An Endeavor of 'Deep-underground Agriculture': Deep-underground Storage in a Gold Mine Impacts the Germination of Canola (Brassica Napus L.) Seeds

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Abstract

Exploring and utilizing the agronomic potential of deep-underground is one of the ways to cope with the challenges of sudden environmental change on agriculture. Understanding the effects of environmental stresses on the morphological and physiological indicators of crop seeds after their storage deep-underground is crucial to developing and implementing strategies for agriculture in the deep-underground space. In this study, we stored canola seeds in envelopes or sealed packages at 0, 240, 690, and 1,410 m in a gold mine. Seeds in envelopes were retrieved at 42, 66, 90, and 227 days of storage, whereas seeds in sealed packages were retrieved at 66 and 227 days of storage. The germination tests were conducted to investigate the effects of storage depth, duration, and packing method on stored and non-stored seeds. Results showed that increased depth and duration led to decreased seed germination rate, with the germination and vigor indexes also descending to varying degrees. Increased hypocotyl length and biomass accumulation suggested that deep-underground environment had more significant compensatory effect on seed germination. For all indicators, the performance of seeds sealed in packages was superior to those stored in envelopes. Regression analysis showed that it was difficult to obtain the optimal value of each indicator simultaneously. The successful germination experiment foreshadowed the possibilities of deep-underground agriculture in the future.

1. Introduction

One of the most challenging issues affecting agriculture is global environmental change (Brilli et al., 2019). Despite the agricultural activity-related adversities such as fertilizer overdose, intensive farming, and etc., sudden environmental change (i.e., solar wind, and nuclear war) may put even more significant threats to modern agriculture (Adegbeye et al., 2020). Therefore, finding alternative methods of farming becomes necessary as these threats may severely affect our society or even civilization.

Nowadays, the exploitation depths of non-ferrous metal mines have exceeded 4,500 m (Liu et al., 2018). Consequently, countries with a history of mining have left a large number of abandoned mining tracks that possibly have the potential for the development of deep-underground agriculture (Mhlongo et al., 2019), which is a new agricultural mode that permits directly planting in the deep-underground environment. Seed storage so that it can be transported and planted later is the first step in the agricultural production process. Understanding seed germination performance after deep-underground storage is of great importance for predicting the success of subsequent deep-underground planting.

In agriculture, suitable seed storage is required to preserve seed viability and improve restoration success (Devi et al., 2019). Generally, favorable storage conditions include appropriate temperature and relative humidity (RH); such conditions reduce the moisture content of seeds (Afzal et al., 2020). (Moravec et al., 2008) showed that when RH is > 55%, seed moisture content increases, which can jeopardize seed storage because seeds are hygroscopic. In addition, given the effect of high temperature on accelerating the respiration of seeds (Faisal et al., 2019), seeds can be severely degraded when RH increases to > 90% (Bakhtavar et al., 2019; Chaengsakul et al., 2019; Pronyk et al., 2006; Redden and Partington, 2019). Furthermore, microbial reproduction accelerates when temperature and RH are high (Wawrzyniak et al., 2018), which can rapidly reduce the seed dry content (Sun et al., 2014). The aforementioned processes would likely accelerate the aging of seeds (Bakhtavar et al., 2019), posing a threat to agricultural production and germplasm protection (Pirredda et al., 2020).

To maintain the genetic diversity of plants, seed banks and botanical gardens around the world preserve more than 16,500 plant species and 7 million plant germplasm resources (Boniecka et al., 2019). One famous example is the Svalbard Global Seed Vault (Westengen et al., 2013) and the largest seed bank in the world is the Millennium Seed Bank (Liu et al., 2020). The USA has also established a National Seed Storage Laboratory in Fort Collins, Colorado (International, 1960), in which valuable seeds are stored for research. However, whether the environmental factors in the deep-underground environment are suitable for seed storage has not been well studied.

Apart from storage environment conditions, storage duration can also influence the viability of seeds. As the storage time increases, seed vigor gradually decreases to zero (Redden and Partington, 2019). Since the degradation of seed vitality during storage is closely related to germination (Boniecka et al., 2019; Lv et al., 2016), changes in vigor inevitably affect the growth of seeds after sowing. In addition to germination rate, growth rate and maximum growth potential are also affected by storage time (Kholibrina et al., 2019). To achieve a high germination rate and better growth performance, it is therefore necessary to determine the appropriate storage time of seeds.

Studies of canola seeds have shown that their chemical composition, morphology, and anatomical structure make them more susceptible to undesirable changes (Wawrzyniak et al., 2018). Keeping vitality after a long-term storage of cruciferous seeds under ideal environmental conditions could be challenging. Therefore, canola seeds could be used as a model for studying seed aging (Boniecka et al., 2019). To investigate the possibility of storing seeds in the deep-underground environment, we conducted a canola seed germination experiment. Multiple linear regression analysis was used to determine the variation trends of canola morphology and physical development under multifactor conditions. Our research aims were as follows: (1) to study the effects of storage depth, duration, and packaging method on canola seed germination performance, and (2) to identify the optimal combination of storage duration and depth conducive to the storage and germination of canola seeds.

2. Materials And Methods

2.1. Measurement of Environmental Parameters

We measured the temperature, RH, air pressure (Testo 480; Testo, AG, Schwaben, Germany), oxygen content (AR8100; SMART SENSOR, HongKong, China), and gamma radiation concentration (AT1123; ATOMTEX, Minsk, Belarus) at four depths (0, 240, 690, and 1,410 m: the entrance of the gold mine is a horizontal tunnel that is about 30 m from the top of the hill, which was considered as 0 m of depth in this study) in a gold mine [Jiapigou Minerals Limited Corporation of China National Gold Group Corporation (42°52'36"N, 127°30'46.2"E)] located in Jilin Province, China, from September 2018 to June 2019. We

recorded each environmental parameter once every 50 m from the shaft and calculated the average value from at least three measurements. Environmental parameters at each depth of seed storage are listed in Table 1.

