Does Deep-Underground Storage Stimulate the Germination of Canola (Brassica napus L.) Seed?

Yuxin He1,2, Chao Liu1, Heping Xie3,4, Jingchen Wang2, Yang Wang2, Wenhua Zhuang1,2 & Xiao Tan1,2

1 State Key Laboratory of Hydraulics and Mountain River Engineering, Sichuan University, Chengdu, China
2 College of Water Resource and Hydropower, Sichuan University, Chengdu, China
3 Guangdong Provincial Key Laboratory of Deep Earth Sciences and Geothermal Energy Exploitation and Utilization, Shenzhen, China
4 Institute of Deep Earth Sciences and Green Energy, Shenzhen University, Shenzhen, China

Correspondence: Chao Liu, College of Water Resource and Hydropower, Sichuan University, Chengdu, China. Tel: 86-028-8540-6946. E-mail: liuchao@scu.edu.cn

Heping Xie, Guangdong Provincial Key Laboratory of Deep Earth Sciences and Geothermal Energy Exploitation and Utilization, Shenzhen, China. E-mail: xiehp@scu.edu.cn

Received: February 27, 2020      Accepted: April 7, 2020      Online Published: May 15, 2020
doi:10.5539/jas.v12n6p23          URL: https://doi.org/10.5539/jas.v12n6p23

The research is financed by the National Natural Science Foundation of China (No. 51709190) and Sichuan Province Science and Technology Support Program (No. 2018SZDZX0027).

Abstract

Agriculture is a crucial area to be considered when exploring and exploiting the use of deep-underground space. We investigated the feasibility of deep-underground seed storage by keeping canola seed in either envelopes or sealed packages at four depths below the Earth’s surface (0, 240, 690, and 1410 m) at a gold mine in northeastern China. We studied the effects of storage depth and duration by conducting germination tests with the stored seed. The results showed that the rate of germination was reduced in seed stored at deeper levels and was also lower at all depths after a more prolonged period of storage. Seeds from sealed packages exhibited better resistance to the deep-underground environment than seeds kept in envelopes. However, measurements of hypocotyl lengths and biomass accumulation revealed that the germination of seeds stored in deep-underground was initially inhibited but recovered well compared with the control as the storage depth increased. The total biomass of the hypocotyl increased as the depth of seed storage deepened, indicating the existence of a compensatory effect on seed germination. The findings suggest that short-term deep-underground storage of seeds in sealed packages would improve the germination performance of cultivated canola in terms of the hypocotyl length and biomass accumulation and might be considered as a pre-sowing strategy.

Keywords: deep-underground environment, canola seed, germination, seed viability

1. Introduction

There is an urgent need to explore and develop a use for deep-underground space due to the predicted exhaustion of resources in the shallow depths of the Earth’s surface (Ranjith et al., 2017; Rezayian, Niknam, & Ebrahimzadeh, 2018), the environmental deterioration because of economic development (Hafeez, Chunhui, Strohmaier, Ahmed, & Jie, 2018), the threat to land due to warfare (Mann, 2018), and most importantly, the increasing demand for resources imposed by the expanding global population (Khanna, Swinton, & Messer, 2018). The basic human requirement for food production requires that we explore the use of the inner Earth space. Hence, a shift from traditional farming to deep-underground agriculture is foreseeable.

The storage of seed so that it can be transported and sold is the first step in the agricultural production process. To maintain genetic diversity, more than 16,500 plant species with 7 million types of plant germplasm are stored in seed banks and botanic gardens worldwide (Boniecka et al., 2019). Long-term storage, however, ages seed and causes delays to germination or damages to the viability of the germplasm. Studies have shown that high temperatures and moisture contents in the seed can lead to mold growth, increase cell membrane permeability,
accelerate lipid peroxidation, reduce levels of antioxidant enzymes, lower ability to synthesize proteins and nucleic acids, and consume nutrients in seeds, etc. (Rodo, 2003; Sathya, 2009a). A high germination rate predicts better canopy development and higher yield (Soleymani, 2019). Therefore, to explore the possibility and feasibility of deep-underground farming systems, understanding the performance of seeds following a period of deep storage is crucial.

