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RESEARCH

Evaluating the risk of living modified organisms on the natural ecosystem by analyzing the overwintering potential of four major species imported into Korea

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Background: Living modified organisms (LMOs) imported into Korea may pose various risks to the domestic natural ecosystem, depending on their seed survival capacity and proliferation potential. In this study, we investigated seed germination and dormancy rates under controlled low temperatures for 12 weeks. In addition, we assessed the overwintering potential of seeds buried at 10 cm soil depth over 48 weeks using non-transgenic seeds of four major LMOs imported into Korea.
Results: *Brassica napus* L. seeds exhibited superior germination and dormancy rates compared to *Glycine max* (L.) Merr., *Zea mays* L., and *Gossypium hirsutum* L. seeds when subjected to incubation at low temperatures (–5°C, –1°C, and 5°C). Seed germination was exclusively recorded for *B. napus* seeds in the field, which reflects the winter environment, for up to 8 weeks after burial. Seeds of both *B. napus* and *Z. mays* remained dormant in the soil for up to 4 weeks after burial; however, *G. max* and *G. hirsutum* seeds did not remain dormant. Germination rates were higher when the seeds were placed in seed bags and buried, whereas dormancy rates were higher when the seeds were buried without seed bags.
Conclusions: These results suggest that *B. napus* could have a higher invasive potential than other plant species, which may affect its survival and spread, ultimately posing a greater threat to the ecosystem. This study provides valuable insights into the immediate need for establishing risk assessments and safety management measures for LMOs (specifically *B. napus*).

Keywords: invasiveness, living modified organism, natural ecosystem, overwintering, risk assessment, transgenic plant

Introduction

Approximately 7–12 million tons of living modified organisms (LMOs) are imported annually into Korea, most of which are LM soybean (*Glycine max* (L.) Merr.), corn (*Zea mays* L.), canola (*Brassica napus* L.), and cotton (*Gossypium hirsutum* L.). These imports are mostly used for feed and food purposes (Korea Biosafety Clearing House [KBCH] 2024). Every year, the Ministry of Environment conducts a risk consultation review to assess the potential impacts of these newly introduced LMOs on natural ecosystems (LMO Environmental Safety Center [LESC] 2024). However, the threat to domestic biodiversity is increasing because of the regular detection of LM seeds and volunteers in natural ecosystems nearby transportation routes, festival sites, and animal feed production factories (Kim et al. 2020c; Lim et al. 2023). In particular, the importance of LMO safety management is underscored by instances where LM seeds are released into the natural environment and their genes might be transferred to wild relatives (Lee et al. 2019).

A comprehensive review of the impacts of LMOs on natural ecosystems considers the risks posed by the host organisms, the risks associated with introduced genes, and the impact of LMOs on the surrounding environment (Fig. 1; National Institute of Environmental Research [NIER] 2011). When reviewing the risk of newly introduced LMOs, the overwintering potential is one of the critical factors in evaluating the risk to natural ecosystems. It is used to determine whether LM seeds can survive and reproduce in