Table 1
Environment parameters at different depths of Jiapigou Gold Mine (Jilin) and laboratory (Chengdu)

Depth (m)	Temperature (°C)	Relative Humidity (%)	CO_2 concentration (ppm)	Air pressure (hPa)
0	11.9 ± 0.2	30.3 ± 0.4	873 ± 10	941.5 ± 1.2
240	14.3 ± 0.3	72.4 ± 0.3	776 ± 7	968.4 ± 2.2
690	21.8 ± 0.3	96.5 ± 0.5	1239 ± 15	1020.2 ± 1.7
1410	28.8 ± 0.1	99.0 ± 0.1	1382 ± 17	1106.6 ± 2.5
Control	17.4 ± 0.5	55.7 ± 2.3	473 ± 7	950.60 ± 7.6

2.2. Research site and treatment

Canola seeds (Dexingyou 12, Chengdu Damei Seeds Co., Ltd.) obtained from the Sichuan Academy of Agricultural Sciences were used as experimental materials. Before the germination test, we sent the seeds to the gold mine for storage. We placed the packaged seeds, packaged in either unsealed envelopes or perfectly sealed packaging, in four horizontal tunnels at depths of 0, 240, 690, and 1,410 m in the mine, respectively. Our rationale was as follows: canola seeds packaged in envelopes could more easily be affected by the external environment, i.e., the moisture content of the seeds would increase in a high-humidity environment, whereas the seeds in a sealed package would be insulated from external humidity and temperature and therefore kept dry. The polyethylene vacuum bags were applied as the sealed packaging. After the air was exhausted by a vacuum machine, the sealed packaging bags were sealed with hot melt glue. On September 29, 2018, 96 packets of canola seeds, including 64 envelopes (16 packages at each depth) and 32 sealed packages (8 packages at each depth), each containing 100 g (> 92,000) of seeds, were equally distributed among the four horizontal tunnels.

To simulate local producers' storage conditions, canola seeds were also stored in their original packaging with breathing holes in the Laboratory of Drainage and Irrigation Engineering at Sichuan University; these canola seeds were considered the control. At 42, 66, 90, and 227 days of storage, four packets of seeds in envelopes were retrieved, respectively, whereas four packets of seeds in sealed packages were collected at 66 and 227 days of storage. Subsequently, the seeds were sent to the laboratory for germination tests.

2.3. Germination test

All seeds were sterilized for 20 min with a 0.1% potassium permanganate solution before being air-dried on filter paper. Germination boxes were sterilized with 75% alcohol solution and then dried in an oven for 1 h. A double layer of filter paper was placed in each germination box and then exposed to UV light for 1 h. Subsequently, 50 healthy seeds were selected from each package and evenly distributed in a germination box (10 rows by 5 columns). Distilled water was used to soak the filter paper, and the germination boxes were covered with plastic wrap. The temperature, RH, and photoperiod of the growth chamber were set to 25°C, 85%, and 18/6 h light/dark, respectively. Under these conditions, we placed all germination boxes in the growth chamber for germination.

A timer was initiated the moment the germination boxes were placed in the growth chamber. During the experiment, the filter paper was saturated with distilled water at 156 and 180 h, respectively. The number of germinated seeds was recorded every 12 h. After 192 h, seed counting was terminated, and the germination rate (GR) and germination index (GI) were calculated using the following equations:

$$GR = \frac{Numberofgerminationseedsin192h}{Totaltestedseeds} \times 100\%$$

1

$$GI = \frac{\sum_{i=1}^{n} GR_i}{n}$$

2

where GR_i is the GR on the i^{th} day and n is the total germination days.

After 180 h of emergence, ten sturdy and well-growing seedlings were selected from each germination box. The hypocotyl length was measured using a caliper. The seedlings were then killed in an oven at 105°C for 15 min before being dried at 65°C for another 72 h until reaching a constant weight. An analytical balance was applied to measure the biomass of the seedlings. The vigor index (VI) and biomass accumulation rate were calculated using the following equations:

$$VI = m_s \times GI$$

3

$$Biomassaccumulationrate = \frac{m_s}{hypocotyllength}$$

4

where, m_s is the biomass of the seedling.

2.4. Statistical analysis

Each parameter was presented as the mean of three replicates. We performed one-way ANOVA and three-way ANOVA analysis on the three parameters of storage duration, storage depth and packaging method, which using the MIXED procedure and a 0.05 significance level via SAS version 9.4 (SAS Institute, 2015, Cary, NC, USA).

In addition, multiple linear regression was applied as a multivariate statistical technique to fit the observation data and identify the linear relationship between two or more independent variables and a single dependent variable (Charulatha et al., 2017). Generally, the form of the multiple linear regression equation was as follows:

$$Y = {}_0 + {}_1X_1 + {}_2X_2 + \cdots + {}_mX_m +$$

5

where, Y is the dependent variable, $X_1 \ldots X_m$ are several independent variables, $\beta 0 \ldots \beta_m$ are regression coefficients, and ε is the random error (Hamil et al., 2018).

3. Results

ANOVA results are shown in Table 2 with the significance levels from three-way ANOVA at $\alpha \leq 0.05$ and $\alpha \leq 0.01$.

Table 2
Effects of packaging method, storage depth, and duration on canola seed germination parameters. The results (P values) of the ANOVA are present for germination rate (GR), hypocotyl length, biomass, biomass accumulation rate, germination index (GI), and vigor index (VI).

Source of variation [a]	GR	Hypocotyl length	Biomass	Biomass accumulation rate	GI	VI
	(%)	(mm)	(g)	(g/mm)		
Package (P)	0	0	0	0	0	0
Depth (D)	0	0	0.004	0.026	0	0
Time (T)	0	0	0	0	0	0
P × D	0	0	0	0	0	0.004
P × T	0	0	0	0	0	0.122
D × T	0	0	0	0	0	0
P × D × T	0	0	0	0	0	0

[a] Package (P) refers to the packaging method, depth (D) refers to the storage depth, and time (T) refers to the storage duration.

3.1. GR

Under the envelope packaging condition, the GR of seeds decreased as storage depth and storage duration increased (Table 3). Over longer storage durations (i.e., > 66 days), the effect of storage depth on GR decreased significantly as storage depth increased. For all storage durations, the GR of seeds was lowest when they were kept at 1,410-m depth. Additionally, the difference between the seeds stored at 1,410 m and the control continued to increase until the seed lost its vigor after 227 days of storage. Notably, mineworkers damaged the samples stored at 240 m; hence, it was not possible to complete the 90- and 227-day experiments. However, we found that the GR of seeds clearly decreased as storage duration increased in deeper environments (i.e., > 690 m). Under sealed packaging conditions, extending the storage depth and prolonging the storage duration did not affect the GR of seeds (Table 3).

Table 3
Morphological parameters of canola seeds under two packaging methods: the effect of storage depth.

