

Article

Seed Germination of Sunflower as a Case Study for the Risk Assessment and Management of Transgenic Plants Used for Environmental Remediation in South Korea

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Abstract: In South Korea, the safety management of living modified organisms (LMOs) is regulated by seven government agencies depending on their use, and the Ministry of Environment is in charge of LMOs to manage environmental remediation and effects on natural ecosystems. This study aimed to develop appropriate research tools to determine the factors affecting the invasiveness of transgenic plants used for environmental remediation. We examined the persistence of sunflower (*Helianthus annuus* L.) as a candidate by comparing the seed viability at different controlled temperatures and soil depths (ranging from 0 to 30 cm). The germination characteristics of seeds significantly differed between cultivars and temperatures. The field trials indicated that seeds buried at a depth of 30 cm mostly decayed within three weeks, whereas those buried at 0 cm persisted for eight weeks but decayed after sixteen weeks, implying a significant interaction between burial depth and seed persistence. At all soil depths, no dormant seeds were detected over one week after burial. These results suggest that sunflower seeds could not be successfully established under our experimental conditions. Since seeds on the soil surface demonstrated the highest rates of germination, such seeds may require particularly careful management to prevent unintended effects on ecosystems.

Keywords: environmental remediation; invasiveness; persistence; seed burial; soil seed bank; sunflower; transgenic plant

1. Introduction

With advances in modern biotechnology, numerous living modified organisms (LMOs) with improved traits have been developed, but their potential risks to human health and the environment remain controversial [1]. Accordingly, to prevent the risks of LMOs to public health and to preserve and sustain the use of biological diversity, the Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity has developed an international standard for a minimum set of requirements for the safe transfer, handling, and use of LMOs [2]. In South Korea, the Transboundary Movement, Etc. of LMOs Act (hereinafter, the LMOs Act), which is a domestic measure to implement the CPB, was enacted in March 2001 and took effect in January 2008 [3]. The LMOs Act regulates the safety management of LMOs by seven government agencies according to the use of LMOs, and the Ministry of Environment (MOE) specializes in the development, production, import, export, distribution, and use of LMOs for environmental remediation (Figure 1). Furthermore, the MOE is responsible for the risk review of LMOs in natural ecosystems.

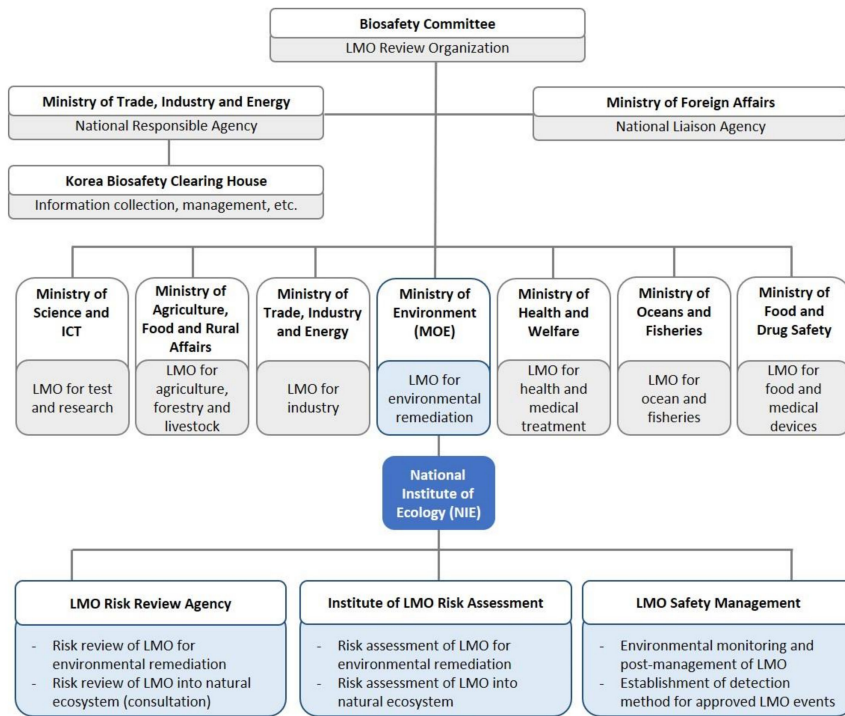


Figure 1. South Korean living modified organisms (LMOs) management system based on the LMOs Act and the roles of the Ministry of Environment and the National Institute of Ecology.

The National Institute of Ecology (NIE), a government-affiliated institute under the MOE, has been delegated affairs concerning the safety management of LMOs, led by the MOE; it was designated as an LMO risk review agency in January 2015 and as an institute of LMO risk assessment in December 2018 [4,5]. The NIE establishes standards and methods for the risk review and assessment of LMOs for environmental remediation. It further reviews, by consultation, the effects of new LMOs on the natural ecosystem. In addition, the NIE investigates the unintentional release of LMOs into natural ecosystems and follows up their spread in the populations of wild relatives. The roles of the MOE and NIE for the risk assessment of LMOs are detailed in Figure 2.

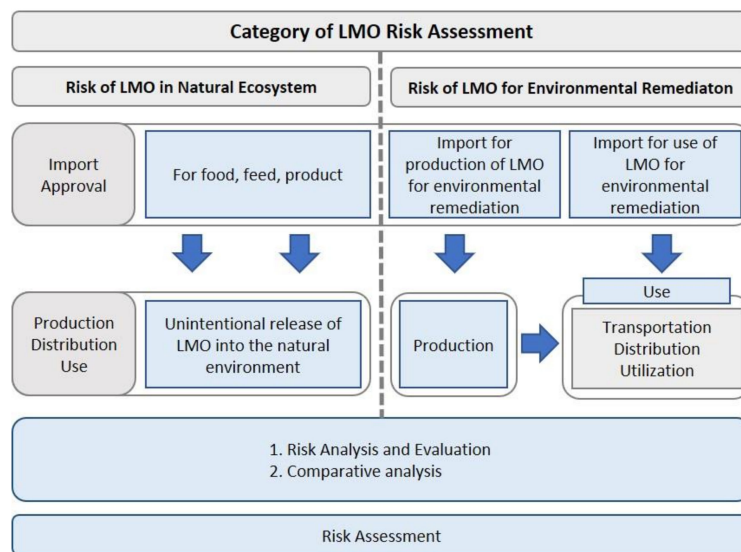


Figure 2. Risk assessment system of living modified organisms (LMOs) led by the Ministry of Environment.