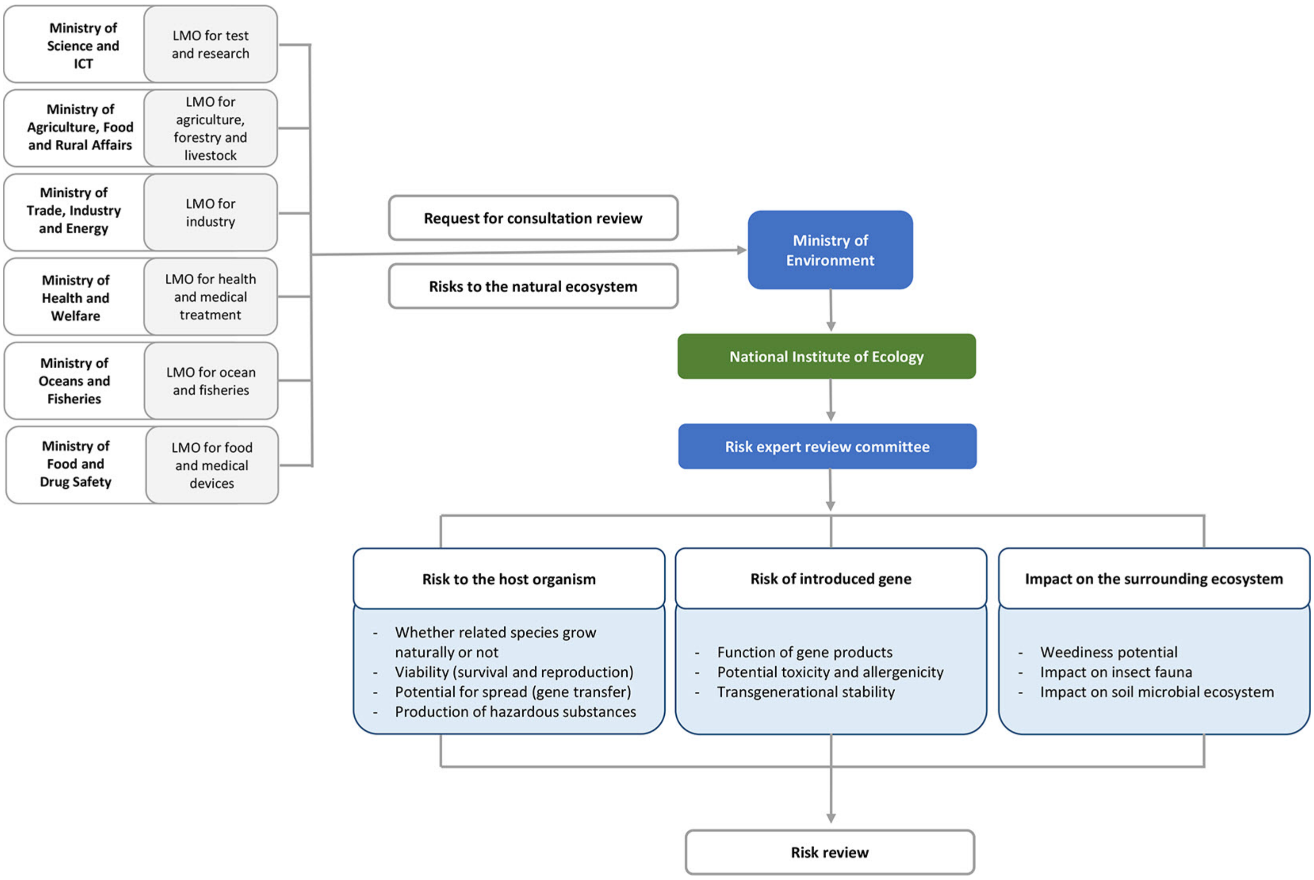

Fig. 1 Risk review system of living modified organisms on the natural ecosystem in South Korea. LMO: living modified organism.

harsh low-temperature environments, which may result in their invasive behavior in the ecosystem (EFSA Panel on Genetically Modified Organisms 2010; Han et al. 2023).

The plants that were tested in various overwintering studies exhibited variations in the number of buried seeds, burial depth, burial period, and burial area. Ko et al. (2022) examined the overwintering ability of eight cruciferous crops, namely, *Brassica juncea*, *B. napus*, *B. oleracea* var. *botrytis*, *B. oleracea* var. *capitata*, *B. oleracea* var. *italica*, *B. rapa* var. *rapa*, *B. rapa* var. *glabra*, and *Raphanus sativus*. These crops were buried at depths of 2 cm and 15 cm in a 25 × 25 cm area, and the investigation lasted for 16 months. Zhang et al. (2019) examined the overwintering ability of weedy rice (*Oryza sativa* f. *spontanea*) seeds. They buried 1,000–3,000 seeds in a 2 × 2 m area at depths of 0–5, 5–10, and 10–15 cm. The investigation lasted for 6 months.

More than 98% of the LMOs imported into Korea from 2009 to 2023 were *G. max* and *Z. mays*, whereas 85.4% of the LMOs detected in the surrounding natural ecosystem such as stockbreeding feed factories, roadsides, ports, and festival planting areas were *B. napus* and *G. hirsutum* (KBCH 2024; Lee et al. 2023). This suggests that the survival and proliferation potential of each LM plant species differs in the natural ecosystem of Korea; therefore, the assessment and management of the risks and safety associated with each plant species needs to be adjusted accordingly.

In this study, we aimed to evaluate the natural ecosystem risk of the four main LM plants imported into Korea and present safety management measures. This was achieved by conducting an overwintering ability test and evaluating the dispersal potential of *G. max*, *Z. mays*, *B. napus*, and *G. hirsutum* seeds as a case study.

Materials and Methods

Plant materials

The plant species utilized in the experiment were *G. max* (cultivar 'Daewon'), *Z. mays* (cultivar 'Shinhwangok'), *B. napus* (cultivar 'Jungmo 7001'), and *G. hirsutum* (cultivar unknown), all of which are extensively cultivated in Korea. The National Institute of Crop Science supplied seeds of *G. max*, *Z. mays*, and *B. napus* for research purposes. These seeds were produced in a seed production field and harvested in the same year and at the same location. The physical characteristics of the seeds for each plant species are presented in Table 1.

Seed germination at low temperatures

Based on the overwintering potential of seeds, the experiment was conducted under two conditions: a controlled environment and a field representing a natural environ-

Table 1 Comparison of the physical characteristics of four different plant seeds

	p-value	*Brassica napus* L.	*Gossypium hirsutum* L.	*Glycine max* (L.) Merr.	*Zea mays* L.
Length (cm)	< 0.001	0.20 ± 0.03^{d}	0.83 ± 0.08^{b}	0.68 ± 0.05^{c}	1.03 ± 0.16^{a}
Width (cm)	< 0.001	0.18 ± 0.03^{c}	0.43 ± 0.05^{b}	0.63 ± 0.08^{a}	0.61 ± 0.10^{a}
Thickness (cm)	< 0.001	0.19 ± 0.02^{d}	0.49 ± 0.05^{c}	0.69 ± 0.05^{b}	0.81 ± 0.10^{a}
Weight (g)	< 0.001	0.24 ± 0.02^{c}	5.10 ± 1.32^{b}	17.87 ± 2.05^{a}	18.69 ± 1.33^{a}

Data are presented as the mean ± standard deviation ($n = 20$), excluding weight. Seed weights of four plant species were calculated as the average weight of 100 seed batches in four replicates. *p*-values are based on one-way analysis of variance, and the values in a column followed by the same letters are not significantly different at the 0.05 level, as determined using Duncan's test.