Treatment	Storage duration	42 days	66 days	90 days	227 days
Unit	Storage depth				
Envelope packaging					
Germination Rate (%)	0 m	97.5 $^{A a}$	96.5 $^{A a}$	96.5 $^{A a}$	96.0 $^{A a}$
	240 m	97.5 $^{A a}$	99.0 $^{A a}$	-	-
	690 m	97.0 $^{A a}$	91.5 $^{B b}$	92.5 $^{A ab}$	72.0 $^{B c}$
	1410 m	92.5 $^{B a}$	69.0 $^{C b}$	58.5 $^{B c}$	0 $^{C d}$
	Control	97.5 ^{A}	97.5 ^{A}	97.0 ^{A}	98.5 ^{A}
Hypocotyl Length (mm)	0 m	21.37 $^{AB b}$	20.84 $^{AB b}$	18.77 $^{B b}$	24.23 $^{A a}$
	240 m	19.30 $^{AB a}$	19.02 $^{B a}$	-	-
	690 m	18.79 $^{B b}$	20.25 $^{AB b}$	19.93 $^{B b}$	26.78 $^{A a}$
	1410 m	20.98 $^{AB b}$	24.35 $^{A ab}$	26.54 $^{A a}$	0 $^{C c}$
	Control	22.43 ^{A}	22.14 ^{AB}	21.09 ^{B}	21.09 ^{B}
Biomass ($\times 10^{-2}$ g)	0 m	3.08 $^{B b}$	3.69 $^{BC a}$	2.95 $^{C b}$	3.92 $^{A a}$
	240 m	3.90 $^{A a}$	3.18 $^{C b}$	-	-
	690 m	3.52 $^{AB b}$	3.55 $^{BC b}$	3.45 $^{B b}$	4.53 $^{A a}$
	1410 m	3.86 $^{A a}$	4.17 $^{A a}$	4.17 $^{A a}$	0 $^{C b}$
	Control	3.35 ^{B}	3.76 ^{AB}	2.99 ^{C}	2.99 ^{B}
Biomass Accumulation Rate ($\times 10^{-2}$ g/mm)	0 m	0.14 $^{B b}$	0.18 $^{A a}$	0.15 $^{AB ab}$	0.18 $^{A a}$
	240 m	0.20 $^{A a}$	0.17 $^{A a}$	-	-
	690 m	0.19 $^{A a}$	0.18 $^{A a}$	0.17 $^{A a}$	0.17 $^{A a}$
	1410 m	0.19 $^{A a}$	0.17 $^{A a}$	0.16 $^{AB a}$	0 $^{C b}$
	Control	0.15 ^{B}	0.17 ^{A}	0.14 ^{B}	0.14 ^{B}
Germination Index	0 m	31.75 $^{A a}$	31.18 $^{A a}$	31.23 $^{A a}$	29.89 $^{A a}$
	240 m	31.75 $^{A a}$	32.34 $^{A a}$	-	-
	690 m	31.30 $^{A a}$	29.14 $^{B b}$	28.58 $^{A b}$	15.48 $^{B c}$
	1410 m	28.07 $^{B a}$	16.92 $^{C b}$	13.41 $^{B c}$	0 $^{C d}$
	Control	31.69 ^{A}	31.98 ^{A}	31.86 ^{A}	31.86 ^{A}
Vigor Index	0 m	12.73 $^{A a}$	14.02 $^{A a}$	13.02 $^{A a}$	13.12 $^{A a}$
	240 m	13.54 $^{A a}$	13.40 $^{A a}$	-	-
	690 m	12.37 $^{A a}$	12.28 $^{A a}$	12.76 $^{A a}$	5.51 $^{B b}$
	1410 m	11.84 $^{A a}$	8.78 $^{B b}$	5.35 $^{B c}$	0 $^{C d}$

[a] One-way ANOVA analysis for $\alpha = 0.05$. There were no data collected or statistical analyses performed for 90 and 227 days of storage at a depth of 240 m since the canola seeds under these conditions were destroyed by the mineworkers. Different capital letters indicate significant differences between individual storage depths for the same storage time. The control represents nonstored seeds. Different lowercase letters indicate significant differences between storage times for the same storage depth. No control was set for the time treatment ($p < 0.05$).

Treatment	Storage duration	42 days	66 days	90 days	227 days
Unit	Storage depth				
	Control	13.38 ^{A}	12.95 ^{A}	13.45 ^{A}	13.45 ^{A}
Sealed packaging					
Germination Rate (%)	0 m		97.5 $^{A\,a}$		99.0 $^{A\,a}$
	240 m		98.0 $^{A\,a}$		97.0 $^{A\,a}$
	690 m		96.5 $^{A\,a}$		95.5 $^{A\,a}$
	1410 m		97.5 $^{A\,a}$		67.5 $^{B\,b}$
	Control		96.5 ^{A}		95.5 ^{A}
Hypocotyl Length (mm)	0 m		19.50 $^{B\,b}$		21.17 $^{BC\,a}$
	240 m		17.96 $^{BC\,b}$		20.48 $^{C\,a}$
	690 m		16.57 $^{C\,b}$		21.75 $^{BC\,a}$
	1410 m		19.44 $^{B\,b}$		26.06 $^{A\,a}$
	Control		23.62 ^{A}		25.02 ^{AB}
Biomass ($\times10^{-2}$ g)	0 m		3.71 $^{AB\,a}$		4.25 $^{B\,a}$
	240 m		4.12 $^{A\,a}$		4.11 $^{B\,a}$
	690 m		3.78 $^{AB\,a}$		4.63 $^{AB\,a}$
	1410 m		4.22 $^{A\,b}$		5.01 $^{A\,a}$
	Control		3.34 ^{B}		4.17 ^{B}
Biomass Accumulation Rate ($\times10^{-2}$ g/mm)	0 m		0.19 $^{A\,a}$		0.20 $^{AB\,a}$
	240 m		0.23 $^{A\,a}$		0.20 $^{AB\,a}$
	690 m		0.23 $^{A\,a}$		0.21 $^{A\,a}$
	1410 m		0.22 $^{A\,a}$		0.19 $^{AB\,a}$
	Control		0.14 ^{B}		0.17 ^{B}
Germination Index	0 m		31.81 $^{A\,a}$		31.43 $^{A\,a}$
	240 m		32.05 $^{A\,a}$		31.26 $^{A\,a}$
	690 m		31.27 $^{A\,a}$		30.21 $^{A\,a}$
	1410 m		31.11 $^{A\,a}$		15.74 $^{B\,b}$
	Control		31.20 ^{A}		30.68 ^{A}
Vigor Index	0 m		15.58 $^{AB\,a}$		12.33 $^{AB\,b}$
	240 m		14.63 $^{AB\,a}$		10.58 $^{B\,b}$
	690 m		14.24 $^{B\,a}$		12.93 $^{A\,a}$
	1410 m		16.47 $^{A\,a}$		8.25 $^{C\,b}$
	Control		15.28 ^{AB}		13.57 ^{A}

[a] One-way ANOVA analysis for $\alpha = 0.05$. There were no data collected or statistical analyses performed for 90 and 227 days of storage at a depth of 240 m since the canola seeds under these conditions were destroyed by the mineworkers. Different capital letters indicate significant differences between individual storage depths for the same storage time. The control represents nonstored seeds. Different lowercase letters indicate significant differences between storage times for the same storage depth. No control was set for the time treatment ($p < 0.05$).