Since 2008, South Korea has been importing LMOs for use as food, feed, or processing, and the total cumulative amount of imported LMOs during the period 2008–2019 was over 110 million tons [6]. Simultaneously, there is an increasing possibility of seed spillage leading to the unintentional escape of LMOs into the natural environment during their transportation and utilization. Although the field cultivation of transgenic plants is not permitted in Korea, transgenic volunteers have been detected around feed factories, livestock barns, small vegetable gardens, and festival places, as well as in ports and along the transportation routes [7–10]. In 2017, a large amount of herbicide-tolerant oilseed rape GT73 and insect-resistant cotton MON531 was found in flower festival-hosting regions and in cultivation fields, respectively [10–12]. These volunteers caused by the unintentional or accidental release of transgenic seeds may adversely affect the variation in natural populations.

The invasion of transgenic seeds into natural ecosystems is a major environmental concern, along with transgene introgression or hybridization [13]. Competitive invaders can disturb the biodiversity in natural habitats, and therefore it is important to detect potential invasiveness early [14]. Raybould [15] suggested that seed production and dispersal are early-stage processes associated with the weediness and invasiveness of transgenic plants. Increased quantities of transgenic seeds could lead to seed dispersal over larger areas and could result in larger seed banks in the disturbed environments [16]. Furthermore, the distribution of soil seed banks across large areas promotes the persistence of volunteer populations during periods of unfavorable growth conditions [17]. However, as the seeds in a soil seed bank may lose their viability in response to high soil temperatures and moisture or unsuitable duration and depth of burial or soil type, seed viability is an important indicator in predicting the potential invasiveness of transgenic plants [18–20].

On the contrary, transgenic plants can be used to remediate disturbed environments, including long-term contaminated sites, and therefore the ecological effects of these plants should be more carefully considered. Although the commercial cultivation of transgenic plants for environmental remediation has not been approved anywhere in the world, several transgenic plants intended to reduce or remove environmental pollutants have been developed [21–24]. Moreover, several transgenic plants for phytoremediation have been approved for experimental field release in the USA and Germany [25–29]. In South Korea, field trials for the environmental release of heavy metal-tolerant transgenic poplar have been conducted in abandoned mine sites [30,31]. As the commercialization of transgenic plants for environmental remediation is imminent, it is necessary to set up a standard for the risk review and assessment of these transgenic plants in natural ecosystems and to prepare for their unintentional escape, especially in the seed state.

Here, we investigated whether the seeds of plants used for environmental remediation can persist in natural ecosystems in South Korea by comparing the viability of seeds under laboratory and field conditions (Figure 3). Sunflower (*Helianthus annuus* L.), a promising candidate for phytoremediation due to its heavy metal-hyperaccumulating efficiency, was considered in this study [32–34]. Sunflower is also a good material with which to examine seed viability, because it produces large amounts of seeds annually, forms persistent seed banks, and is often observed in disturbed areas [16,35]. The unintentional spillage of LMO seeds may occur during seed transportation and utilization throughout the year; therefore, we conducted our study during the spring season, when seeds are most likely to germinate. Our results could support the development of appropriate research tools to determine factors affecting the invasiveness of transgenic plants used for environmental remediation.



Figure 3. Method for testing the viability of seeds under laboratory and field conditions.

2. Materials and Methods

2.1. Plant Materials

Five sunflower cultivars, Jaeraejongja (height, 1.6–1.8 m; standard type), Jaeraejong (height, 1.0–1.6 m; semi-dwarf type), Jaeraejong1 (height, 0.5–1.0 m; dwarf type), Jaeraejong2 (height, <0.5 m; extreme dwarf type), and Jaeraejong3 (height, <0.5 m; extreme dwarf type) [36], were purchased from Danong Co. (Namyangju, Korea). The physical characteristics of their seeds, including length, width, thickness, and weight, are presented in Table 1.

Table 1. Physical characteristics of five sunflower cultivar seeds.

Cultivars	Seeds				Seed Category ¹
	Length (mm)	Width (mm)	Thickness (mm)	Weight (mg)	
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	
Jaeraejongja	12.3 ± 0.6 ^a	6.0 ± 0.9 ^a	3.9 ± 0.3 ^a	84.5 ± 18.3 ^a	Large
Jaeraejong	9.5 ± 0.1 ^b	4.4 ± 0.6 ^b	2.6 ± 0.4 ^c	40.8 ± 10.9 ^c	Medium
Jaeraejong1	7.9 ± 0.9 ^d	4.3 ± 1.0 ^b	3.0 ± 0.6 ^b	38.1 ± 13.0 ^c	Small
Jaeraejong2	9.2 ± 1.1 ^c	4.4 ± 0.8 ^b	3.1 ± 0.5 ^b	49.6 ± 15.9 ^b	Medium
Jaeraejong3	6.6 ± 0.7 ^e	3.4 ± 0.5 ^c	2.4 ± 0.4 ^d	20.6 ± 5.2 ^d	Small

Note: data are means ($n = 5$) ± standard deviations, and each replicate consisted of 20 seeds. *p*-values are based on one-way ANOVA, and values in a column followed by same letters are not significantly different at the 0.05 level using Duncan's test. ¹ Seed samples were classified into three categories according to the method of Gupta and Das [37]: large (length > 10 mm; Jaeraejongja), medium (8 < length < 10 mm; Jaeraejong, Jaeraejong2), and small (length < 8 mm; Jaeraejong1, Jaeraejong3).