ment (Han et al. 2023). To verify the impact of low temperature on the germination rate, seeds were sown in Petri dishes (125 × 125 mm; SPL Life Sciences Co., Ltd., Pocheon, Korea) containing filter paper (Whatman filter paper). The dishes were then placed in incubators set at –5°C, –1°C, and 5°C. Germination and dormancy rates were investigated for 1, 2, 4, 8, and 12 weeks in accordance with the International Rules for Seed Testing (International Seed Testing Association [ISTA] 2010). After placing 20 seeds in each Petri dish, 20 mL distilled water was added. When the radicle length of the seed reached or exceeded 2 mm, it was considered germinated, and the initial germination rate was calculated. The remaining seeds were germinated at 25°C for 7 days, and the final germination rate was calculated. The tetrazolium (TZ) test was performed on intact seeds that failed to germinate after 7 days of incubation to calculate the dormancy rate. The normal germination rates of the seeds were confirmed with data obtained from culturing 20 seeds of each plant species in four replicates at 25°C for 7 days.

Seed burial experiment

The overwintering experiment in the field representing natural environmental conditions was conducted in a confined field (36°01′43.0″ N, 126°43′23.7″ E; elevation: 20 m above sea level [a.s.l.]) at the National Institute of Ecology in Seocheon-gun, South Korea, in accordance with our previous research (Han et al. 2023; Nam and Han 2020). The germination rates of seeds buried in seed bags and those buried without them were investigated to evaluate the overwintering potential of LMO seeds when intentionally or unintentionally released into the environment. Seeds were placed in randomized blocks with three replicates and 144 plots in each block. Twenty seeds each, regardless of whether they were in seed bags or not, were buried at a depth of 10 cm in a 50 × 50 cm plot in December 2022. The buried seeds were recovered after 2, 4, 8, 16, 24, and 48 weeks to determine their germination rates. The ungerminated seeds were subjected to a 7-day incubation period at 25°C in a Petri dish containing filter paper to determine their indoor germination rates. The TZ test was used to investigate the dormancy rates of seeds that failed to germinate indoors.

During the experimental period, the soil temperature and moisture were measured at soil depths of 10, 20, 30, 40, and 50 cm using a HOBO instrument (U30-NRC-10-S100; Onset Computer Co., Pocasset, MA, USA) that was installed in the field. Air temperature and humidity data were obtained from the Gunsan Meteorological Observatory (36°00′19.1″ N, 126°45′40.9″ E; elevation: 23.2 m a.s.l.) of the Korea Meteorological Administration (KMA 2024).

Statistical analysis

All statistical analyses were performed using SAS Studio (version 3.8; SAS Institute Inc., Cary, NC, USA). The data were subjected to analysis of variance (ANOVA) at a significance level of 5%. When the ANOVA results indicated a significant difference between the means, Duncan's multiple comparison test was applied to ascertain the difference between means. The effects of plant species, presence of seed bags, low temperature, incubation, burial periods, and their interactions on seed germination and dormancy were examined by ANOVA using a general linear module.

Results

Changes in seed germination and dormancy of four plant species at low temperatures

When the four plant species, *G. max*, *Z. mays*, *B. napus*, and *G. hirsutum*, were cultured at the optimal germination temperature of 25°C, the germination rates were 98.8%, 96.3%, 95.0%, and 71.3%, respectively (data not shown). The germination and dormancy rates of the four plant species cultured at controlled low temperatures (–5°C, –1°C, and 5°C) are shown in Table 2 and Figure 2. The initial and final germination and dormancy rates of the seeds differed significantly depending on the plant species, incubation temperature, and incubation duration (Table 2). In addition, significant interaction effects between species × temperature, species × duration, temperature × duration, and species × temperature × duration were found to affect initial and final germination and dormancy rates.

At low temperatures of –5°C and –1°C, *B. napus* seeds did not germinate; however, at 5°C, the germination rate was 36.7% after 2 weeks of culturing and increased to 83.3% after 4 weeks of culturing (Fig. 2). The final germi-

Table 2 Results of a general linear model for seed germination and dormancy at controlled low temperatures

	Initial germination	Final germination	Dormancy
Species	< 0.001	< 0.001	< 0.001
Incubation temperature	< 0.001	< 0.001	0.021
Incubation duration	< 0.001	< 0.001	0.048
Species × temperature	< 0.001	< 0.001	< 0.001
Species × duration	< 0.001	< 0.001	< 0.001
Temperature × duration	< 0.001	< 0.001	0.005
Species × temperature × duration	< 0.001	< 0.001	0.009

Seeds of four plant species (*Glycine max* (L.) Merr., *Zea mays* L., *Brassica napus* L., and *Gossypium hirsutum* L.) were cultivated at three different temperatures (–5°C, –1°C, and 5°C) for 12 weeks. Data are presented as the mean ± standard deviation ($n = 3$); p-values were calculated using a general linear model-based method.

