equation; forty years on. *Seed Science and Technology*, *50* (1), 1-20.

Guloksuz, T. & Demir, I. (2012). Vigor tests in geranium, salvia, gazania and impatiens seed lots to estimate seedling emergence potential in modules. *Propagation of Ornamental Plants*, *12*, 133-138

Groot, S.P.C., (2022). Seed maturation and its practical implications. *Seed Science and Technology*, *50*(1), 141-151

ISTA. (2020). *International Rules for Seed Testing*. International Seed Testing Association, Bassersdorf, Switzerland.

Justice, O. L., & Bass, L. N. (1978). *Principles and Practices of Seed Storage*. United States Department of Agriculture, Washington DC: Agriculture Handbook 506.

Mavi, K., Demir, I., & Matthews, S. (2010). Mean germination time estimates the relative emergence of seed lots of three cucurbit crops under stress conditions. *Seed Science and Technology*, *38*, 14-25.

McDonalds, M.B. (1999). Seed deterioration: physiology, repair and assessment. *Seed Science and Technology*, *27*, 177-237.

McDonalds, M.B. (2005). *Flower Seed Longevity and Deterioration*. Flower Seeds Biology and Technology, UK: CABI Publishing,

Nadarajan, J.; Walters, C.; Pritchard, H.W.; Ballesteros, D. & Colville, L. (2023). Seed Longevity—The Evolution of Knowledge and a Conceptual Framework. *Plants*, *12*, 471.

Schmidt, D. (2002). Presentation at International Seed Federation Congress. Chicago, Illinois.

Walters, C. (2015). Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using thresholds responses to water loss. *Planta*, *242*, 397-406