2.2. Seed Germination Test

According to the International Rules for Seed Testing [38], fifty seeds from each cultivar were placed into a 125 × 125 mm Petri dish (SPL Life Sciences Co., Ltd., Pocheon, Korea) on Whatman filter paper moistened with 20 mL of distilled water and randomly assigned to eight replications (Figure 3). The seeds were then incubated in a growth chamber (Sanyo MLR-352H; Panasonic Healthcare Co., Ltd., Oizumi, Japan) under two conditions: (i) a constant temperature of 25 °C with darkness and (ii) alternating temperatures of 30/20 °C under a 16 h light/8 h dark cycle. Germinated seeds were counted and removed daily for up to 7 d. Seeds were considered to have germinated when their radical length reached more than 2 mm. Germination was calculated as the percentage of seeds that germinated out of the total number of seeds.

After 7 d of incubation, a tetrazolium (TZ) test was conducted on intact non-germinated seeds to check their viability [37]. The number of viable and non-viable seeds was counted, and the proportion of germinated, dormant, and dead seeds for each cultivar was scored by combining the results of the incubation test and the TZ test.

2.3. Seed Burial Experiment

Field experiments were conducted at a confined field located at the NIE, Seocheon-gun, South Korea (36°01'43.0" N, 126°43'23.7" E; elevation: 20 m), based on the modified method of Alexander and Schrag. [35] (Figure 3). Two sunflower cultivars, Jaeraejongja and Jaeraejong3, were selected for this study. Three fully randomized blocks were set up, and 96 plots (size: 50 cm × 50 cm) were established within each block in April 2020. Fifty seeds mixed into 50 cm³ of sterilized sand were enclosed in a seed bag (12 cm × 16 cm) made of nylon mesh (size: 0.3 mm) and secured using a cord. These bags were buried in the center of a plot at depths of 0, 2, 5, 10, 15, and 30 cm. During the experiment period, the plots were left undisturbed.

Seed bags were retrieved 1, 2, 3, 4, 6, 8, and 16 weeks after burial. The mixture of seeds and sand was placed on a 2-mm sieve to separate the sand and seeds, and the filtered seeds were washed with distilled water. The number of germinated and dead seeds was counted, and germination was calculated as the percentage of germinated seeds out of the total number of seeds.

Intact ungerminated seeds were then incubated in a growth chamber (Sanyo MLR-352H) at a constant temperature of 25 °C under darkness for 7 d, as described above. After 7 d, the seeds that germinated were counted, and a TZ test was performed for the remaining hard seeds. For seed burial experiment, the proportion of germinated seeds were scored by dividing the initial number of seeds germinated after burial and that germinated after the 7-day incubation test.

2.4. Soil Physico-Chemical Properties

The daily soil temperature and moisture levels in the field were recorded at 10, 20, 30, 40, and 50 cm soil depths over 16 weeks with HOBO Temp data loggers (U30-NRC-10-S100; Onset Computer Co., Pocasset, MA, USA). The daily air temperature and precipitation were taken from the daily weather report for the Gunsan Meteorological Station (36°00'19.1" N, 126°45'40.9" E; 23.2 m above sea level) [39]. Climate data for the period between 1 January 2010 and 31 December 2019 in South Korea were obtained from the Korea Meteorological Administration [40].

The soil chemical and physical characteristics were analyzed according to the National Institute of Agricultural Science and Technology (NIAST) soil and plant analysis methods [41]. Samples were collected from 5, 15, and 30 cm soil depths in the experimental field. The soil moisture content was determined based on the difference in weight between the initial samples and after drying the samples at 105 °C until a constant weight was reached. The soil pH and electrical conductivity (EC) were determined using a pH meter (Starter 3100, Ohaus, NJ, USA) and EC meter (Orion Star A329, Thermo Scientific, Waltham, MA, USA), respectively. The organic matter content was estimated based on the weight lost after the samples were kept in a crucible at 450 °C for 45 min. The total nitrogen content was determined using the Kjeldahl method, and the available phosphorus content was determined via the Lancaster method using a spectrophotometer (UV-1800; Simadzu, Japan). The cation exchange capacity (CEC) was calculated after saturating the samples with 1 N NH₄OAc (pH 7.0), and exchangeable cations (Ca²⁺, K⁺, Mg²⁺, and Na⁺) were measured via inductively coupled plasma optical-emission spectroscopy (5100; Agilent, Santa Clara, CA, USA). The soil texture was determined using the hydrometer method.

2.5. Statistical Analysis

All the analyses were performed using STATISTICA (version 8.0; StatSoft Inc., Tulsa, OK, USA) or SAS Studio (version 3.8; SAS Institute Inc., Cary, NC, USA). Data were analyzed via analysis of variance (ANOVA) and tested at a 5% significance level. If the ANOVA showed significant differences between

means, Duncan's multiple comparison test was used to determine their differences. To analyze the effects of sunflower cultivar, duration of burial, depth of burial, and their interactions on germination rates, ANOVA was performed using a general linear model (GLM) module. The regression analysis function of Sigmaplot (version 12.5; Systat Software Inc., San Jose, CA, USA) was used to obtain best-fit curves.