Canola is one of the most productive oilseed crops. The oil and plants are commonly used for human consumption, livestock feed, and biofuel production (Issariyakul & Dalai, 2014). As one of the most common oil crops, the canola yield in China reached \(1.33 \times 10^7\) tons in 2017 (China National Grain & Oils Information Center, 2019). The storage of canola seeds, however, has certain specificities compared with other crops. As the seed coat is brittle and thin, the tissue is loose, and the grain is small, the seeds are more likely to absorb moisture, and thus, the moisture content of the seed is easily affected by environmental humidity. Cruciferous crops have a relatively short seed life even under excellent storage conditions, which makes canola seed an extremely important model for studying seed aging (Boniecka et al., 2019).

Canola seeds are nearly round in shape, dense, and contain a high level of oil, which intensifies respiration in these seeds compared with those of other crops. Therefore, the nutrients in the seeds are quickly consumed, and a high level of heat is released. The presence of mold often represents the spoilage of seeds, resulting in the failure to germinate properly. The loss of germination at a rate of 5 to 20% or the appearance of visible mold are factors often used to establish safe storage guidelines for crop seeds. Mills and Sinha (1980) compared safe storage periods in rapeseed containing different water contents at different temperatures in farm storage tanks. The results demonstrated that rapeseed with a water content of 8.5% or lower could be stored for five months without mold growth because mold activity was lower when the water content was less than 10%. However, a study of the canola storage guidelines found that even if the moisture content of canola seeds was below 8%, some mold species grew at temperatures of 30 °C to 40 °C (Sathya, 2009b).

The deep-underground environment could be considered as a pre-treatment strategy prior to sowing. Chemical soaking, physical heating, and radioactive exposure have all been used as seed pre-treatments before sowing or crushing for oil (Clarke & Moore, 1986; Wroniak, Rekas, Siger, & Janowicz, 2016). Previous studies have indicated that dry heat can be used to sterilize seeds (Hong & Kang, 2016; Park, Ahn, & Kang, 2018) and help them to break dormancy (Tabi, Ebongue, Ntsomboh, & Youmbi, 2017; Timple, Hay, Mercado, Borromeo, & Sta Cruz, 2018), and consequently, to increase the germination rate. Radioactive exposure, on the other hand, was reported to harm seeds in storage by causing genomic changes to seed proteins and biochemical alternations to the seed composition (Jan et al., 2018). Therefore, deep-underground storage may stimulate the germination of seeds by providing the constant warm temperatures and minimally radioactive surroundings. However, studies related to this topic are limited and not well documented.

To determine the possibility of deep-underground seed storage and the potential of using this strategy as a pre-treatment before sowing, we conducted deep underground canola seed storage experiments and germination tests. The objectives of our research were to: 1) study morphology and physical development by which canola seeds germinate following storage at various depths of deep-underground using two packaging methods; and 2) investigate the possibility of using the deep-underground environment to pre-treat seeds for better germination.

2. Materials and Methods

2.1 Research Site and Treatment

We chose a gold mine [Jiapigou Minerals Limited Corporation of China National Gold Group Corporation (42°52′36″N, 127°30′46.2″E)] in Jilin Province, China, to store the seeds. The mine reaches a depth of approximately 1500 m (about 820 m below the sea level) and contains several horizontal tunnels at different depths (Liu et al., 2018). In this study, four horizontal tunnels at depths of 0, 240, 690, and 1410 m containing suitable space for storing seeds were selected. Table 1 presents the elevation, temperature, humidity, background radiation, and air pressure measured at each seed storage location.

Table 1. Environment parameters at different depths of Jiapigou Gold Mine, Jilin, China