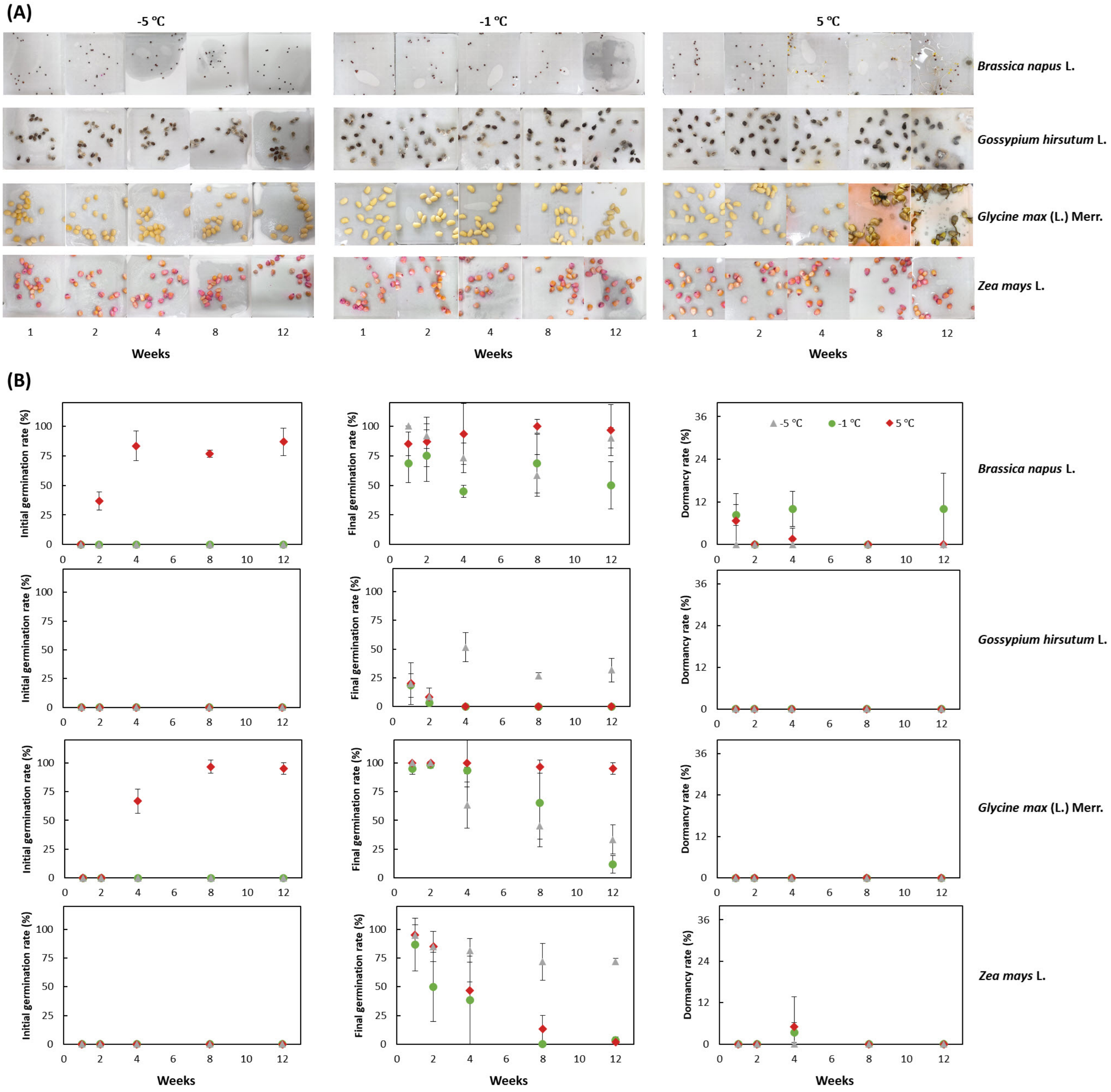

Fig. 2 Changes in the germination of seeds incubated at low temperatures (A). Initial and final germination and dormancy rates of four plant species seeds (B). Seeds of four plant species were cultured in various low-temperature regimes (–5°C, –1°C, and 5°C) for different durations (1, 2, 4, 8, and 12 weeks). The initial germination rate was equal to the germination rate at low temperature, and the final germination rate was calculated by adding the initial germination rate to the germination rate obtained by transferring ungerminated seeds at low temperature to 25°C. The data are presented as the mean ± standard deviation ($n = 3$). The temperatures are color-coded (gray, –5°C; light green, –1°C; red, 5°C).

nation rates of the seeds cultured at −1°C, −5°C, and 5°C were 45.0%–75.0%, 58.3%–100%, and 85.0%–100%, respectively, after being transferred to the optimal germination temperature of 25°C for 7 days. Dormancy was confirmed only in the seeds cultured at −1°C and 5°C, with dormancy rates of 8.3%–10% and 1.7%–6.7%, respectively. The seeds cultured at −5°C did not become dormant.

The initial germination rates of *G. hirsutum* seeds cultured at −5°C, −1°C, and 5°C for 12 weeks were 0% at all temperatures. In the first week of culture, the final germination rates of seeds cultured at −1°C and 5°C were 18.3% and 20.0%, respectively. In the second week, the germination rates decreased to 3.3% and 8.3%, respectively. From the fourth week onwards, the germination rate was 0%. The final germination rate of *G. hirsutum* seeds cultured at −5°C peaked at 51.7% in the fourth week of culture. In other cases, the germination rate was either 8.3% or 31.7%. The dormancy rate of *G. hirsutum* seeds was 0% at all temperatures.

