HortiS (2024) 41(1):1-5
http://doi.org/10.16882/HortiS.1403403

HORTICULTURAL STUDIES

RESEARCH PAPER

The Effects of Oxygen Availability in the Seed Container during Storage on Seed Germination in Tomato, Onion, Cabbage, and Marrow Seeds

İbrahim DEMİR[1] Neslihan KADIOĞLU[1]

[1] Ankara University Faculty of Agriculture Department of Horticulture, 06110, Ankara, Türkiye

Article History
Received 02 November 2023
Accepted 11 December 2023
First Online 01 January 2024

Corresponding Author
E-mail: kadiogluneslihan96@gmail.com

Keywords
Germination
Longevity
Oxygen
Seedling
Vegetable seeds

Abstract

This research was conducted to test the effect of oxygen content (low O_2, high O_2, air) during hermetic seed storage at 20±2°C over 8 and 12 months on seed germination and seedling root and shoot length in tomato, onion, cabbage, and marrow seeds. Samples with low oxygen storage had higher seed germination as well as longer root and shoot lengths than both control and high oxygen storage. When the storage period extended from 8 to 12 months, the germination percentages also reduced. However, these results varied among the species. The greatest advantage of low oxygen storage was obtained in tomatoes, which exhibited 15% and 9% higher germination compared to the control after 8 and 12 months of storage, respectively. The longest root and shoot lengths of 6.4 cm and 11.6 cm, respectively, were obtained from the low oxygen storage treatments. A similar positive effect of low oxygen storage was observed in onion and cabbage seeds but not in marrows. Results indicated that oxygen level in the packets during storage can be an effective component to maintain high seed germination and seedling growth potential (seed vigour). The difference in the effect on different species is a matter of further research.

1. Introduction

The storage environment greatly influences seed longevity. The principal environmental factors affecting seed deterioration and seed survival are temperature, seed moisture (relative humidity), and oxygen pressure (Ellis and Hong, 2007; Schwember and Bradford, 2011; Han et al., 2021). In orthodox seeds, the relationship between seed longevity, temperature, and moisture are determined over wide ranges in different species (Ellis and Roberts, 1980; De Vitis et al., 2020; Hay et al., 2022). Seeds of agronomic crops are in general produced in large amounts and stored in open storage (in cloth bags, paper bags etc.) where the seed moisture eventually equilibrates with ambient relative humidity and temperature, and oxygen is freely available at atmospheric concentration during storage. In vegetables however, particularly hybrid cultivars, seeds are very valuable and are mostly kept in hermetic packets in rather small amounts compared to cereals or agronomic crop seeds. Here, the gaseous environment in the packets can be regulated during the storage. The role of oxygen in seed storage has not been investigated as much detail as seed moisture or temperature, since there was an assumption that its effect on longevity in air dry storage was modest. The importance of oxygen on seed storage was first emphasized by Roberts and Abdalla (1968) in rice. More recently however, there are an increasing number of studies in which the deleterious effect of oxygen on longevity has been clearly demonstrated (Barzali et al., 2005; Gonzales-Benito et al., 2011; Schwember and Bradford, 2011; Groot et al., 2012; Groot et al., 2015; Buijs et al., 2020; Pirredda et al., 2020; Prasad et al., 2022; Tahir et al., 2023). The damaging effect of oxygen on seed germination during storage has been associated with damage to macromolecules (DNA, RNA and proteins) (Sano et al., 2016), ROS enzymes and lipid peroxidation (Tahir et al., 2023), and accumulation of point mutations (Kranner et al., 2011).

Seed deterioration and ageing during storage have always been of major concerns in plant production. Vegetable seeds such as tomatoes, cabbages, and marrows are produced through transplants, and very small differences in seed germination during storage can result in great differences in their performance in the greenhouse or field. Seed vigour describes overall performance of the seed lots in a non-optimum sowing environment (ISTA, 2022). Seed vigour denominates the differences in performance of those seed lots that have similar seed germination percentages in stress environments. Therefore, it can be an important measurement criterion to obtain strong transplants for plant production. In this study, we investigated the effect of oxygen content in the packets on seed germination and vigour in tomato, cabbage, onion, and marrow seeds.