3. Results

3.1. Seed Germination at Different Controlled Temperatures

The sunflower seed germination rates differed significantly according to the cultivar and temperature (Figure 4). Overall, the cumulative germination was high in Jaeraejong2 and Jaeraejongja, regardless of the temperature conditions. Days to reach almost complete germination varied by cultivars under both temperature conditions. Under constant temperatures, the total germination during the test period was 84.0%, 78.5%, and 74.0% for Jaeraejong2, Jaeraejong3, and Jaeraejongja, respectively (Figure 4a,c). However, the total germination for Jaeraejong and Jaeraejong1 was 26.8% and 29.0%, respectively, under constant temperatures. Under alternating temperatures, the total germination for Jaeraejong2 and Jaeraejongja was 85.8% and 80.3%, respectively (Figure 4b,d). However, that of Jaeraejong3, Jaeraejong1, and Jaeraejong was 50.8%, 36.5%, and 22.3%, respectively. Differences in germination rates under constant and alternating temperatures were found for Jaeraejong3 ($p < 0.05$). The overall germination rate of Jaeraejong3 seeds was 35.4% lower under alternating temperatures than under constant temperatures.

The seed viability determined by the TZ test also varied among the sunflower cultivars and temperature conditions (Figure 4e,f). Under a constant temperature, the highest proportion of dormant seeds was observed for the Jaeraejongja cultivar, while under alternating temperatures, Jaeraejong3 seeds demonstrated the highest rate of dormancy. The proportion of dormant Jaeraejongja and Jaeraejong3 seeds was 16.5% and 11.5%, respectively, under a constant temperature, whereas that for Jaeraejong3 and Jaeraejong1 it was 18.0% and 12.8%, respectively, under alternating temperatures. Differences in the proportion of dormant seeds under constant and alternating temperatures were found for Jaeraejong2 and Jaeraejong3 ($p < 0.05$). The proportion of dormant Jaeraejong2 seeds was reduced by 62.9% under alternating temperatures compared to that under constant temperatures, whereas for Jaeraejong3 the proportion of dormant seeds increased by 56.5% under alternating temperatures.

3.2. Viability of Seeds Buried at Various Soil Depths

In our laboratory experiment, the germination rate of Jaeraejongja did not differ under constant and alternating temperatures, whereas that of Jaeraejong3 differed significantly under the two temperature regimes. In addition, the seed size and weight and plant height clearly differed between Jaeraejongja and Jaeraejong3. Therefore, based on these differences in the seed physical properties, plant phenotypic traits, and germination characters, we selected the Jaeraejongja and Jaeraejong3 cultivars to study the viability of seeds buried in the soil. In South Korea, the mean annual temperature and precipitation for the period of 2010–2019 were 13.0 °C and 1264.4 mm, respectively (Figure 5a,b) [40]. During the test period, the mean daily air temperature and precipitation were 11.2–26.1 °C and 0–61.8 mm, respectively (Figure 5c,d) [39]. In addition, the mean daily soil temperature was 14.7–27.7 °C, and daily soil moisture was 14.8–45.4% at a depth of 10 cm (Figure 5e,f). Soil temperature decreased as the soil depth increased, and the difference in temperature between soil depths of 10 cm and 30 cm was approximately 2.9 °C. Soil moisture increased on days with precipitation, and elevated soil moisture was maintained longer as the soil depth increased. Throughout the test period, the differences in daily soil temperature and moisture were statistically significant with varying soil depths, though the soil moisture content at nine and twelve weeks after burial did not differ significantly. The soil physio-chemical properties did not statistically differ between soil depths of 5, 15, and 30 cm (Table 2).