Glycine max seeds did not germinate at the low temperatures of −1°C and −5°C. At 5°C, 66.7% of seeds germinated in the fourth week of culture, while more than 96.7% of seeds germinated in the eighth week. The final germination rate of *G. max* seeds cultured at 5°C was 66.7%–96.7% for 1–12 weeks. However, the final germination rate of seeds cultured at −1°C was 93.3%–98.3% until the fourth week of culture; however, it decreased rapidly to 65.0% in the eighth week and 11.7% in the twelfth week. The final germination rate of seeds cultured at −5°C was 100% for the first and second weeks; however, it decreased to 33.3%–63.3% from the fourth to twelfth weeks. *Glycine max* seeds had a dormancy rate of 0%, similar to that of *G. hirsutum* L. seeds.

The initial germination rate of *Z. mays* cultured at low temperatures for 12 weeks was 0%, regardless of the temperature. The final germination rate of seeds cultured at −5°C was relatively high, at 95.0% for the first week of culture and 71.7%–81.7% for the second to twelfth weeks. The final germination rate of seeds cultured at −1°C was 86.7% for the first week of germination but decreased to 50.0% and 0% for the second and eighth weeks, respectively. The final germination rate of seeds cultured at 5°C was 85.0%–95.0% for the first 2 weeks of culture; however, it decreased to 46.7% from the fourth week. By the eighth and twelfth weeks, the final germination rates were 13.3% and 1.7%, respectively. Dormancy was only observed in 3.3%–5.0% of *Z. mays* seeds cultured at −1°C and 5°C.

Evaluation of the overwintering potential of the seeds of four different plant species buried with and without seed bags

Seeds placed in seed bags and those not placed in seed bags were buried at a depth of 10 cm, and germination and dormancy rates were investigated for 48 weeks. The average air temperature was 0.2°C after 2 weeks of burial but dropped to −0.7°C after 4 weeks (Fig. 3). The average air temperature increased to 3.2°C after 8 weeks of burial and 21.6°C after 24 weeks. It then dropped to −4.7°C after 48

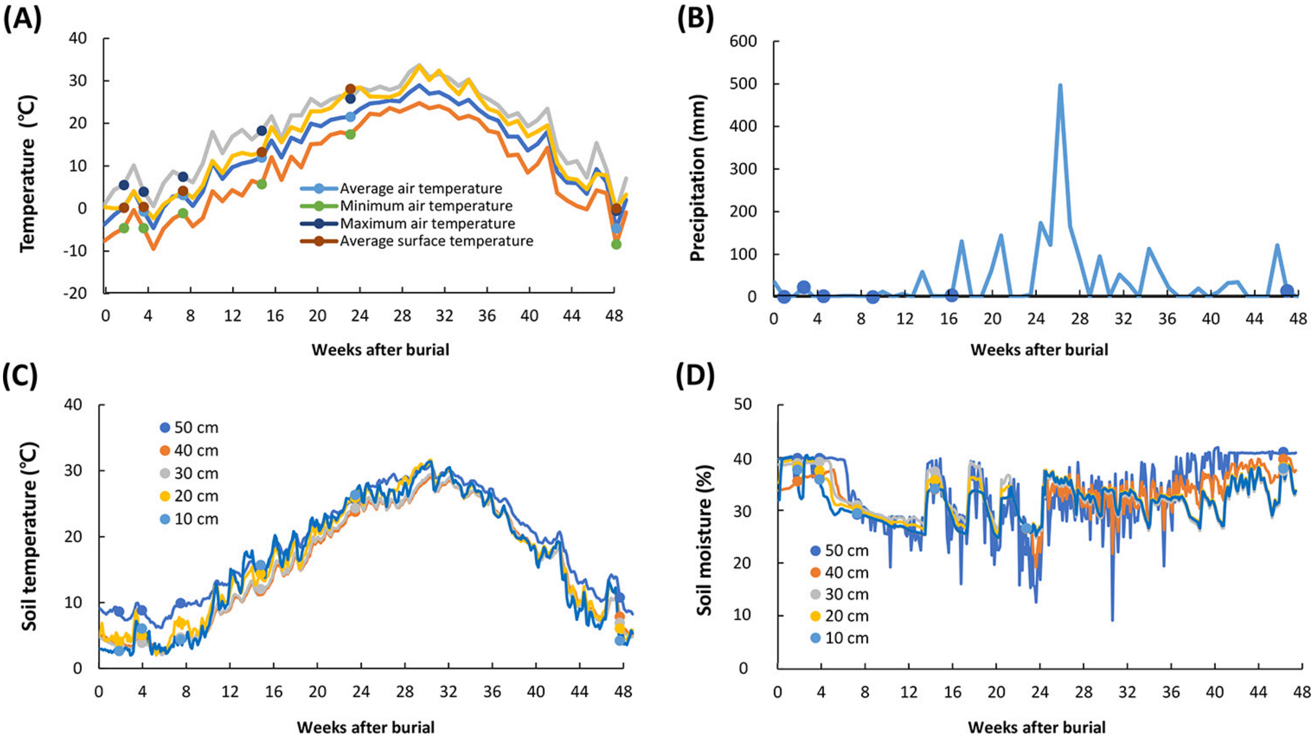

Fig. 3 Meteorological changes in the field during the experimental period. Variations in (A) air temperature, (B) precipitation, (C) soil temperature, and (D) soil moisture. Soil temperature and moisture were measured at depths of 10, 20, 30, 40, and 50 cm.