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>CO₂ concentration (ppm)</th>
<th>Air pressure (hPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.9</td>
<td>30.3</td>
<td>873</td>
<td>941.5</td>
</tr>
<tr>
<td>240</td>
<td>14.3</td>
<td>72.4</td>
<td>776</td>
<td>968.4</td>
</tr>
<tr>
<td>690</td>
<td>21.8</td>
<td>96.5</td>
<td>1239</td>
<td>1020.2</td>
</tr>
<tr>
<td>1410</td>
<td>28.8</td>
<td>99.0</td>
<td>1382</td>
<td>1106.6</td>
</tr>
</tbody>
</table>
Canola seeds (Dexingyou 12, Chengdu Damei Seeds Co., Ltd., a major variety used by local producers) were acquired from Sichuan Academy of Agricultural Sciences. Two packing methods with three replications, including unsealed envelopes and perfectly sealed plastic bags, were adopted in this study. Each package contained about 100 g of seeds. Seeds in the envelopes were fully exposed to the surrounding environment at different depths. The seeds in the sealed plastic bags, however, were protected from humidity. Seeds in both envelopes and sealed plastic bags, with 16 and eight replications of each, respectively, were stored at 0, 260, 690, and 1410 m below ground on Sept 29th, 2018. Meanwhile, seeds in the original commercial plastic bag with small breathing holes were placed in the laboratory at Sichuan University, treated as the blank control group (CK), to simulate the normal storage condition used by local producers.

Four replications of seeds stored in envelopes were retrieved from the different depths on Nov. 10th, Dec. 4th, Dec. 26th, 2018, and May 14th, 2019, which were 42, 66, 90, and 227 days after the initiation of the experiment, respectively, while the seeds stored in sealed packages were only retrieved on Dec. 4th, 2018, and May 14th, 2019. The seeds were taken to the Laboratory of Drainage and Irrigation Engineering, Sichuan University, for germination tests and physiological biochemistry experiments.

### 2.2 Germination Test

After retrieving, seeds were surface sterilized in 0.1% potassium permanganate solution for 20 min and air-dried on filter paper. Germination boxes (13 × 19 × 4.5 cm in size) were wiped with 75% alcoholic solution and oven-dried for one h. A double layer of filter paper was placed in each germination box. The germination boxes were then exposed to UV light for one h. Fifty healthy seeds from each pack were evenly placed on the filter paper in a germination box. The boxes were then wrapped with plastic wrap to prevent water evaporation (Derakhshan, Bakhshandeh, Siadat, Moradi-Telavat, & Andarzian, 2018). Germination was carried out in a growth chamber at 25 °C with a humidity of 85% under a photoperiod of 18 h light/6 h dark. Distilled water was added until the filter paper became saturated at 156 h and 180 h after the onset of germination.

Germination time was recorded once the boxes were placed in the growth chamber. In this study, the point at which the seed showed an emerging cotyledon was considered to be the start of germination (Koch & Seeliger, 1988). Germinated seeds were counted every 12 h until 192 h after the initiation of germination to calculate the germination rate (GR) and germination index (GI).

**GR** was calculated using the following equation:

\[
GR = \frac{\text{Number of germinated seeds in 192 h}}{\text{Total tested seeds}} \times 100\%
\]  (1)

**GI** was calculated using the following equation:

\[
GI = \frac{\sum_{i=1}^{n} GR_i}{n}
\]  (2)

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

To calculate the biomass of the seedling, ten healthy and well-developed young plants were selected from each germination box at 180 h after the onset of the germination process. The plants were measured for hypocotyl length using a vernier caliper and then treated at 105 °C for 15 min and then moved to an oven at 65 °C for another 72 h until the weight stabilized. The biomass of the seedling was then weighed using an analytical balance. Then the biomass was used to calculate the vigor index (VI) of seeds.

**VI** and biomass accumulation rate were calculated using the following equations:

\[
VI = m_s \times GI
\]  (3)

\[
\text{Biomass accumulation rate} = \frac{m_s}{\text{Hypocotyle length}}
\]  (4)

where, \( m_s \) is the biomass of the seedling.

### 2.3 Statistical Analysis

All data were statistically analyzed using Mixed Procedure in SAS version 9.4 (SAS Institute, 2015, Cary, NC, USA). Storage depth was the fixed effect and the random replication effect. Least square mean separation for each treatment during the sampling period was at the \( P = 0.05 \) significance level (SAS Institute, 2015). Since the main purpose of this study was to verify the impacts of deep-underground on seed vitality, treatments were compared by package method each sampling period and were not compared across package methods or across the storage durations.
3. Results