2. Material and Methods

The seeds of tomato (*Solanum lycopersicum* cv. Falcon), onion (*Allium cepa* cv. Elit), cabbage (*Brassica oleracea* var capitata cv. Yalova), and marrow (*Cucurbita pepo* L. cv. Sakız) were obtained from commercial seed companies. Total seed germination percentages were recorded as 91, 93, 84, and 100% in tomato, onion, cabbage and marrow seeds, respectively. The seed moisture content of the seeds tested was determined as 7.0, 7.7, 6.2, and 6.6%. Initial laboratory germination tests of seeds were conducted with three replicates of 50 seeds in each species. The seeds were placed between wet filter paper (10 ml distilled water, 20 × 20 cm, Filtrak, Germany). The papers were then placed in plastic bags. Germination tests were carried out in the dark at 20°C for onions and cabbages and 25°C for tomatoes and marrows. Standard germination (normal and abnormal seedlings) was evaluated after 14 days for tomatoes, after 12 days for cabbages and onions, and seven days for marrow seeds. Seeds that produced a 2 mm radicle emergence were counted every day at the same time and considered as total germination (TG). At the final count, normal seedling percentages (NG, seedlings with developed root and shoots) were also determined (ISTA, 2022).

Six samples were prepared for each species, each containing 150 seeds. A total of 900 seeds for each species were weighed and equilibrated to 75% relative humidity in plastic cups (360 mm long × 180 mm wide × 120 mm deep) by placing them on top of wire mesh over a saturated NaCl solution (41 gram per 100 ml) for three days at room temperature in the dark. At the end of the equilibration, the seed moisture content at which seeds were stored was calculated by weighing (Basak et al., 2006). The seed moisture contents of the seeds at storage were 10.6, 12.4, 9.6, and 8.2% for tomato, onion, cabbage and marrow, respectively. A hundred and fifty seeds per sample were placed in a glass jar (22 ml) and low O_2 (Nitrogen) nitrogen (Kasweld EN ISO 2503) or high oxygen gas (100% High O_2) was added by using oxygen (UN1072, Kasweld oxygen EN ISO 2503) tubes. The caps of the jars were closed tightly and covered well with cling film. The control seeds were stored in air (21% O_2). The samples were placed at 20±2°C in the dark plastic bags. Seed quality tests were conducted in three samples (control, low O_2, high O_2) of each species. Germination tests after each storage duration or period were conducted as described above, and total and normal seed germination percentages were determined. At the end of the germination tests, 15 seedlings (five seedlings × three replicates) were selected randomly, and root and shoot lengths measured as cm in each treatment sample, species and storage period.

Means of total and normal germination percentages and shoot and root length in the seed samples in each species were compared at the 5% level by Duncan's multiple range tests by using the SPSS package program (IBM version 25). Angular transformation for percentages was carried out before analyses.

3. Results and Discussion

Total germination percentages (radicle emergence, 2 mm) after the fourth-day or the final count were not significantly different in either the treatments or in any species (Table 1) after eight and 12 months of storage. A single significance in the fourth-day radicle emergence percentage was observed in high O_2 storage in onion seeds after eight months compared to the other two treatments. In this sample, radicle emergence percentages declined to 55%, while control and low O_2 were 69 and 68%, respectively (Table 1).

Low oxygen storage influenced normal seed germination percentages in tomatoes and cabbages in both storage periods. In onions, the effect was clearer at eight months of storage but was not significant after 12 months. Neither the control nor any of the oxygen content levels exhibited significant differences in marrow seeds during the storage period. When tomato seeds were stored at low oxygen level, seed germination was 15 and 18% higher after eight months than that of the control and high oxygen stored seeds (P<0.05). Corresponding differences in 12 months of storage were 9 and 10%, respectively (Table 1). Even though low oxygen-stored cabbage seeds had the highest seed germination values of 71 and 75%

Table 1. Changes in radicle emergence (2 mm long) of 4th day and final counts of tomato, onion, cabbage, and marrow seeds stored at 20±2°C in the glass jars with low and high O_2 and air (control) over 8 and 12 months.