weeks. The average precipitation during the experimental period was 39.9 mm, with a maximum weekly precipitation of 496.7 mm in July. During the entire experimental period after seed deposition, the soil temperatures measured at depths of 10–50 cm remained above zero. The soil temperatures at a depth of 10 cm were 2.6°C, 6.0°C, and 4.4°C after 2, 4, and 8 weeks of burial, respectively. The soil temperature increased significantly to 15.7°C after 16 weeks of burial and 26.3°C after 24 weeks. It then dropped back to 4.2°C after 48 weeks of burial. The temperature difference during the burial period did not significantly increase with greater soil depth. Soil moisture was 38% at a depth of 10 cm 2 weeks after burial and remained at 27%–38% for the entire experimental period. In contrast to the soil temperature, soil moisture differences increased as soil depth increased.

The initial and final germination rates of the embedded seeds differed significantly based on the presence or absence of seed bags, the plant species, and the embedding period (Table 3). In addition, we observed significant interaction effects of seed bag × species, seed bag × duration, species × duration, and seed bag × species × duration on the initial and final germination rates. Significant differences were only found among species ($p < 0.001$), burial periods ($p < 0.001$), and species × duration interactions ($p < 0.001$).

Figure 4 displays the germination and dormancy rates of seeds buried in seed bags and those not buried in bags over 48 weeks. Germination was exclusively observed for *B. napus* seeds buried in soil. The germination rates of *B. napus* seeds in the seed bags were 21.7% and 18.3% in the fourth and eighth weeks, respectively. Conversely, the germination rate of *B. napus* seeds not buried in seed bags was 11.7% in the eighth week. After subjecting the non-germinating *B. napus* seeds buried in seed bags to 25°C incubation for 7 days, 85.0% germinated in the second week, whereas 10.0% and 43.3% germinated in the fourth and eighth weeks, respectively. When culturing the *B. napus* seeds buried in the soil without being placed in seed bags, the germination rates were 10%, 1.7%, 10%, and 3.3% at 2, 4, 8, and 16 weeks, respectively, at 25°C. Dormant seeds were observed in 1.7% of the seeds buried in the soil without being placed in a seed bag and confirmed in seeds buried 4 weeks after being placed.

None of the *G. hirsutum* seeds germinated in the soil during the experimental period. When these seeds were subsequently transferred to the optimal germination temperature of 25°C, only 1.7% of the seeds buried in seed bags germinated after 2 weeks.

Similar to the *G. hirsutum* seeds, the *G. max* seeds did not germinate in the soil, either when planted inside or outside of seed bags. When these seeds were transferred to 25°C in the laboratory and cultured, only 48.3% of the *G. max* seeds buried in seed bags germinated after 2 weeks.

Zea mays seeds did not germinate in the soil; however, when transferred to the optimal germination temperature of 25°C, 35.0% and 11.7% of the seeds inside and outside the seed bags, respectively, germinated in the second week after burial. Regardless of the presence of seed bags, dormant seeds were observed in *Z. mays* until the fourth week following burial. The dormancy rates of *Z. mays* seeds buried in the seed bags were 6.7% and 23.3% in the second and fourth weeks following burial, respectively. Conversely, the dormancy rates of *Z. mays* seeds that were not buried in the seed bags were 6.7% and 25.0% in the second and fourth weeks following burial, respectively.

Discussion

The potential for LM crops to negatively impact natural ecosystems stems from the possibility that LM pollen can cross-pollinate with wild relatives in the vicinity. This can result in the stable integration of the transgene into wild relatives, enhancing their stress resistance and increasing seed production. Consequently, the abundance of wild populations may increase while that of valuable species may decrease (Raybould 2010). Therefore, when reviewing the risks associated with a new LMO in Korea, the germinability and vitality of LM seeds, which serve as the foundation for this potential, are evaluated.

In this study, we carried out indoor experiments at winter temperatures of –5°C, –1°C, and 5°C using non-transgenic seeds of the four LM plant species most commonly

Table 3 General linear model results for the germination and dormancy of seeds buried in a field

	Initial germination	Final germination	Dormancy
Seed bag	0.008	< 0.001	0.690
Species	< 0.001	< 0.001	< 0.001
Burial duration	< 0.001	< 0.001	< 0.001
Seed bag × species	< 0.001	< 0.001	0.984
Seed bag × duration	0.002	< 0.001	0.958
Species × duration	< 0.001	< 0.001	< 0.001
Seed bag × species × duration	< 0.001	< 0.001	1.000

Seeds of four plant species (*Glycine max* (L.) Merr., *Zea mays* L., *Brassica napus* L., and *Gossypium hirsutum* L.) were buried at a depth of 10 cm in the soil, with or without seed bags, and left for 48 weeks. Data are presented as the mean ± standard deviation ($n = 3$); p-values were calculated using a general linear model-based method.