3.1 Germination Rate

The GR of seeds stored in envelopes and sealed packages are shown in Figure 1 (I-IV). As shown in the figure, generally, the GR of seeds in the envelopes decreased with increasing storage depths for all four sampling periods. The decreasing trend in germination became more dramatic as the storage period lengthened. After 42 days of storage, a significant reduction in germination (about 5% lower than the CK treatment) occurred only at a depth of 1410 m. The GRs at all four treatments and the CK were greater than 90%. When the storage period increased to 66 days, the deep-underground environment impacted seed germination both at 690 m and 1410 m. After storage under these conditions, the GRs were 7% and 29.5% lower than those for the CK treatment, respectively. A similar trend was observed following the 90- and 226-day storage periods. The GR following storage at 1410 m was the lowest among all treatments. Furthermore, the difference between seeds from 1410 m and the CK widened until the seeds lost viability after 226 days of underground storage. Unfortunately, the samples at 240 m were damaged by mine workers, and therefore, we had to abandon the data for the last two sampling periods.

![Figure 1](image_url)

**Figure 1.** The germination rate (GR) and germination index (GI) of canola seeds stored at different depths with the various duration of storage. I-IV are the results of seeds stored in envelopes for 42, 66, 90, and 227 days, respectively. V-VI are the results of seeds stored in sealed packages for 66 and 227 days, respectively.

**Note.** Upper-case letters are for the GR, and lower-case letters are for the GI. Treatments with different letters indicate significant differences at the \( P = 0.05 \) level within the same sampling time. The absence of letters indicates no significant differences among treatments for that sampling time. Samples in the envelope from 240 m stored for 90- and 226-days were damaged by local mine workers.

The GRs for the seeds in the sealed packages are displayed in Figure 1 (V-VI). The GR was not affected by the deep underground environment in the short-term at any of the study depths. At the 66-day sampling point, no
statistical differences in germination were found among treatments. At this time point, GRs for all treatments were greater than 97%. This pattern persisted for all storage periods up to 226 days for all treatments except storage at 1410 m, under which treatment the GR decreased to about 70%. Therefore, the viability of the seeds in sealed packages decreased slower than that for seeds in the envelopes according to the germination results.

3.2 Germination Index
GI was affected by both the storage depth and duration. Deeper storage tended to decrease the GI faster compared with shallow storage. As shown in Figure 1 (I-IV), for seeds stored in envelopes, only a depth of 1410 m impeded the germination of seeds stored for 42 days. An impact on germination, however, was seen in seeds stored at 690 m when the storage period was extended to 66 days. A similar trend was also observed in seeds stored for 90 and 226 days. The decreasing GI was seen in seeds stored at 0 m when the storage time was increased to 226 days. Furthermore, the GI decreased to 0 at 1410 m, indicating a total loss of viability. Therefore, the longer storage period and deeper storage depth both influenced the germination of envelope-stored seeds. Generally, the deep-underground environment reduced the vitality of the seeds if they fully exposed to the environment.

Figure 1 (V-VI) shows the GI of the canola seeds stored in sealed packages. After a short storage period, the GI was not affected by the storage depth using the sealed package method. At 66-day of storage, the GIs at all depths were more than 30, indicating fast germination at the early stage and high germination rate by the end of the experiment. Similar results were seen following 226-day of storage. There were no significant differences in GI among the CK, and seeds stored at 0, 240, and 690 m depths. The only reduction in GI was observed for seeds stored at 1410 m. This was about 50% compared to the other treatments, demonstrating a negative impact of the deep-underground storage environment on the seed germination process. Interestingly, this negative influence did not impact seeds kept in shallow storage (i.e., 0, 240, and 690 m). Therefore, for the sealed package method, neither the short-term storage period nor shallow storage depth had a negative impact on germination speed and final GR.

3.3 Germination Process
The results of the germination process are given in Figure 2. In seeds packaged in envelopes, a slower germination process was observed when the duration of storage and depth increased. After 42 days, seeds from 1410 m grew slower during the middle-stage of germination compared to plants from the other treatments. However, the final GR was not statistically different from other treatments, including the CK. This reduced germination speed gradually appeared in seeds stored at shallower depth as the duration of storage increased. The final GR also decreased compared to the CK. As the duration of storage extended to 226 days, the seeds stored at 1410 m lost vitality completely and, therefore, the VI was zero throughout the experiment.