Species	4th day radicle emergence percentage					
	8 month			12 month		
	Control	High O_2	Low O_2	Control	High O_2	Low O_2
Tomato	80±8.0 a*	80±2.0 a	87±5.8 a	76±3.5 a	76±5.3 a	82±6.9 a
Onion	69±1.2 a	55±8.1 b	68±7.2 a	54±2.0 a	40±13.1 a	47±3.1 a
Cabbage	78±8.0 a	78±2.0 a	79±8.3 a	74±3.5 a	75±3.1 a	80±7.2 a
Marrow	95±2.3 a	98±2.0 a	95±2.3 a	99±1.2 a	99±1.2 a	99±1.2 a
Species	Final count radicle emergence percentage					
	8 month			12 month		
	Control	High O_2	Low O_2	Control	High O_2	Low O_2
Tomato	92±6.9 a	92±3.5 a	97±2.3 a	90±5.3 a	91±4.2 a	94±5.3 a
Onion	91±4.2 a	91±1.2 a	93±4.6 a	87±1.2 a	79±7.0 a	79±2.3 a
Cabbage	81±9.0 a	81±5.0 a	84±8.0 a	79±3.1 a	80±3.5 a	85±9.9 a
Marrow	95±2.3 a	98±2.0 a	95±2.3 a	99±1.2 a	99±1.2 a	99±1.2 a

* Mean values were given with SEM and the means with different letters in the same line and species in the same storage period were significantly different (*P<0.05*).

Table 2. Changes in normal germination percentages, 4th day root length and shoot length of tomato, onion, cabbage, and marrow seeds stored at 20±2°C in the glass jars with low and high O_2 and air (control) over 8 months.

Treatments	Germination (%)			
	Tomato	Onion	Cabbage	Marrow
Control	77±4.2 b*	73±2.3 b	66±7.2 a	85±4.6 a
High O_2	74±5.3 b	74±2.0 b	65±5.0 a	86±3.5 a
Low O_2	92±3.5 a	84±3.5 a	75±8.1 a	87±4.2 a
Treatments	Root length (cm seedling^{-1})			
	Tomato	Onion	Cabbage	Marrow
Control	3.2±1.1 b	3.9±0.8 b	6.1±1.7 a	5.4±0.8 b
High O_2	3.5±1.5 b	3.7±0.9 b	3.7±1.0 c	6.0±0.6 a
Low O_2	6.4±1.6 a	5.2±0.8 a	5.0±1.4 b	5.2±0.7 b
Treatments	Shoot length (cm seedling^{-1})			
	Tomato	Onion	Cabbage	Marrow
Control	8.5±1.6 b	6.2±0.7 b	6.2±0.6 a	3.6±0.6 a
High O_2	7.6±1.5 b	5.1±0.9 c	4.5±0.8 b	3.9±0.6 a
Low O_2	11.6±1.2 a	7.7±1.1 a	6.8±1.1 a	3.5±0.6 b

* Mean values were given with SEM and the means with different letters in the same column and species in the same criterion were significantly different (*P<0.05*).

after eight and 12 months of storage, the differences between the control and high oxygen content level stored seeds were not statistically significant. Onion seeds that were stored at low oxygen level had the highest value of 84% among the treatments after eight months, but the difference was in favour of control seeds, at 71% after 12 months of storage. Thus, onion seed germination was more changeable compared to tomato and cabbage seed (Table 1 and 2) as the seed storage period was extended. The normal seed germination of marrow did not vary significantly when stored under conditions of air, low oxygen or high oxygen. Seed germination varied between 85 and 87% after eight months and 77 and 84% after 12 months of storage. There was no statistically significant difference among the treatments after either eight or 12 months (Table 2 and 3).

Earlier studies on the effect of oxygen on longevity have indicated that oxygen plays an effective role in seed germination in barley, peas and broad beans (Roberts and Abdalla, 1968), lettuce (Ibrahim et al., 1983), rye (Barzali et al., 2005), sesame (Ellis and Hong, 2007), onion (Schwember and Bradford, 2011), brassica species (Gonzales-Benito et al., 2011), celery (Groot et al., 2015) and, rice (Tahir et al., 2023) seeds. It appears that the effect of low oxygen was seen to be positive in various type of plant seeds. These findings are in agreement with our results (Table 2 and 3). However, the species react differently. Our results indicated that marrow seeds were not affected by the presence of oxygen during storage, while tomatoes had the most positive response to reduced oxygen storage (Table 3). Obviously, there are various factors that may be responsible for that. One may be the chemical composition of the seed, as marrow is very oily and the seeds are larger as compared to the other seeds.