A comparison of the GRs between the two packaging methods at different storage depths is shown in Fig. 1a. Generally, significant differences only appeared at deeper depths (i.e., 690 and 1,410 m). For the 66-day sampling period, seeds in the sealed package had a significantly greater GR than that of seeds in

envelopes stored at a depth of 1,410 m. When the storage duration was prolonged to 226 days, similar phenomena were observed at both 690 and 1,410 m depths.

3.2. GI

GI was affected by both storage depth and duration. For both packaging methods, shallower depths and shorter storage periods did not significantly reduce the GI (Table 3). Notably, the seeds stored at 240 m for 66 days had the maximum GI value (32.34) of all treatments. However, a downward trend in GI values was gradually noticeable and then more rapidly declined as seeds were stored at deeper depths. Moreover, extending storage duration accelerated this downward trend. Generally, deeper depths and extended storage durations negatively affected the final GI of the seed.

Similar to GR, there was no significant difference in the GI between seeds stored in sealed packages and in envelopes when the storage depth was shallow [i.e., 0 and 240 m; Fig. 1b]. At storage depths of 690 and 1,410 m, the GI of seeds stored in the sealed package was significantly greater than that of seeds stored in the envelopes. The reduction in GI became more evident as the storage duration increased and storage depth deepened.

3.3. Hypocotyl length

For envelope packaging, the hypocotyl length of the canola is generally presented as a U-shaped curve at different storage depths (Table 3). This phenomenon was pronounced after 60 and 90 days of seed storage. Similarly, this parabolic shape appeared under sealed packaging conditions. Results showing an increase in hypocotyl length at deeper depths indicate that the deep-underground environment could compensate for losses due to low GR. In addition, the effect of storage duration on hypocotyl length was positively correlated with storage duration (Table 3). The only exception occurred in seeds packaged in envelopes for 227 days at a depth of 1,410 m; here, hypocotyl length could not be measured due to the loss of seed viability. Correspondingly, the increased hypocotyl length that occurred with extended storage duration could compensate for the loss caused by low GR.

The effects of packing methods on hypocotyl length are shown in Fig. 1c. Generally, at the same storage depth and over the same storage duration, there was no significant difference between the hypocotyl length of seeds stored in envelopes and sealed packages. However, we observed that seeds stored in envelopes had a longer hypocotyl length than those stored in sealed packages when seeds were stored at 1,410 m for 66 days. Seeds in envelopes stored at 1,410 m for 226 days failed to germinate; therefore, hypocotyl length was not measured.

3.4. Biomass Accumulation during the Germination Process

Similar to hypocotyl length, the accumulation of biomass of canola seeds in envelope packaging during the germination process also decreased at first and then increased for all storage depths (Table 3). The largest biomass accumulation often occurred at deeper storage depths. For example, the largest biomass appeared at 1,410 m (0.042 g) and 690 m (0.045 g) for seeds stored over 90 and 227 days, respectively. However, for seeds in sealed packaging, there was a positive correlation between biomass accumulation and storage depth (Table 3). This trend of increasing biomass with increasing storage depth indicates that the deep-underground environment has the potential to promote seedling performance, which would function with the elongation of hypocotyl length to compensate for the loss of yield caused by low GR. In addition, the variation in biomass for seeds stored in envelopes over time was generally similar to the variation in hypocotyl length over time (Table 3). The greatest biomass accumulation occurred in seeds stored for 227 days at 1,410 m (4.54), which was 8.87–53.90% higher than the biomass accumulation detected under other storage durations. For seeds in sealed packages (Table 3), the biomass accumulation at shallow depths (i.e., < 690 m) was only slightly increased by extending storage duration and results were statistically similar. In a deeper storage environment (i.e., > 690 m), biomass accumulation and storage duration showed a positive correlation.

Generally, the packing method did not play an important role in hypocotyl biomass accumulation during the germination process (Fig. 1d). However, significant differences were observed at 240 m with 66-day storage. Seeds stored in the sealed packages tended to develop stronger hypocotyls during the germination process compared with the hypocotyls of seeds stored in envelopes.

3.5. Rate of Biomass Accumulation

The biomass accumulation rate of seeds stored in envelopes was affected by storage duration and storage depth (Table 3). For envelope packaging over 42 days, the biomass accumulation rate at storage depths of 240, 690, and 1,410 m was significantly higher than that at 0 m and for the control treatment. This suggests that the deep-underground environment may stimulate seed vigor.

Furthermore, under the sealed packaging condition, a positive correlation was observed between the rate of biomass accumulation and storage depth. Similarly, considering the influence of storage duration on the rate of biomass accumulation, we observed a positive correlation in the shallowest environment (i.e., 0 m) and a negative correlation in the deeper environments (i.e., > 690 m) for seeds stored in the envelope packaging. As for sealed packaging conditions (Table 3), extending the storage duration led to a slight decrease in biomass accumulation rate at all three storage depths (i.e., 240, 690, and 1,410 m), and these data did not differ statistically.

As shown in Fig. 1e, the packaging method played a critical role in biomass accumulation rates. For both sampling periods, a significantly greater biomass accumulation rate was measured for seeds stored in the sealed packages when they were stored deeper than 240 m, whereas the biomass accumulation rates of seeds stored at 0 m have no statistical difference between packing methods. Furthermore, the hypocotyl biomass accumulation rates of seeds stored for 66 days at 1,410 m did not differ statistically between packing methods. Nevertheless, for all treatments without statistical differences between packing methods, sealed-packaged seeds tended to have a greater biomass accumulation rate than those packed in envelopes.