For the seeds in the sealed package, the deep underground environment did not influence the germination speed and rate in the short-term (Figure 2 V-VI). However, long-term storage (226 days) caused a significant reduction in germination speed and rate for seeds stored at 1410 m. Canola seeds from 1410 m reached their highest GR at 84 h to 132 h, which is about 36 h later than that for seeds from the other treatments, in which most germination occurred 48 h to 72 h after the germination experiment was initiated.
3.4 Hypocotyl Length

In general, a U-shaped pattern was observed in the plot of canola hypocotyl lengths after 180 h of germination for seeds treated at different depths and stored in envelopes (Figure 3 I-IV). The hypocotyl length decreased with the increasing storage depth and then increased again when the storage depth was beyond 690 m. This pattern was particularly true of seeds stored for 66 and 90 days, although the data for seeds stored at 240 m for 90-days is missing. Following 66-days of storage, hypocotyl lengths of seeds stored at 1410 m were about 10% longer than those of the CK. This increased to 26% longer for seeds stored for 90-days indicating that the deep-underground had a positive effect on hypocotyl growth during the germination process. Furthermore, the longer hypocotyl may compensate for the low GR and eventually result in the same or even greater yield.

The graph of hypocotyl lengths for seeds stored in sealed packages shows a parabolic pattern with increasing storage depth (Figure 3 V-VI). This pattern was measured at both 66- and 90-day sampling periods. The shortest hypocotyls were measured for seeds stored at 690 and 240 m over two sampling periods, respectively. After that, the hypocotyl lengths increased with increasing storage depth, indicating a stimulation effect of the deep-underground environment on seeds. A similar observation was found in the envelope-stored seeds that the parabolic shape was seen in the graph of the measurements at all sampling times. These findings demonstrate a potential environmental impact on seed viability, which could lead to an initial reduction in germination, which is compensated for by the longer hypocotyl.
Figure 3. The hypocotyl length and biomass accumulation of canola seeds stored at different depths with the various duration of storage. I-IV are the results of seeds stored in envelopes for 42, 66, 90, and 227 days, respectively. V-VI are the results of seeds stored in sealed packages for 66 and 227 days, respectively.

Note. Upper-case letters are for the hypocotyl length, and lower-case letters are for the biomass accumulation. Treatments with different letters indicate significant differences at the $P = 0.05$ level within the same sampling time. The absence of letters indicates no significant differences among treatments for that sampling time. Samples in the envelope from 240 m stored for 90- and 226-days were damaged by local mine workers.

3.5 Biomass Accumulation During the Germination Process

As shown in Figure 3 I-IV, generally, during the germination process, the accumulation of biomass for the seed in the envelope decreased at first with increasing storage depth and then increased after the storage depth exceeded 690 m. When the storage duration was 42 days, both 240 and 1410 m treatments resulted in significantly greater biomass accumulation. When the storage duration increased to 66 days, however, the biomass for germinated seeds stored at 240 m was the lowest among all treatments, while seeds stored at 1410 m showed the greatest accumulation of biomass. A similar trend was also observed in seeds stored for 90 days. Although the data for 240 m was missing, both 690 and 1410 m seeds accumulated greater biomass during germination than the CK and 0 m treated seeds. When the storage period increased to 226 days, seeds from 1410 m totally lost vitality and were unable to sprout. However, seeds from 690 m accumulated the most biomass although there was no significant difference among the CK, 0 m, and 690 m treatments.

The biomass accumulation during the germination process generally increased with the increasing storage depth at both sampling periods for the seed in the sealed package treatments (Figure 3 V-VI). The seeds from the 1410 m treatment demonstrated the greatest accumulation in biomass at both sampling periods and were significantly greater than that seen for the CK treatment. The results for seeds from the sealed packages at 66-days of storage were close to those for the envelope-packaged seeds at 42-days from 240 and 1410 m, which developed the greatest biomass during the germination process. A general trend of greater biomass accumulation with
increasing storage depth indicated a potential stimulation of seed germination. This biomass increment could compensate for the yield loss due to the lower GR found in seeds stored at 1410 m for 226 days.