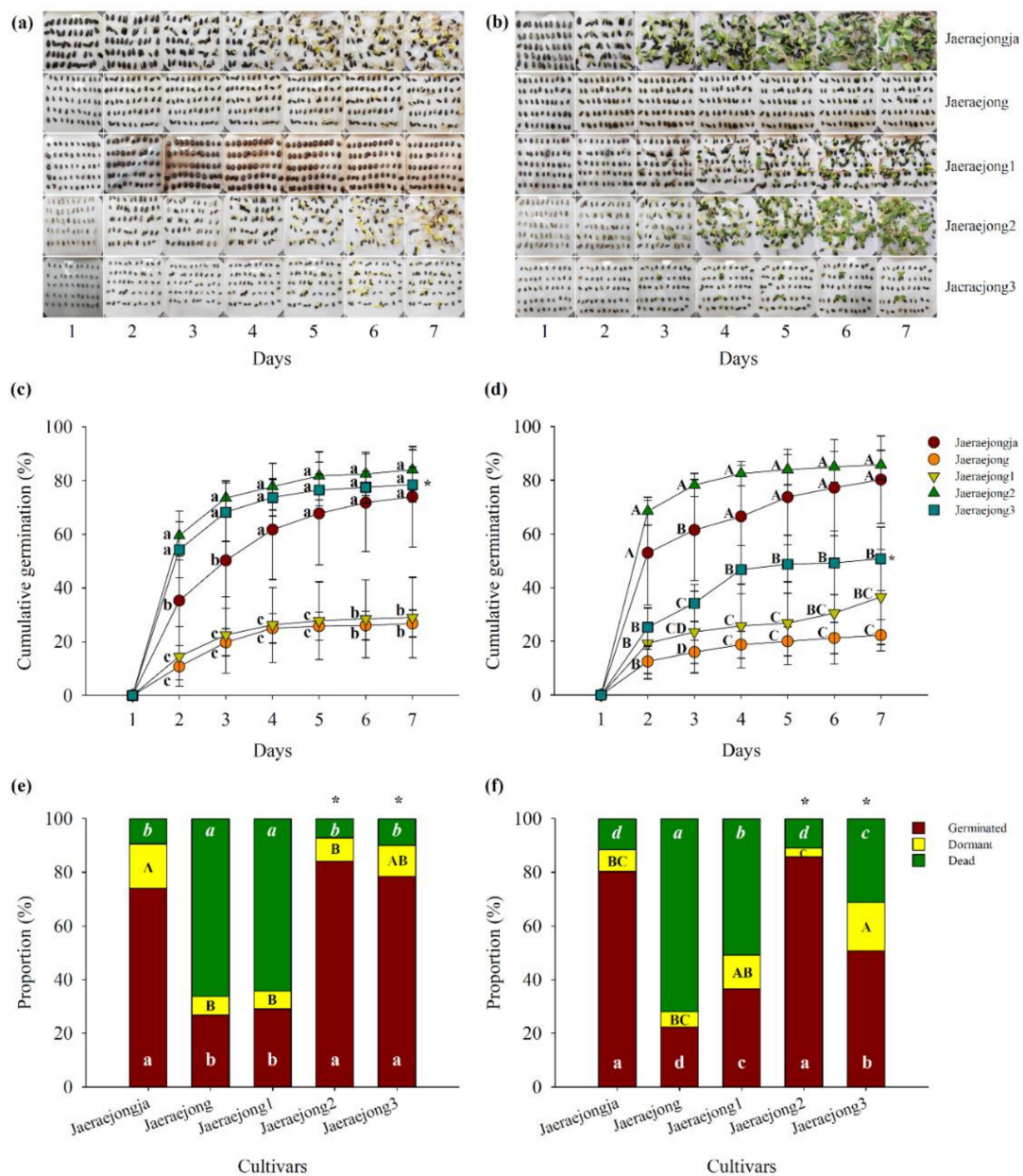


Figure 4. Changes in the germination of seeds of five sunflower cultivars under (a,c) a constant temperature of 25 °C and (b,d) alternating temperatures of 30/20 °C over 7 d. The proportion of germinated, dormant, and dead seeds of the five sunflower cultivars under (e) a constant temperature of 25 °C and (f) alternating temperatures of 30/20 °C. Data are presented as means ($n = 8$) \pm standard deviations. Different letters indicate significant differences among cultivars at the $p < 0.05$ level by one-way ANOVA. Asterisks indicate significant differences at the $p < 0.05$ level between outcomes under constant and alternating temperature.

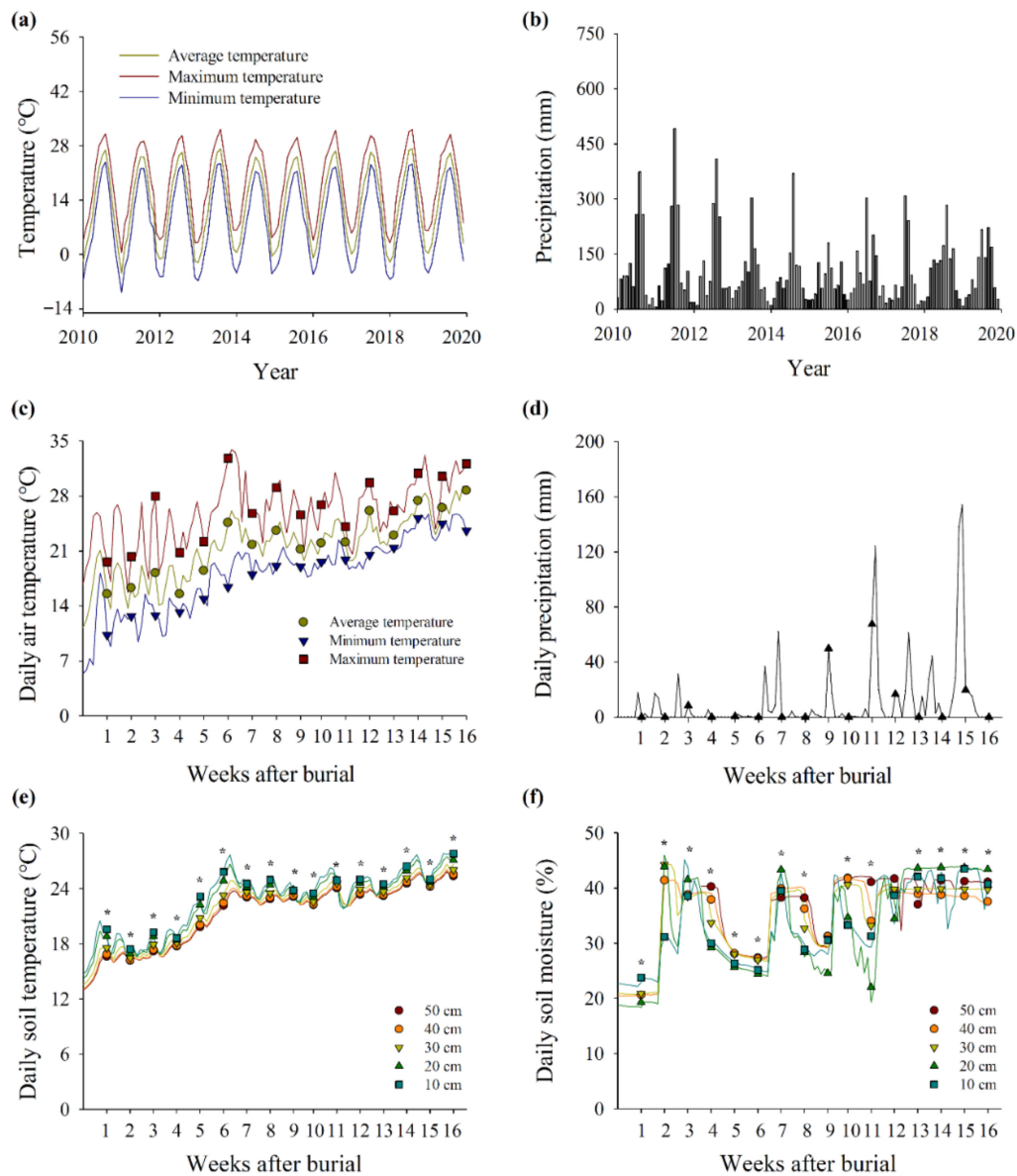


Figure 5. (a) Long-term temperature and (b) precipitation data between 1 January 2010 and 31 December 2019 in South Korea [40]. Variations in the (c) daily air temperature and (d) precipitation [39]; (e) daily mean soil temperature and (f) moisture in the field trial site during the burial period from April to August 2020. The soil temperature and moisture were measured at the depths of 10, 20, 30, 40, and 50 cm. Asterisks indicate significant differences among soil depths at the $p < 0.05$ level by one-way ANOVA.