3.6. Vigor index

The VI of seeds stored in envelope packages was affected by storage duration and storage depth (Table 3). Increased storage depths significantly reduced the VI of seeds, indicating that the deep-underground environment negatively influenced the vigor of seeds stored in envelopes, similar to the effects shown on GR and GI. In the sealed packaging condition, the VI reached a maximum value (16.47) at 1,410 m over a 66-day storage period. In contrast, after 227 days of storage, a negative correlation existed between VI and storage depth. In addition, longer storage durations tended to reduce the VI of canola compared with the VI measured at shorter storage durations. At 0 m, statistical differences were not detected among treatments. When storage depth increased to 690 m, the only reduction in VI occurred after 227 days of storage, which was about 40% compared with the VI of other treatments. A similar trend was observed in seeds stored at 1,410 m. Overall, a negative correlation existed between the VI and storage duration under sealed packaging conditions (Table 3). Except for at a storage depth of 690 m, statistical differences existed at other depths.

Packaging methods significantly affected VI when seeds were stored at deep levels over long durations (Fig. 1f). Seeds stored in sealed packages at 1,410 m for 66 days and those stored at 690 or 1,410 m for 226 days had significantly greater VIs than those measured in envelope-stored seeds. Statistically, shallow storage depths and short storage duration did not affect the VI of canola seeds no matter which packaging method was chosen.

3.7. Germination process

Figure 2 shows the results of the germination process. For envelope packaging, the germination process became slower with increased storage depth and duration. After 42 days, the growth rate of seeds stored at 1,410 m during germination was slower than the growth rate observed with other treatments at 60–132 h. The germination speed of seeds stored at shallower depths gradually decreased as storage duration was extended. Under sealed packaging conditions, however, only long-term storage significantly influenced the germination process. For instance, deeper storage depths significantly reduced the germination speed of seeds: canola seeds at 1,410 m reached their highest GR at 84–132 h, which was 36 h later than the GR peak for other treatments.

3.8. Combined effects of GR, GI, hypocotyl length, biomass, biomass accumulation rate, and VI

To understand the effects of storage duration and storage depth on the morphological parameters of canola, and to identify the optimal combination of storage duration and storage depth, we used multiple linear regression to determine the relationships among the dependent (GR, GI, hypocotyl length, biomass, biomass accumulation rate, and VI) and independent (storage duration and storage depth) variables. The results of this multiple linear regression with testing via ANOVA are shown in Table 4.

Table 4
Regression equations between storage duration and storage depth inputs and germination rate, hypocotyl length, biomass, biomass accumulation rate

Response variable Y (envelope) / Z (seal)		Regression equation
Germination Rate	Y_1	$Y_1 = 96.039 - 0.041T + 0.042D + 2.87 \times 10^{-4}T^2 - 2.48 \times 10^{-5}D^2 - 3.37 \times 10^{-4}TD$
	Z_1	$Z_1 = 94.876 + 0.024D + 1.07 \times 10^{-4}T^2 - 1.05 \times 10^{-5}D^2 - 1.33 \times 10^{-4}TD$
Hypocotyl Length	Y_2	$Y_2 = 27.246 - 0.117T - 0.04D + 2.61 \times 10^{-5}D^2 + 6.07 \times 10^{-4}TD + 2.06 \times 10^{-6}T^3 - 4.04 \times 10^{-9}D^3 - 1.56 \times 10^{-6}T$
	Z_2	$Z_2 = 19.152 - 0.008D + 4.04 \times 10^{-5}T^2 + 4.83 \times 10^{-6}D^2 + 2.26 \times 10^{-5}TD$
Biomass	Y_3	$Y_3 = 0.043 - 1.74 \times 10^{-4}T - 2.38 \times 10^{-5}D + 7.88 \times 10^{-7}TD + 3.15 \times 10^{-9}T^3 + 1.59 \times 10^{-11}D^3 - 1.94 \times 10^{-9}T^2D$
	Z_3	$Z_3 = 0.038 - 1.23 \times 10^{-6}D + 6.82 \times 10^{-8}T^2 + 1.47 \times 10^{-9}D^2 + 2.36 \times 10^{-8}TD$
Biomass Accumulation Rate	Y_4	$Y_4 = 0.001 + 1.57 \times 10^{-6}T + 1.47 \times 10^{-6}D + 1.60 \times 10^{-9}T^2 - 6.26 \times 10^{-10}D^2 - 8.05 \times 10^{-9}TD$
	Z_4	$Z_4 = 0.002 + 8.10 \times 10^{-7}T - 1.29 \times 10^{-9}T^2 - 4.40 \times 10^{-10}D^2 - 9.15 \times 10^{-10}TD$
Vigor Index	Y_5	$Y_5 = 14.101 - 0.017T + 0.042D + 3.11 \times 10^{-3}D + 5.69 \times 10^{-5}T^2 - 2.49 \times 10^{-6}D^2 - 4.13 \times 10^{-5}TD$
	Z_5	$Z_5 = 16.09 - 0.015D + 2.81 \times 10^{-5}D^2 - 3.6 \times 10^{-7}T^3 - 1.04 \times 10^{-8}D^3 + 1.74 \times 10^{-7}T^2D - 4.85 \times 10^{-8}TD^2$
Germination Index	Y_6	$Y_6 = 36.175 - 0.091T + 0.007D + 2.76 \times 10^{-4}T^2 - 6.29 \times 10^{-6}D^2 - 8.77 \times 10^{-5}TD$
	Z_6	$Z_6 = 30.411 + 0.013D + 3.94 \times 10^{-5}T^2 - 6.16 \times 10^{-6}D^2 - 6.67 \times 10^{-5}TD$

[a] T and D represent the storage duration and storage depth, respectively.

We could not obtain appropriate equations with great precision to estimate biomass, biomass accumulation rate, and VI under sealed packaging conditions. The relationships among these three parameters, storage duration, and storage depth were not significant ($p > 0.05$) even when a quadratic or higher power

equation was used for fitting. However, the GR, hypocotyl length, and GI resulting from the two packaging methods were successfully fitted, as evidence by high R^2 values and $p < 0.05$ for both methods (Table 4).

We also calculated the storage duration and storage depth that would maximize the above-mentioned parameters based on the acquired regression equations (Table 5). However, it was challenging to obtain the maximum GR, hypocotyl length, biomass, biomass accumulation rate, VI, and GI of canola seeds simultaneously. In the envelope packaging condition, canola seeds reached the maximum GR (100%) at 233.1 m and 37.05 days, whereas the GI was maximized (37.89) with 0-day storage at 520.77-m depth. The longest hypocotyl (29.02 mm) was obtained over 227 days at a depth of 390.91 m. The greatest biomass accumulation (0.05 g) was achieved over 227 days at 397.79 m of depth. After 0 days of storage, the maximum biomass accumulation rate (0.0023 g/mm) was obtained when the storage depth was 1,168.13 m, whereas the maximum VI (15.07) was obtained at 623.2 m. Under sealed packaging conditions, canola seeds were stored at 490.30 m for 53.44 days to reach the maximum GR (100%), whereas the GI was maximized (36.37) with 0 days of storage at a depth of 1030.79 m. The largest hypocotyl length (26.06 mm) was measured at 227 days of storage and a depth of 1,410 m. The greatest biomass accumulation (0.05 g) was achieved over 227 days of storage at 1,410-m depth. After 0 days of storage, the maximum biomass accumulation rate (0.0024 g/mm) was obtained when the storage depth was 920.75 m, whereas the maximum VI (19.71) was obtained at 1401.84 m (Table 5).