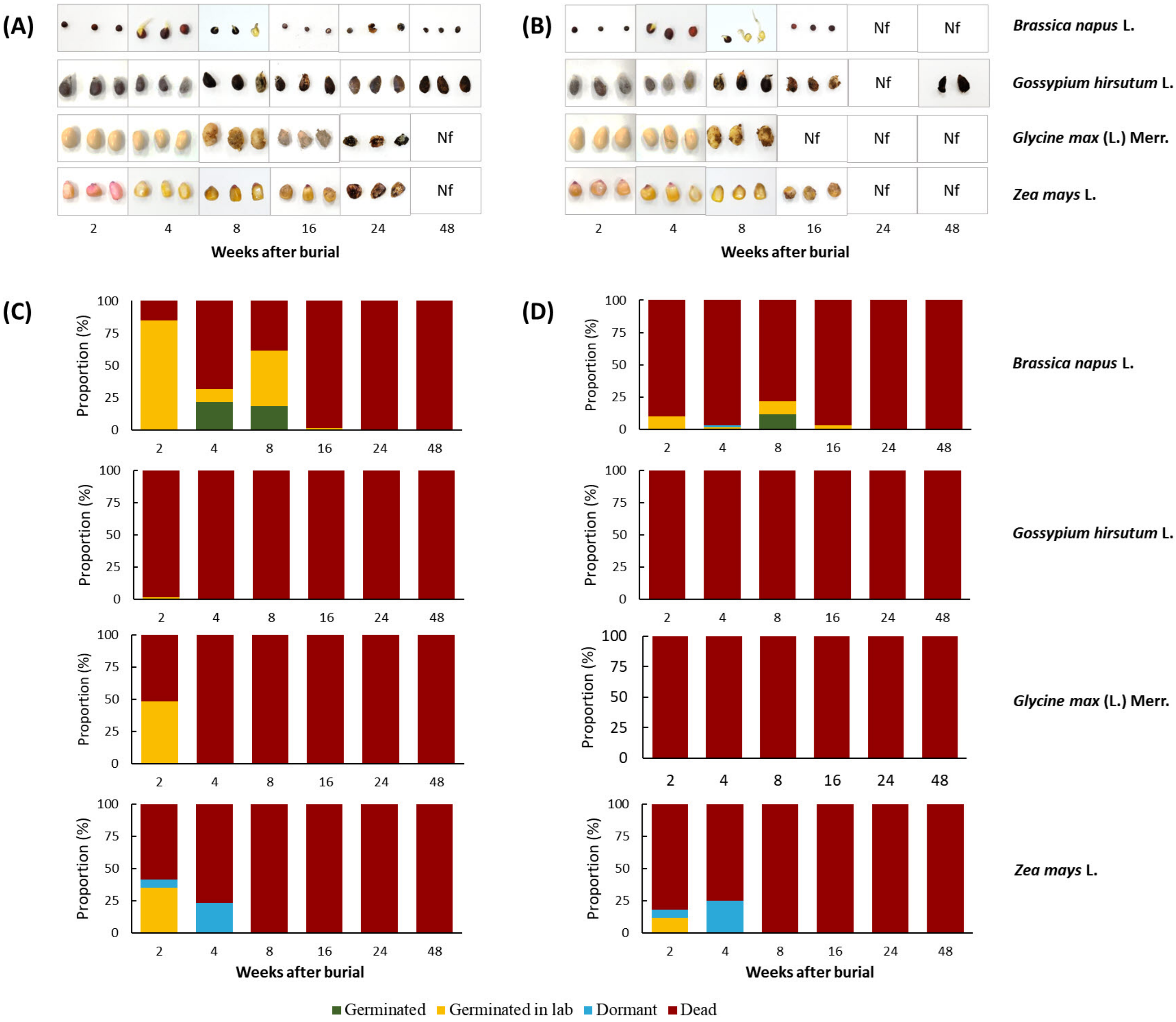

Fig. 4 Changes in germination of seeds buried in soil (A) with and (B) without seed bags. Proportions of dead, germinated, laboratory-germinated, and dormant seeds of the four plant species buried at a depth of 10 cm in soil (C) with and (D) without seed bags over 48 weeks. The data are presented as the mean ± standard deviation (*n* = 3). Colored areas and symbols indicate seed state (green, germinated; yellow, germinated in the laboratory; blue, dormant; red, dead). Nf: not found.

imported into Korea. All four plant seeds did not germinate at –1°C and –5°C; however, *B. napus* and *G. max* seeds germinated at 5°C. When seeds that did not germinate at the low temperatures were transferred to the optimal germination temperature of 25°C, all seeds germinated. Although the germination rate of *B. napus* was high over 12 weeks, that of *G. max* and *Z. mays* decreased over time. Nevertheless, the germination rate of *G. hirsutum* seeds was low even at the optimal germination temperature of 25°C. Seed dormancy was only observed in *B. napus* and *Z. mays* seeds cultured at –1°C and 5°C. These results indicate that although the germination capacity of *G. max* and *Z. mays* decreases over time, *B. napus*, *G. max*, and *Z. mays* have a higher probability of germination and survival when temperatures increase in the subsequent year after winter. In particular, *B. napus* and *Z. mays* exhibited dormant seeds at low temperatures, suggesting that they pose greater threats to natural ecosystems.