Table 2. Soil physicochemical properties at different soil depths.

Soil Characteristics	<i>p</i> -Value	Soil Depth (cm)		
		5	15	30
Moisture (%)	0.285	12.2 ± 0.3	13.3 ± 2.9	10.8 ± 0.4
pH	0.086	6.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1
EC (ds m ⁻¹)	0.706	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Organic matter (%)	0.399	4.4 ± 0.7	4.0 ± 0.2	4.8 ± 0.8
Total N (mg kg ⁻¹)	0.330	580.9 ± 204.6	424.9 ± 185.3	363.4 ± 91.0
P ₂ O ₅ (mg kg ⁻¹)	0.677	19.0 ± 7.6	23.0 ± 7.7	23.6 ± 4.0
CEC (cmol ⁺ kg ⁻¹)	0.479	13.7 ± 1.6	12.4 ± 0.8	12.7 ± 1.3
Exchangeable Ca (cmol ⁺ kg ⁻¹)	0.748	6.7 ± 1.0	6.4 ± 0.4	6.3 ± 0.4
Exchangeable K (cmol ⁺ kg ⁻¹)	0.313	0.4 ± 0.3	0.2 ± 0.0	0.2 ± 0.0
Exchangeable Mg (cmol ⁺ kg ⁻¹)	0.978	4.5 ± 0.8	4.4 ± 0.4	4.4 ± 0.3
Exchangeable Na (cmol ⁺ kg ⁻¹)	0.784	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Sand (%)	0.501	56.3 ± 3.1	57.8 ± 2.1	55.5 ± 1.3
Silt (%)	0.604	26.3 ± 2.0	24.6 ± 3.0	25.9 ± 0.8
Clay (%)	0.397	17.3 ± 1.2	17.7 ± 1.5	18.7 ± 0.6
Soil texture		Sandy Loam	Sandy Loam	Sandy Loam

Note: data are means ($n = 3$) ± standard deviations. *p*-values are based on one-way ANOVA tests. EC: electrical conductivity; CEC: cation exchange capacity.

The germination and viability of seeds buried in soil were significantly affected by burial depth ($p < 0.001$), duration ($p < 0.001$), and cultivar ($p < 0.001$) (Table 3). In addition, significant interactions were found between duration × cultivar ($p < 0.001$), duration × depth ($p < 0.001$), cultivar × depth ($p < 0.001$), and duration × cultivar × depth ($p < 0.001$).

Table 3. General linear model (GLM) results for seed germination and viability obtained for two sunflower cultivars at six burial depths and seven burial durations.

Source	SS ¹	DF ²	MS ³	F ⁴	<i>p</i> ⁵
Duration of burial	103,582.9	6	17263.8	3061.6	<0.001
Cultivar	1676.6	1	1676.6	297.3	<0.001
Depth of burial	3993.4	5	798.7	141.6	<0.001
Duration × cultivar	4293.7	6	715.6	126.9	<0.001
Duration × depth	11,482.1	30	382.7	67.8	<0.001
Cultivar × depth	446.5	5	89.3	15.8	<0.001
Duration × cultivar × depth	5928.5	30	197.6	35.0	<0.001
Error	947.3	168	5.6	–	–

Note: ¹ SS, Sum of Squares; ² DF, Degrees of Freedom; ³ MS, Mean Square; ⁴ F, F-statistic; ⁵ *p*, *p*-value.

Jaeraejongja seeds decayed within eight weeks and Jaeraejong3 seeds decayed within four weeks at burial depths of 2–30 cm (Figure 6). However, some Jaeraejongja and Jaeraejong3 seeds buried at 0 cm germinated even after eight weeks. Except at the 15 and 30 cm burial depths, Jaeraejongja seeds maintained a relatively high viability until four weeks after burial, and Jaeraejong3 seeds showed a relatively high viability for three weeks. Differences in germination rates between Jaeraejongja and Jaeraejong3 were found after three and four weeks of burial.