Table 5
Maximum germination rate, hypocotyl length, biomass, biomass accumulation rate, vigor index, and germination index of canola seed corresponding to the optimal duration and depth.

Response variable Y (envelope) / Z (seal)		Maximum value of response variable	Storage duration/day	Storage depth/m
Germination rate (%)	Y_1	100	37.05	233.10
	Z_1	100	53.44	490.30
Hypocotyl length (mm)	Y_2	29.02	227	390.91
	Z_2	26.06	227	1410
Biomass ($\times 10^{-2}$ g)	Y_3	0.05	227	397.79
	Z_3	0.05	227	1410
Biomass accumulation rate ($\times 10^{-2}$ g/mm)	Y_4	0.0023	0	1168.13
	Z_4	0.0024	0	920.75
Vigor index	Y_5	15.07	0	623.20
	Z_5	19.71	0	1401.84
Germination index	Y_6	37.89	0	520.77
	Z_6	36.37	0	1030.79

4. Discussion

4.1. Effects of storage depth and duration on the GR and germination process of canola seed packaged in envelopes or sealed packets

Seed germination largely determines the success of seedling establishment; therefore, it has become the fundamental goal in the pursuit of high yield, stable crops (Hatzig et al., 2015). In addition, seed germination is also an indicator reflecting the advantages and disadvantages of deep-underground seed storage, which can be considered the first step toward deep-underground agriculture. Generally, our experimental results showed that the GR of seeds decreased as storage depth (except at 240 m) and duration increased under both packaging methods. Under envelope packaging conditions, the seeds were non-viable when stored at 1,410 m for 227 days. Under similar storage conditions, the GR of seeds in the sealed package also declined sharply, indicating that the temperature in the deep-underground environment was not the main reason for reduced seed vitality. We reasoned that this decremental phenomenon was due to the decline in seed vigor caused by seed aging. Previous studies have shown that the storage of seeds under high pressure and oxygen conditions could accelerate seed aging (Groot et al., 2012). Therefore, although substance exchange with the environment was prevented by sealed packaging, seed vigor still decreased significantly with the prolonged storage duration, which was reflected in the GR. Furthermore, we speculate that the most important factor influencing canola seeds was RH, which increased with storage depth. An increase in humidity increases seed moisture content and accelerates the deterioration of seeds (Modi and Bornman, 2004); in turn, this accelerates the inactivation process, as mentioned above.

For envelope-packaged seeds at a 0-m storage depth, the GR did not significantly decrease as the storage period was extended. We attribute this phenomenon to ambient temperature and RH (11.9°C and 30.3% at 0 m, respectively), which likely met the appropriate storage conditions for canola seed. Previous studies have indicated that 70% RH in the external environment (Aragão et al., 2019) and a temperature of 10°C–20°C in storage surroundings did not threaten canola seed quality (Sun et al., 2014). In addition, seeds are known to be viable for about 25–30 years when stored at − 3.5°C (Solberg et al., 2020). Therefore, suitable seed moisture and temperature can maintain seed vigor and produce a relatively stable and well-performing GR within short-term storage periods. In the shallower storage environment tested here (i.e., 0, 240, and 690 m), the sealed packaging appeared to produce almost ideal storage conditions with isolation from the external environment. Additionally, the GR of canola seeds in sealed packaging conditions was higher than that of seeds stored in envelopes when seeds storing at 1,410 m for 66 days or at 690 and 1,410 m for 227 days.

The germination curve produced in this study reflected the variation in GR over time. The curve was delayed by increasing storage depth and storage duration for both packaging methods. Sealed-packaged seeds germinated faster than did envelope-packaged seeds, which indicates that the material exchange between the seeds and the environment was important. During the exchange process, water may be most critical since it slows down the germination process of seeds and reduces GR. In addition, other factors in the deep-underground environment, such as temperature, air pressure, and gravitational acceleration, might have affected the germination process of canola seeds.

4.2. Effects of storage depth and duration on the GI and VI of canola seeds packaged in envelopes or sealed packets

GI can effectively characterize the germination speed of seeds, which is one of the critical factors for healthy seed germination (Finch-Savage et al., 2010). Overall, for the two packaging methods, as storage depth and duration increased, the GI gradually decreased. Since the GI can accurately reflect the GR and germination speed, the changing trend in GI was similar to that of GR. In turn, the factors that affect the GR also affected the GI. For instance, a high RH environment causes an increase in seed moisture, which aggravates seed degradation and inactivation (Capilheira et al., 2019), which in turn reduces GR. Furthermore, extending the storage duration exacerbates these processes, resulting in a higher likelihood of seed degradation and inactivation. Therefore, long-term storage in locations that are not conducive to seed storage always reduces the GI. Even when the envelope-packaged seeds were stored at 0 m and the sealed packages were stored at a shallow depth (i.e., 0, 240, or 690 m), there were no statistical differences among the data, although a slight decrease in GI values was observed. Such small decreases could be caused by the aging of seeds with prolonged storage, even though the environmental parameters were acceptable.

VI is a comprehensive reflection of seedling biomass and germination speed, which is substantially affected by seed degradation. For seeds in envelope packaging, conditions including high temperature, an accumulation of microorganisms, and high RH resulting in increased seed moisture may reduce the ability of seeds to germinate (Capilheira et al., 2019; Jian et al., 2019; Modi and Bornman, 2004). Consequently, we found that VI declined most drastically over longer storage durations in deeper locations. Similarly, a downward trend was also observed for seeds in sealed packaging, even though substance exchange with the surroundings was prevented, which again suggests an association with seed aging. (Solberg et al., 2020)indicated that seed vigor and storage period always have a negative relationship, i.e., as storage duration increases, the vigor of seeds always decreases.