3.6 Rate of Biomass Accumulation

The rate of increase in hypocotyl length for seeds packaged in envelopes was not affected by storage depth or duration (Figure 4 I-IV). However, we did notice that this rate for seeds stored at 240, 690, and 1410 m for 42 days was significantly greater than that seen in seeds stored at 0 m and the CK treatments. This observation indicated a potential stimulation of seed viability by the deep-underground environment. This trend did not last as the storage period prolonged. The only exception was seen in seeds stored for 90 days at 690 m in which the rate of hypocotyl growth was significantly greater than that seen for the CK treatment. However, there were no statistical differences among underground storage treatments. Therefore, we conclude that the potential stimulation effects were valid only for a short period. Prolonged storage duration could damage the seed and eventually sacrifice the vitality.

Figure 4. The biomass accumulation rate and vigor index (VI) of canola seeds stored at different depths with the various duration of storage. I-IV are the results of seeds stored in envelopes for 42, 66, 90, and 227 days, respectively. V-VI are the results of seeds stored in sealed packages for 66 and 227 days, respectively

Note. Upper-case letters are for the biomass accumulation rate, and lower-case letters are for the VI. Treatments with different letters indicate significant differences at the P = 0.05 level within the same sampling time. The absence of letters indicates no significant differences among treatments for that sampling time. Samples in the envelope from 240 m stored for 90- and 226-days were damaged by local mine workers.

Generally, for the seeds in sealed packages, the biomass accumulation rate increased with increasing storage depth at both sampling periods. In samples stored for 66 days, all storage depths positively impacted the rate of biomass accumulation during the germination process. The increments in accumulation rates were greater than
50% compared to the CK for all 240, 690, and 1410 m treatments. A similar trend was also found for seeds following 266-days of storage. The accumulation of biomass for seed stored at 690 m was the greatest among all treatments and significantly greater than that for the CK. Although statistical differences were not measured among the CK, 0, 240, and 1410 m treatments, greater accumulation rates were found for all treatments compared with the CK. Greater rate of increase in hypocotyl length might indicate stronger sprouts. These results indicated that a deeper storage depth could stimulate some inherent abilities of the seed and consequently result in better germination.

3.7 Vigor Index

Generally, VI decreased with increasing storage depth and prolonged storage period for the seeds stored in envelopes (Figure 4 I-IV). At the 42-day sampling period, there were no statistical differences measured among seeds stored at all depths. When the storage time was increased to 66 days, the VI at 1410 m decreased significantly compared to the other treatments. The trend was the same at 90 days when the VI at 1410 m was the lowest among all treatments. Seeds stored for 226 days at 1410 m lost viability, and the VI decreased to 0. Meanwhile, the VI of seeds at 690 m was about half of that for the CK and 0 m treatments, indicating a significant reduction in vitality. As for the GI, the prolonged storage time and deep-underground environment had negative impacts on the canola seed VI in the envelope packages.

The VI of the canola seed in sealed packages is shown in Figure 4 V-VI. The only statistical difference was seen between seeds stored for 66 days at 690 and 1410 m. Among all storage depths, the VI reached a maximum (16.47) at 1410 m. Although a gentle decreasing trend in VI was measured as the storage depth increased from 0 to 690 m, no statistical differences were found compared to the CK at these depths. Unlike the VI of seed stored for 66 days, the VI after 226-day showed a general decreasing trend as the storage depth increased. However, the VI of seeds stored at 690 m depth was not significantly different from the CK and was significantly greater than that measured for seeds stored at 240 and 1410 m. The greatest VI (13.57) was measured in the CK, and the lowest value (8.25) was found in seeds stored at 1410 m.