Secondly, seed coat structure can be a factor as marrow seed coat is harder in relation to water and gaseous exchange between the seed and its environment. Some earlier papers indicated such effects in controlled gas atmospheric storage conditions (Gonzales-Benito et al., 2011; Han et al., 2021). Ellis and Hong (2007) indicated that the effect of oxygen was more prominent at lower seed moisture contents than at higher contents. In this study, we equilibrated the seeds with saturated NaCl solutions (75% RH), which gave about 10.6, 12.4, 9.6, and 8.2% of seed moisture in tomato, onion, cabbage, and marrow. These moisture

Table 3. Changes in normal germination percentages, 4th day root length, and shoot length of tomato, onion, cabbage, and marrow seeds were stored at 20±2°C in the glass jars with low and high O_2 and air (control) over 12 months.

Treatments	Germination (%)			
	Tomato	Onion	Cabbage	Marrow
Control	76±2.0 ab*	71±3.1 a	66±2.0 a	81±3.1 a
High O_2	75±7.0 b	60±3.5 a	62±8.7 a	84±6.0 a
Low O_2	85±5.0 a	61±11.4 a	71±11.0 a	77±3.1 a
Treatments	**Root length (cm seedling^{-1})**			
	Tomato	Onion	Cabbage	Marrow
Control	3.2±0.8 b	3.9±0.9 b	5.0±1.0 a	4.7±1.1 a
High O_2	3.5±1.1 b	3.5±0.7 b	3.6±0.6 b	4.6±0.9 a
Low O_2	5.0±2.0 a	4.6±0.7 a	4.6±1.2 a	4.4±0.5 a
Treatments	**Shoot length (cm seedling^{-1})**			
	Tomato	Onion	Cabbage	Marrow
Control	7.9±1.0 b	5.8±0.7 b	5.2±0.5 a	3.0±0.5 a
High O_2	6.7±1.3 c	5.0±0.7 c	3.7±0.4 b	3.0±0.5 a
Low O_2	8.8±1.0 a	6.9±0.8 a	5.5±1.1 a	3.2±0.4 a

* Mean values were given with SEM and the means with different letters in the same column and species in the same criterion were significantly different (*P<0.05*).

contents are relatively higher than what is used in commercial storage.

Schwember and Bradford (2011) observed the negative influence of oxygen presence at higher seed moistures in lettuce and onion seeds during artificial ageing. Tahir et al. (2023) also mentioned that at higher moisture levels (14%) the availability of oxygen in storage is more harmful to rice seed lifespan than is low seed moisture level (12%). It appears to be that oxygen may have an effect at any seed moisture, but the relative impact may be different (Groot et al., 2015). High seed moisture and the natural proportion of oxygen available during storage may accelerate the ageing process of the seeds and negatively affect seed germination as explained by De Vitis et al. (2020). The deteriorative effect of oxygen on seed germination was determined to be related to damage to macromolecules (DNA, RNA and proteins) (Sano et al., 2016), ROS (Tahir et al., 2023), and the accumulation of point mutations (Kranner et al., 2011).

The current study indicated that the root and shoot lengths were higher in seeds stored with low oxygen compared to both controls and those with high oxygen in tomatoes and onions after 8 and 12 months of storage (Table 2 and 3). The same as to normal germination percentages lower oxygen levels affected root and shoot lengths more in tomato and onion seeds than in cabbage and marrow seeds (Table 2 and 3) after both storage periods. In these two species, low O_2 storage increased both root and shoot lengths significantly (P<0.05) compared to control and high O_2 storage. The difference varied, but in tomato, it went up to 3.2 and 3.1 cm in low O_2 compared to that of the control after eight months of storage (Table 2). The difference in onion seeds was lower. In cabbage and marrow seeds, decreased oxygen composition had no effect on either root or shoot lengths. The differences in seedling lengths indicated that lowering the oxygen content increased seed vigour as reflected in seedling size. Vegetables are produced through transplants, and longer root and seedling size result in better stand establishment and larger seedling size (Demir et al., 2008). Therefore, the present results, which show a more positive effect on plant size in low oxygen storage may be valuable in preserving seed vigour, particularly for vegetable seeds in which large industrial transplant production is important (Zulfikar, 2021).

4. Conclusion

This study indicated that lower oxygen content during storage affected seed germination, and vigour (normal germination, seedling root and shoot length) in tomato, onion, and cabbage seeds. The effect was not pronounced in marrow seeds. It appeared to be that the effect of oxygen was somehow species dependent. Meanwhile, further research on the topic combining various seed moisture and temperature environments need to be explored in the future experiments.

References

Barzali, M., Lohwasser, U., Niedzielski, M., & Börner, A. (2005). Effects of different temperatures and atmospheres on seed and seedling traits in a long-term storage experiment on rye (*Secale cereale* L.). *Seed Science and Technology*, 33:713–721.