4.3. Effects of storage depth and duration on the hypocotyl length, biomass, and biomass accumulation rate of canola seed packaged in envelopes or sealed packets

The elongation of the hypocotyl is a crucial feature of the seedling stage; with better development of the seedling, a satisfactory yield can be obtained (Luo et al., 2017). In the present study, the maximum hypocotyl length usually appeared after more extended storage periods for seeds in both envelopes and sealed packages. However, other studies have shown that the aging of seeds caused by long-term storage can reduce the GR and lead to a decline in initial seedling growth (Rajjou et al., 2008; Stanisavljevic et al., 2011), which was contrary to our results. The main reason for this discrepancy is as follows: in the previous studies, seed storage lasted 990 days or several years, whereas our seeds were stored for a maximum of 227 days; the latter may not result in severe vitality loss through seed aging. In addition, the difference in storage surroundings may also have contributed to the difference in results. For both packaging methods, a decreasing trend in hypocotyl variation followed by an increasing trend was observed as storage depth increased (except for envelope packaging over 227 days). This suggests that other factors in the deep-underground environment played roles in compensating for the later growth of the seeds. However, these factors have yet to be adequately studied. Therefore, we can only speculate that the beneficial effect is due to high pressure or low radiation in the deep-underground setting.

A compensatory effect of the deep-underground environment on hypocotyl length was observed during all storage durations for both packaging methods when considering the influence of storage depth. A positive correlation between hypocotyl length and storage depth was observed for treatments other than those over 66 days of storage. (Bass et al., 1988) stated that the seedlings that germinated in advance could put consistent pressure on the seedlings that germinated later, delaying their germination and physical development. Therefore, even with a high GR under short-term conditions, the seedlings that grew in the later stages were under pressure due to differences in germination time, which resulted in a smaller average hypocotyl length.

In general, we found that biomass gradually increased as storage depth and duration were extended regardless of the packaging method used. Furthermore, biomass and hypocotyl length showed similar variation with storage time, which is similar to the results reported by (Luo et al., 2017). Therefore, the lengthening of the hypocotyl with storage depth and duration may explain the observed increase in biomass accumulation. However, we did find that the biomass of envelope-packaged seeds stored at 0 m for 66 days was higher than the biomass of seeds stored for 90 days. The seedlings of the former had better lodging resistance capabilities. Considering the variation in hypocotyl length and biomass with storage depth and duration, we reasoned that a compensatory effect on seeds existed in the deep-underground environment; the extension of storage depth and duration play a positive role in this compensatory effect.

The biomass accumulation rate is a simple calculation of biomass divided by hypocotyl length, reflecting the biomass accumulation per unit length of the hypocotyl. In our study, we found that with the extension of storage duration, the biomass accumulation rate gradually decreased for seeds stored in envelope packaging (except for at a depth of 0 m). This phenomenon may have arisen during long-term storage in which the extension of hypocotyls was more sensitive than biomass to the environmental factors in deep-underground. In contrast, the “stress response” of biomass was not as apparent as that of hypocotyl length. Therefore, although biomass accumulation increased with storage duration, it increased to a much lesser extent than did hypocotyl length. Under the sealed conditions in which substance exchange with the external environment was prevented, the biomass accumulation rate did not significantly change as storage duration was extended. However, the slight decrease over time still showed that the compensatory effect was more evident in hypocotyl length elongation. In terms of the effect of storage depth on the biomass accumulation rate, we found that all treatments produced greater biomass accumulation rates than that observed in the control. Contrary to biomass and hypocotyl length data, the greatest biomass accumulation rate occurred at a depth of 1,410 m, which further explained the sensitivity of the hypocotyl to the compensatory effect of the deep-underground environment.

4.4. The combined effects of storage duration and storage depth

In our analysis, it was difficult to obtain the maximum values for the experimental indicators simultaneously. In general, over short-term storage durations and at shallow storage depths (i.e., 66 days at 240 m), increased values of GR, biomass accumulation rate, VI, and GI were measured. In contrast, over long-term storage periods at deeper storage depths, greater hypocotyl length and biomass accumulation were observed (i.e., 227 days at 690 or 1,410 m). For canola, hypocotyl length and biomass accumulation are closely related to the final yield (Li et al., 2019; Luo et al., 2017), which is the concern of most producers. Therefore, to increase hypocotyl length and biomass accumulation, storing seeds in a deeper location for a shorter period would be reasonable. However, the vigor of seeds after storage in these conditions is often low, which inevitably leads to a decrease in GR. An effective way in which to deal with this problem would be increasing the seeding rate with the aim of obtaining a higher GR, although this approach would come at an additional cost (Stanisavljevic et al., 2020). In addition, specific pretreatment of canola seeds could be an effective method; for example, thermal pretreatment can effectively break seed dormancy and thereby increase the GR and final yield (Xu et al., 2020).

Overall, our research showed that deeper storage of seeds usually increased the compensatory effect of the deep-underground environment. Due to the limitations of the experimental conditions, it is unclear whether deeper storage depths (i.e., > 1,410 m) would produce greater compensatory effects. However, in an extremely deep environment, the storage duration must be accurately controlled, at least for envelope packaging. The sealed packaging maintained the positive effects of the deep-underground setting after 227 days of storage, but if the storage duration were to be extended longer, it is unknown whether this stimulating effect could be maintained.

5. Conclusion

When canola seeds were stored in a deep-underground environment, prolonging storage duration and increasing depth seemed to compensate for reduced seed vigor by increasing hypocotyl length and biomass accumulation. Generally, the deep-underground environment inhibits seed germination and reduces seed vitality; moreover, increasing depth and duration of storage exacerbates this suppression effect. According to our germination test results, inhibition of germination was weakened while the compensatory effect was enhanced when the material exchange with the external environment was blocked via sealed packaging of seeds. The results of regression analysis and optimal value prediction showed that under envelope packaging conditions, ensuring the existence of vitality, storing in a deep place for a relatively long duration could obtain the maximum hypocotyl length and biomass accumulation, which were closely related to the final yield. Similar conclusions can be drawn from the analysis of seeds in sealed packaging conditions, even when the storage conditions prevented substance exchange between the seeds and the deep-underground environment. However, high yield is typically obstructed by a low GR, which can be resolved by increasing the sowing rate. Under this premise, storing seeds in envelope packaging at 690 m for 227 days or in sealed packaging at 1,410 m for 227 days can be considered optimal if considering only the current experimental conditions. Although some seed vigor is lost due to the deep-underground storage, the increase in hypocotyl length and biomass can compensate for this loss. Future field trials could be used to verify the stimulating effect of the deep-underground environment. Furthermore, successful germination indicates a possible farming potential in the deep-underground space. Our results suggest a worth-trying agricultural method when the environment becomes more challenging for farming in the near future.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files]

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Conceptualization, C.L., L.L. and Y.H.; methodology, Y.W. and Y.H.; validation, Y.W. and J.W.; formal analysis, Y.W. and J.W.; investigation, Y.W. and J.W.; resources, C.L., L.L. and Y.H.; data curation, Y.W.; writing—original draft preparation, Y.W.; writing—review and editing, Y.H.; visualization, Y.W.; supervision, C.L., L.L. and B.T.; project administration, Y.H., X.T. and B.T.; funding acquisition, C.L. and Y.H. All authors have read and agreed to the published version of the manuscript.