4. Discussion

Seed germination is the most critical stage in seedling establishment and crop yield determination (Rezayian et al., 2018). From this study, the GR for the envelope-packaged seeds decreased with the increase in storage depth and time. Following storage at 1410 m for 66 days, the GR was lower than 80%, which is considered to be the safety level (Sun, Jian, Jayas, & White, 2014). After storage for 226 days, the seeds were completely deactivated. Meanwhile, the GR for seeds in sealed packages was significantly reduced in the 1410 m treatment after 226 days, indicating that temperature was not the main factor influencing the final GR. Although there is evidence that high pressure and oxygen content accelerate seed aging, this change is more due to changes in oxygen concentration (Groot, Surki, de Vos, & Kodde, 2012), and the effect of gas pressure itself on seed storage has not been studied in depth. The biggest influencing factor in this study might be attributed to gas exchange, which resulted in increased humidity in the seed storage environment. The increase of humidity would cause the water content of the seed to rise, which could increase the cytoplasmic viscosity of the seed, destroy its “glassy state”, affect the low molecular mobility and high stability of the cytoplasm, and change the protective mechanism of the enzymes in the seed (Groot et al., 2012), thus accelerating the process of seed deterioration (Modi, 2004). In addition, due to the high ambient temperature in the deep-underground, the increase in humidity might also lead to accelerated reproduction in microbial populations, which could cause a reduction in the quality of the seed dry matter and accelerate the deterioration process. Meanwhile, microbial communities can attack germplasm, leaving seeds unable or less likely to germinate (Modi, 2004). Also, research has shown that an increase in oxygen can cause chromosomal aberrations in the seed during cell division, which induces DNA oxidative damage during storage. High moisture content in the deep-underground could be another factor causing a reduction in the GR. Karunakaran et al. (Karunakaran, Muir, Jayas, White, & Abramson, 2001) found that there was a water content threshold for seeds stored at 25 °C; the wheat GR did not change within 70 d if the moisture content was less than 16% but gradually decreased over time when the moisture content was higher than 17%. The GR change in our experiment may have been caused by the same reason. The relative humidity of 93.3% to 98.8% increases the water content of the seed to a critical value in a short period of time, causing it to germinate. The rate greatly reduced over time, and the decline in the GR of the envelope-packaged seeds stored at 690 m could also be explained in this way. We speculate that when the storage period is lengthened if the seed moisture content increases to a certain critical value, germination will occur. The rate will show a rapid decline in the short term.
The GI is a simple calculation of the area under the curve of the GR and can accurately reflect germination speed and rate. In canola, slow germination increases the sensitivity of seedlings to pathogens, delays maturity, and reduces yield; therefore, germination speed plays an equally important role to the final GR (Schwinghamer, Souleimanov, Dutilleul, & Smith, 2015). Like the GR, the GI decreased rapidly at the beginning of the experiment and tended to slow down gradually as the duration of storage lengthened. The GI of seeds packed in envelopes was both impacted by the storage depth and duration. Deeper storage led to the greater moisture content in the seeds and higher temperatures that rapidly decreased the GI. Without any substance exchange between the environment and seeds, the GI tended to be maintained at a higher level longer, as it was not impacted during a short storage period (66 days). Longer duration of storage (226 days) could reduce the GI of the seeds, although the seeds were not directly exposed to the deep-underground environment.

The seed VI comprehensively illustrates the germination ability of the seed and the biomass of the seedling, which can truly reflect the aging and deterioration of the seed. Due to the negative impact of the deep-underground environment on seed GR and speed, the overall canola seed VI showed a downward trend with the increase of storage depth and storage time. This downward trend was also measured for the sealed package treatments in which substance exchange with the environment was prevented. However, the VI of seeds from the sealed packages stored for 66 days at 1410 m was the greatest among all treatment, indicating possible stimulation by the deep-underground environment. In practice, the loss of VI due to low GR could be compensated for by polyculture at the time of sowing.

The curve of the germination process reflected the GR change over time. As the depth of storage increased and the storage time was prolonged, the germination curve appeared to be delayed. This phenomenon was observed for both packaging methods. Although the germination process was delayed after the influence of substance exchange was removed by using the sealed package, the overall germination process of seeds in the sealed packages was faster than those in the envelopes. This phenomenon indicated that the substance exchange between seeds and with the underground atmosphere played a major role in the germination process. Among the substances exchanged, water might be the most crucial factor slowing down the germination process and reducing the GR. This is particularly true for the seeds in envelopes in which the water molecules could directly interact with the seeds. Moreover, other abiotic factors (i.e., gravity, background radiation rate, temperature, and air pressure) could also affect the germination process of the canola seeds.