Basak, O., Demir, I., Mavi, K., & Matthews, S. (2006). Controlled deterioration test for predicting seedling emergence and longevity of pepper (*Capsicum annuum* L.) seed lots. *Seed Science and Technology*, 34:723-734.

Buijs, G., Willems, L.A.J., Kodde, J., Groot, S.P.C., & Bentsink, L. (2020). Evaluating eppo method for seed longevity analyses in Arabidopsis. *Plant Science*, 301:110644.

Demir, I., Ermis, S., Mavi, K., & Matthews, S. (2008). Mean germination time of pepper seed lots (*Capsicum annuum)* predicts size and uniformity of seedlings in germination tests and transplant modules. *Seed Science and Technology*, 1:21-30.

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

Ellis, R.H., & Roberts, E.H. (1980). Improved equations for the prediction of seed longevity. *Annals of Botany*, 45:13–30.

Ellis, R.H., & Hong, T.D. (2007). Seed longevity – moisture content relationships in hermetic and open storage. *Seed Science and Technology*, 35:423–431.

Gonzales-Benito, M.E., Perez-Garcia, F., Tejeda, G., & Gomez-Campo, C. (2011). Effect of the gaseous environment and water content on seed viability of four *Brassicaceae* species after 36 years storage. *Seed Science and Technology*, 13:443-451.

Groot, S.P.C., Surki, A.A., De Vos, R.C.H., & Kodde, J. (2012). Seed storage at elevated partial pressure of oxygen, a fast method for analysing seed ageing under dry conditions. *Annals of Botany*, 110:1149–1159.

Groot, S.P.C., De Groot, L., Kodde, J., & Van Treuren, R. (2015). Prolonging the longevity of ex situ conserved seeds by storage under anoxia. *Plant Genetic Resources*, 13:18-26.

Han, B., Fernandez, V., Pritchard, H.W., & Colville, L. (2021). Gaseous environment modulates volatile emission and viability loss during seed artificial ageing. *Planta*, 253:106.

Hay, F.R., Rezaei, S., & Buitink, J. (2022). Seed moisture isotherms, sorption models, and longevity. *Frontiers in Plant Science*, 13.

Ibrahim, A.E., Roberts, E.H., & Murdoch, A.J. (1983). Viability of lettuce seeds. *Journal of Experimental Botany*, 34:631-640.

ISTA. (2022). International Rules for Seed Testing. International Seed Testing Association, Welliselen, Switzerland.

Kranner, I., Chen, H., Pritchard, H.W., Pearce, S.R., & Birtic, S. (2011). Internucleosomal DNA fragmentation and loss of RNA integrity during seed ageing. *Plant Growth Regulation*, 63:63–72.

Pirredda, M., Gonzales-Benito, M. E., Martin, C., & Mira, S. (2020). Genetic and epigenetic stability in rye seeds under different storage conditions: ageing and oxygen effect. *Plants*, 9:393.

Prasad, C.T.M, Kodde, J., Angenent, G.C., De Vos, R.C.H., Diez-Simon, C., Mumm, R., Hay, F.R., Siricharoen, S., Yadava, D.K., & Groot, S.P.C. (2022). Experimental rice seed aging under elevated oxygen pressure: Methodology and mechanism. *Frontiers in Plant Science*, 13:1050411.

Roberts, E.H., & Abdalla, F.H. (1968). The influence of temperature, moisture and oxygen on period of seed viability in barley, broad beans, and peas. *Annals of Botany*, 32:97–117.

Sano, N., Rajjou, L., North, H.M., Debeaujon, I., Marion-Poll, A., & Seo, M. (2016). Staying alive: Molecular aspects of seed longevity. *Plant and Cell Physiology*, 57:660–674.

Schwember, A.R., & Bradford, K.J. (2011). Oxygen interacts with priming, moisture content and temperature to affect the longevity of lettuce and onion seeds. *Seed Science Research*, 21:175–185.

Tahir, A., Afzal, I., Khalid, E., Razzaq, M., & Arif, M.A.R. (2023). Rice seed longevity in the context of seed moisture contents and hypoxic conditions in the storage environment. *Seed Science Research*, 33:39-49.

Zulfikar, F. (2021). Effect of seed priming on horticultural crops. *Scientia Horticulturae*, 286:11097.