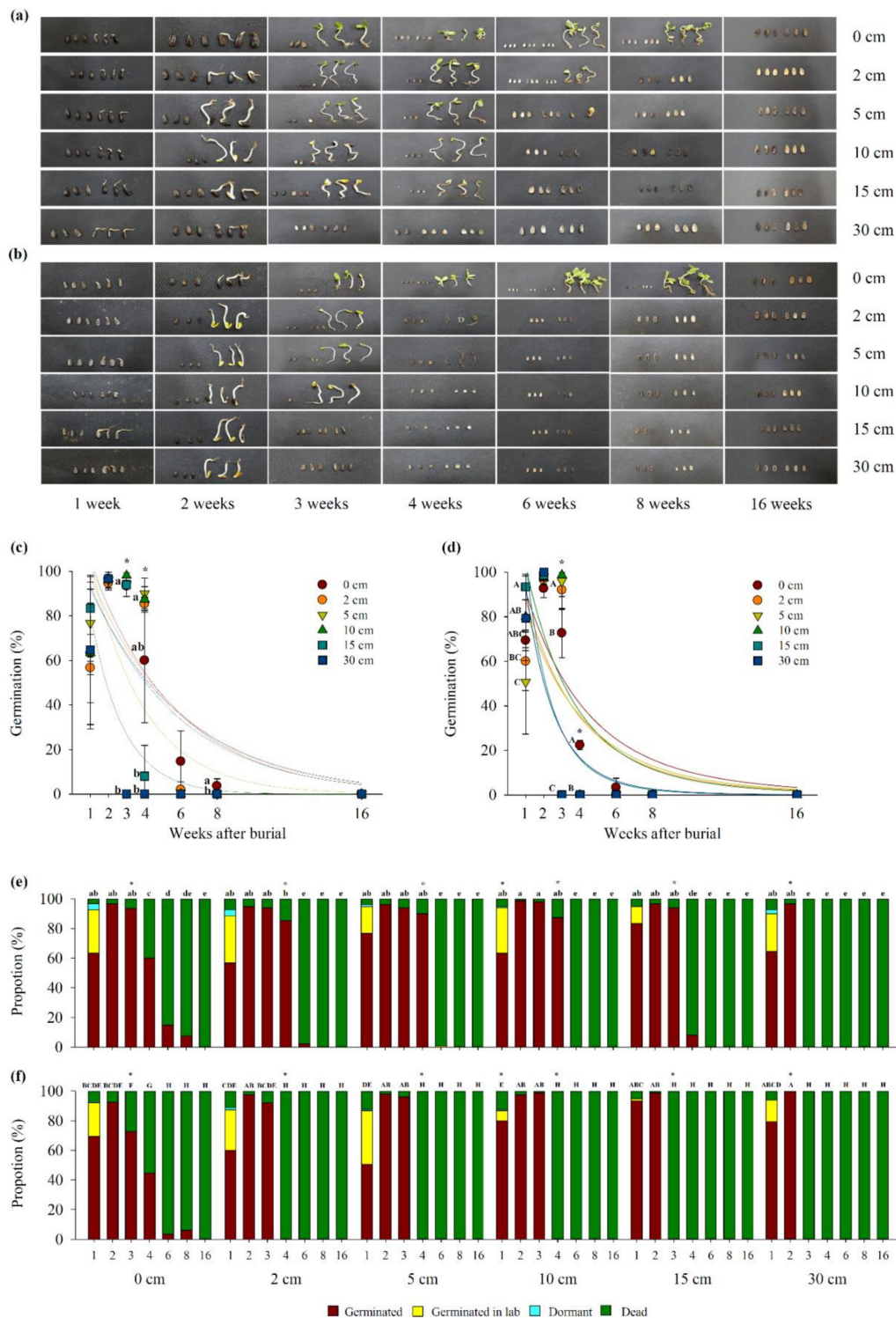


Figure 6. Changes in the germination of seeds of (a) *Jaeraejongja* and (b) *Jaeraejong3* and the exponential decay curves of (c) *Jaeraejongja* and (d) *Jaeraejong3* for seeds that were viable for 1, 2, 3, 4, 6, 8, and 16 weeks after burial at soil depths of 0, 2, 5, 10, 15, and 30 cm. The proportion of germinated, dormant, and dead (e) *Jaeraejongja* and (f) *Jaeraejong3* seeds buried for 1, 2, 3, 4, 6, 8, and 16 weeks at soil depths of 0, 2, 5, 10, 15, and 30 cm. Data are presented as means ($n = 3$) \pm standard deviations. Different letters indicate significant differences at the $p < 0.05$ level among (c,d) burial depths by one-way ANOVA and (e,f) the interaction of duration \times depth by two-way ANOVA. Asterisks indicate significant differences at the $p < 0.05$ level between the *Jaeraejongja* and *Jaeraejong3* cultivars.

For Jaeraejongja seeds, no significant differences between viability at different burial depths were observed at one and two weeks after burial (Figure 6a,c). Germination after one week of burial varied from 56.7% to 83.3% depending on burial depth, but after two weeks of burial it exceeded 94.7% in all burial depths. After three weeks of burial, clear differences were observed based on burial depth. Specifically, no seeds germinated at depths of 30 cm, while 93.3–98.0% of those buried at other depths germinated after three weeks of burial. After four weeks of burial, the seed germination rapidly declined by 8.0% at a depth of 15 cm, while 60.0–90.0% germination rates were recorded at depths of 0–10 cm. Germinated seeds were not found at 15 and 10 cm depths after six weeks of burial. After eight weeks of burial, the germination rate was only 7.3% at a depth of 0 cm, and no seeds germinated at the other depths.

For the Jaeraejong3 seeds, the highest total germination rate was found at a depth of 15 cm (93.3%), while the lowest was observed at a depth of 5 cm (50.7%) after one week of burial (Figure 6b,d). After two weeks of burial, germination was more than 92.7% at all burial depths, similar to the results for Jaeraejongja. After three or more weeks of burial, germinated seeds were not observed at depths of 15 cm and 30 cm, while germination rates of 72.7–98.7% were recorded at depths of 0–10 cm. The germination rate was 44.7% at a depth of 0 cm, but no seeds germinated at 2–30 cm soil depths after four weeks of burial. The germination at a depth of 0 cm was significantly reduced to 3.3% after six weeks of burial.

Of the retrieved seeds, intact ungerminated seeds were found for both the Jaeraejongja and Jaeraejong3 cultivars up to two weeks after burial (Figure 6e,f). However, such seeds were not detected at any soil depth after three weeks of burial. The proportion of dormant seeds were 0.0–4.0%, and they were only observed within one week of burial. The highest proportion of germinated and dormant seeds was found at a depth of 10 cm after two and three weeks of burial for Jaeraejongja, while these proportions were highest after two weeks of burial at a 30 cm depth for Jaeraejong3. Differences in the proportion of germinated and dormant seeds between Jaeraejongja and Jaeraejong3 were found at depths of 0 cm and 15 cm after three weeks of burial, at depths of 2 cm and 5 cm after four weeks of burial, at depths of 10 cm after one and four weeks of burial, and at depths of 30 cm after two weeks of burial.