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Figures

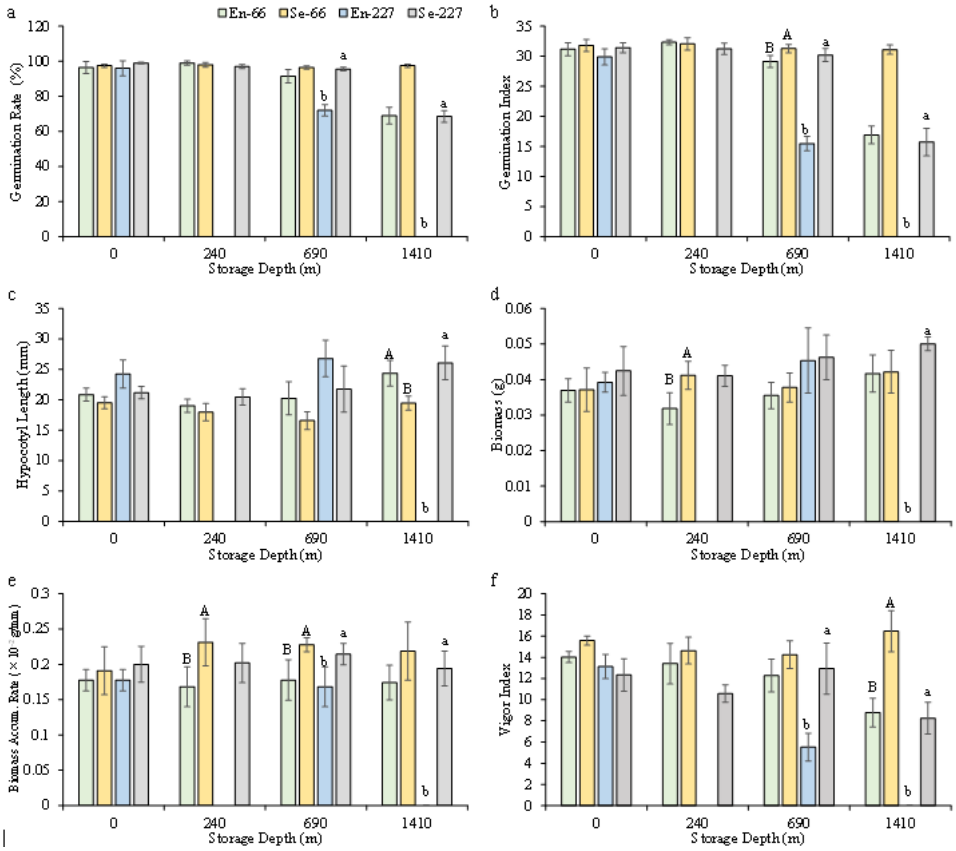

Figure 1

Comparisons of seed germination parameters of canola seeds stored at different depths for 66 and 227 days with different packing methods (P<0.05). Different capital letters indicate the difference between different packaging methods after 66-days of seeds storing. Different lowercase letters indicate the difference between different packaging methods after 227-days of seeds storing.

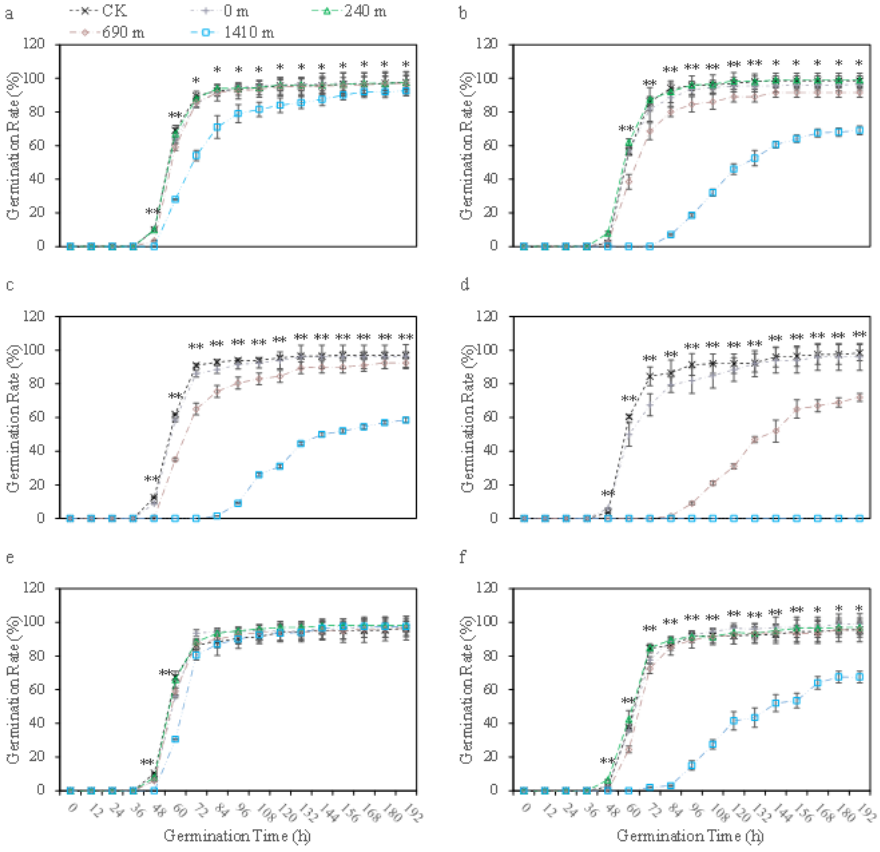

Figure 2

The overall germination process of canola seeds stored in envelopes (a–d) and sealed packages (e, f) at different depths with various durations of storage. a–d show the results of seeds stored in envelopes for 42, 66, 90, and 227 days, respectively. e and f show the results of seeds stored in sealed packages for 66 and 227 days, respectively. * and ** indicate significant effect at the 0.05 and 0.01 probability level between the control and different treatments. There were no data collected or statistical analyses performed for 90 and 227 days of storage at a depth of 240 m since the canola seeds under these conditions were destroyed by the mineworkers.