The hypocotyl length and biomass of the germinated seeds grown under the same conditions can, to some extent, reflect the growth ability of the plant at the bud stage. The hypocotyl length of both envelope-packed and sealed seeds first decreased and then increased with the increase of the storage depth. Due to the slower germination speed of seeds stored in deep ground (i.e., 1410 m), the growth rate of the seedlings accelerated. This trend was most evident in the test of envelope-packed seeds stored for 90 days and sealed seeds stored for 226 days. Envelope-packaged seeds stored at 1410 m for 90 days developed significantly greater hypocotyl length compared with the CK group. Unfortunately, these seeds completely lost vitality when the storage duration was lengthened to 226 days. Otherwise, we might be able to observe a trend showing that seeds stored deep underground could develop longer hypocotyls in the germination stage. For the envelope-packed seeds, the deep-underground environment seemed to have a selective effect—only the vigorous, strong, and healthy seeds could survive there. This is particularly true when the storage depth was greater than 240 m when the underground environment had the most negative effect on seed germination. For the sealed seeds, the U-shape pattern in germination was measured after storage for 66- and 226 days. The deep-underground environment seems to have a compensatory effect during the germination process for sealed packages from which substance exchange with the underground environment was excluded. Therefore, air pressure, temperature, background radiation, and even gravitational acceleration rate might be the reasons for the compensatory effect.

The compensatory effect of the deep-underground environment on biomass accumulation was observed at all storage periods for both package methods. A generally increasing trend in biomass accumulation with increasing storage depth was found for every duration of storage of envelope-packed seeds, except the 66-day period. Without the impact of substance exchange, sealed seed showed the same trend, where the biomass accumulation at 1410 m was the greatest and significantly greater than the CK at both sampling periods. The reason for this compensatory effect remains unknown and might be related to other deep-underground environmental factors.

To better understand the accumulation of biomass, we introduced the biomass accumulation rate factor, which reflects biomass accumulation over the unit length of the hypocotyl. As can be seen from the data, the biomass accumulation rate under all treatments was greater than that of the CK group. This pattern was particularly true when the duration of storage was short. Unlike the hypocotyl length and biomass data, for which the seeds stored at 1410 m always had the best performance during the germination process, seeds stored at 240 m showed a
better biomass accumulation rate for both types of packaging when the storage duration was 66 days. A potential stimulation of germination by the deep-underground environment was observed in this study, especially when the storage depth was deep (i.e., > 240 m) and the storage period was short (i.e., < 90 days). This is particularly true in terms of hypocotyl length and biomass accumulation, which also suggested a potential pre-sowing treatment strategy for greater yield.

5. Conclusion

The deep-underground environment seemed to compensate for the seed vitality loss due to the enhanced environmental stress as the depth increased in this study. This phenomenon was obviously displayed in terms of hypocotyl length and biomass accumulation. The deep-underground environment appeared to compensate for the inhibitory effect of increased temperature and moisture content on seed germination. Generally, the deep-underground environment inhibited the germination of envelope-packed seeds by lowering the GR, decreasing seed vitality, and slowing the overall germination process, especially after more extended periods of storage and increased depth. However, the deep-underground environment somehow stimulated the germination of sealed seeds in terms of the hypocotyl length and biomass accumulation, for which substance exchange between seeds and the surrounding environment was prohibited. This was particularly true when the duration of storage was short. The stimulation of germination suggested that seed pre-treatment in deep storage before sowing could be beneficial. Further on-farm whole growth period studies will be conducted to verify this theory. Different types of seeds (i.e., endosperm seed, endosperm-free seed, monocotyledon seed, and dicotyledon seed) will be tested to screen for the best adaptive plants for deep-underground agriculture. Additionally, future studies will focus on the impacts of the deep-underground environment on proteins, hormones, and enzymes of seeds, followed by investigations of gene expression.

Acknowledgements

The authors express their thanks to Jiapigou Minerals Limited Corporation of China National Gold Group Corporation for providing experimental site and assistance during the research. We also appreciate Yike Xie from West China Hospital, Sichuan University, for retrieving the samples from the gold mine and sending them back to the laboratory. The authors gratefully acknowledge grant support from the National Natural Science Foundation of China (No. 51709190) and Sichuan Province Science and Technology Support Program (No. 2018SZDZX0027). We also thank our colleagues from West China Hospital and College of Life Science, Sichuan University, for providing their valuable suggestions on this project.

References