4. Discussion

Seed germination and dormancy dynamics are involved in population persistence, intra- and interspecific competition, and plant fitness [42]. These factors are largely affected by temperature and are species-dependent [43]. Non-dormant sunflower seeds are reported to germinate well under a broad range of temperatures (5–40 °C), with the optimum temperature being 25 °C [44]. We found that the germination rates of five sunflower cultivars ranged from 26.8% to 84.0% at constant temperatures of 25 °C and from 22.3% to 85.8% at alternating temperatures of 30/20 °C in the laboratory experiment. In addition, the proportion of dormant seeds differed according to cultivar under both temperature conditions. In particular, the overall germination rate and the proportion of dormant Jaeraejong3 seeds were 35.4% lower and 56.5% greater, respectively, under alternating temperatures than constant temperatures, implying that seed viability could be strongly affected by temperature conditions. For weeds, seed size has been reported to contribute to differences in seed germination and persistence in response to variety of environmental factors [45]. Larger seeds were more likely to produce seedlings in shaded conditions, and smaller seeds were more sensitive to various environmental conditions [46,47]. Therefore, the increased proportion of dormant seeds and decreased proportion of germinated seeds under alternating temperature conditions for the Jaeraejong3 cultivar, which featured the smallest seeds used in the present study, may be attributable to seed size. Further, Khalifa et al. [48] noted that sunflower hybrids could successfully adapt to a wide range of climates, because genetic variations were associated with different germination temperatures. We also found that sunflower seed germination and dormancy are highly cultivar-dependent, suggesting that some sunflowers have

the potential to survive in diverse habitats and environmental conditions and could be appropriate for use in remediation projects.

Soil seed banks play an important role in stabilizing and ensuring species survival; they also have the potential to favor competitive invaders within the population dynamics of many ecosystems [49]. However, seed banks are only functional if the seeds are deposited in a completely viable state and are ready to germinate in a timely manner [50]. Traba et al. [51] stated that studies on the relationship between burial depth and seed emergence offer important insight into the actual functionality of a soil bank. In many plant species, the seed germination and seedling emergence decrease when the burial depth increases [52,53]. In contrast, canola seeds buried at a deeper soil depth could persist longer than those buried at shallow depths in fields [54,55].

In this study, we examined the viability of seeds from two sunflower cultivars buried at various soil depths (0, 2, 5, 10, 15, and 30 cm) with different degrees of temperature and moisture in a field. The results indicated that the germination among Jaeraejongja and Jaeraejong3 seeds buried at depths of 2–10 cm significantly declined after six and four weeks of burial, respectively, whereas the germination among seeds buried at a depth of 15 cm was reduced after four and three weeks of burial, respectively. At a depth of 30 cm, the germination of both cultivars decreased after two weeks of burial. Benvenuti and Macchia [56] suggested that reduced seed germination under excessive soil depth may result from secondary dormancy, an ecological adaptation mechanism that may serve to prevent germination under unsuitable conditions for seed survival and persistence. The absence of oxygen or light, fluctuations in soil temperature and moisture, and the presence of volatile or allelopathic inhibitors are known to regulate the induction of secondary dormancy in buried seeds [57,58]. However, in the present study, dormant seeds were only detected in 0–4% of seeds up to one week after burial, after which all the ungerminated seeds decayed.

Chantre et al. [59] demonstrated that seed decay increases as burial depth and period increase, and decay was further elevated by complementary water supply. Seed decay is highly affected by the activity of soil microorganisms influenced by the soil environment, including the soil temperature, moisture, and physicochemical properties [60,61]. Several studies have shown that the impact of fungicidal treatments on the survival of buried seeds is more pronounced in relatively wetter conditions [62,63]. In this study, the sunflower seed decay was markedly elevated as the burial depth and period increased. In particular, soil moisture significantly increased at two weeks after burial in conjunction with an increased decay rate of the seeds buried at a depth of 30 cm. Even at other soil depths, most of the buried seeds decayed within eight weeks. These results indicate that sunflower seeds buried in spring could lose their viability over a short period without the induction of secondary dormancy, suggesting that these seeds might not be successfully established in the ecosystem.

On the other hand, the appearance of seedlings from buried seeds can positively correlate with larger seed sizes [64]. Although seed germination decreases with increased burial depth, the germination rate of large-sized seeds is higher than that of small seeds [65]. Furthermore, seedling survival at different burial depths has been noted to be greatly enhanced as the seed weight increases [66]. Several studies have revealed that the differences in germination and seedling emergence are due to seed size, derived from differences in energy reserves [47,67]. In our results, the seed decay at burial depths of 2–15 cm was more rapid for the Jaeraejong3 seeds than for the Jaeraejongja in an inverse proportion to the seed size and mass, reflecting a close relationship between seed viability and seed size.

5. Conclusions

Based on the laboratory and field results, we concluded that the germination of sunflower seeds vary strongly with the cultivars and environmental conditions. The unintentional release of transgenic seeds during their transportation and utilization could result in the formation of soil seed banks in natural environments and the subsequent proliferation of volunteer plants. In this study, we compared the germination of seeds of five sunflower cultivars at different temperatures. Furthermore, the viability of seeds buried at various soil depths (ranging from 0 to 30 cm) in the spring sowing season was

examined under undisturbed field conditions over sixteen weeks. The seed germination substantially varied with the cultivar and temperature. Field trials showed that most sunflower seeds lost their viability within eight weeks rather than entering secondary dormancy, although affected by the burial depth. In addition, smaller seeds buried at a depth of 2–15 cm were found to decay faster than larger seeds. Further studies, including those on the overwintering viability of seeds buried during the autumn harvest season and the effects of different soils, locations, and climate conditions, will provide valuable insights into the potential invasiveness of sunflower seeds in a range of environments. Our results suggest that each transgenic plant requires individual assessment procedures depending on the specific environmental conditions. The seeds spilled on the soil surface in spring should be meticulously handled and managed to prevent their unintended effects on the ecosystem. Our study could serve as a useful reference for future studies seeking to develop appropriate research tools to determine factors affecting the invasiveness of transgenic plants used for environmental remediation.

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