In contrast to controlled laboratory environments, natural environments exhibit large temperature fluctuations between day and night, variations in soil moisture content in response to rainfall, and diverse microbial distributions influenced by soil composition and depth. These factors can significantly affect seed germination and dormancy (Han and Nam 2022; Han et al. 2023; Nam and Han 2020). The seed vitality of transgenic *G. max* seeds was maintained for 2–4 months in a field in Korea and contingent upon various environmental factors, such as soil temperature and moisture at the time of release and burial depth; therefore, it only temporarily formed a soil seed bank and had a low likelihood of becoming a weed (Kim et al. 2020b; Ko et al. 2016). *Brassica napus* seeds have been reported to remain viable for 16 months in undisturbed fields in Korea, successfully producing seeds and overwintering; however, *B. napus* plants did not have an advantage in competition over other wild plants because of reduced overall growth and survival rates (Ko et al. 2022).

In the unmanaged fields representing the natural environment, seed germination after winter was only observed in *B. napus* for up to 8 weeks following burial. When intact non-germinated seeds in soil were incubated in the laboratory at 25°C, germination was only observed 2 weeks after burial for *G. hirsutum*, *G. max*, and *Z. mays*. Dormant seeds were exclusively detected in *B. napus* and *Z. mays* for up to 4 weeks after burial. This effect was greater in seeds buried without seed bags than in those buried in seed bags. Although soil temperatures were above freezing during the experimental period, all four species of plant seeds had low seed germinability and viability, making them unlikely to invade the natural ecosystem.

Moravcová et al. (2022) investigated the dynamics of seed germinability and viability in 21 invasive and 38 naturalized herbs. They found that seed-banking strategies differed between invasive alien and naturalized species, which significantly affected plant establishment and spread. The study revealed that invasive species utilized high seed viability in the first few seasons, whereas naturalized species extended their seed viability and germinability over time to maintain naturalized populations. Furthermore, the persistence of soil seed banks has been correlated with the life history and seed size of different plant species. Specifically, annual and biennial plants tend to have higher persistence in soil than perennials. In addition, herbaceous plants with small seeds have been more persistent than those with large seeds (Thompson et al. 1993, 1998). All the plants used in our study were annuals and biennials. Notably, *B. napus* has the smallest seed size, indicating the greatest potential to form a long-term seed bank in Korea. Several studies have demonstrated that *B. napus* plants can survive in undisturbed fields for more than 11 years, and volunteers of herbicide-tolerant transgenic *B. napus* have been found at field trial sites for up to 15 years after harvesting (Belter 2016; Lutman et al. 2003).

In addition to the persistence of LM seeds, the ability of surviving LM volunteers to transfer their genes to wild relatives and disperse populations among wild relatives is an important consideration when assessing the risks to natural ecosystems (Chèvre et al. 1997). However, the risk of gene transfer from LMOs to wild species may vary among plant taxa because of variations in native plant distributions in different countries. Although close relatives of the four plant species are present in the agricultural environments of Korea, wild relatives capable of cross-pollinating with *Z. mays* and *G. hirsutum* are rarely encountered in natural ecosystems. Conversely, *B. juncea* and *G. soja*, which are close relatives of transgenic *B. napus* and *G. max*, are widely distributed in natural ecosystems (National Institute of Biological Resources [NIBR] 2024). Gene transfer from transgenic *G. max* to *G. soja* has not been detected in field trials in Korea; however, gene transfer from transgenic *G. max* to non-transgenic *G. max* has been observed up to 13.1 m away, although it decreases with increasing distance (Kim et al. 2019, 2020a). Moreover, it has been reported that gene transfer from transgenic *B. napus* to the closely related species *B. juncea* may have occurred in the actual natural ecosystem of Korea (Lee et al. 2023).

Consequently, our study, which considered the seed survival and dispersal potential of LMOs, indicates that transgenic *B. napus* poses a greater risk to the biodiversity of the domestic ecosystem than transgenic *G. hirsutum*, *Z. mays*, and *G. max* when assessing the impacts of newly introduced LMOs. However, generalizing the results of this case study to non-transgenic seeds may be limited. This is because actual LMOs can be resistant to various biotic/abiotic stresses owing to improved traits, and as a result, may have a competitive advantage over wild plants. Long-term and extensive research using LM seeds should be conducted in the immediate future to establish risk assessments and safety management measures for each plant species, thereby safeguarding the biodiversity of natural ecosystems in Korea.

Conclusions

Brassica napus seeds exhibited higher germinability and viability than *G. hirsutum*, *Z. mays*, and *G. max* seeds at controlled low temperatures. In fields representing natural winter environments, *B. napus* seeds exhibited the highest germination rates. In addition, dormancy was higher for both *Z. mays* and *B. napus* seeds. However, the introduction of transgenic *B. napus* is anticipated to pose the highest potential risk to the natural environment because wild relatives of *B. napus* are more widely distributed in the natural ecosystem of Korea. Practically, although their continued generation has not yet been verified, transgenic *B. napus* volunteers have consistently been observed in natural ecosystem over the past decade (Lee et al. 2023). Our study offers an empirical basis for future research that utilizes LM seeds, which will yield more profound knowledge regarding the potential risks that newly introduced LMOs may pose to natural ecosystems.

Abbreviations

LMO: Living modified organisms
TZ: Tetrazolium

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Authors' contributions

KHN designed the study and wrote the manuscript. SMH performed the experiments. SJC, JWL, and JK participated in data analysis. All the authors have read and approved the final version of the manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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