Introduction

In Korea, the ambient air CO$_2$ concentration is substantially higher than the global average. The average CO$_2$ concentration for 2016 was recorded as 409.9 ppm, which was an increase of 39.2 ppm (10.6%) relative to the annual average of 370.7 ppm for 1999, and 6.6 ppm higher than the global average of 403.3 ppm for the same year as documented by World Meteorological Organization (WMO) [1]. The mean yearly temperature for South Korea in 2016 was 13.6°C, which was 0.6°C higher than the recent 10 years (2007-2016) and 1.1°C higher than the current climatological normal (1981-2010) [2]. Baseline scenarios, those without additional mitigation (RCP 8.5), result in an increase in the average temperature of the Korean Peninsula to 6.0 degrees at the end of the 21st century [3].

Climatic change, such as warming and alteration of precipitation regimes, is causing shifts in species distributions [3, 4] and phenologies [5]. These changes can also alter forest composition; for example, warming could increase the growth rate of established individuals or select for warm-adapted species. Population demographics of woody plants established within intact successional plant communities are likely to be constrained by factors that alter seed germination and seedling establishment, and ultimately, recruitment of individuals into the population [6-8].

Plant seed germination is a crucial stage in the life cycle of plants, and the successful establishment of plants largely depends on successful germination [9]. Generally, seed germination tends to be highly unpredictable over space and time.

However, climate has a large influence on plant recruitment [10-12]. For example, with shortening winters [13], seeds may remain partially dormant in spring and need an extended time to germinate [14]. The alteration of temperature and water supply due to global climate change could preclude, delay, or enhance regeneration from seeds [15].

Abstract: Effects on seed germination characteristics of 17 tree species were investigated under elevated temperature and CO$_2$. Seeds of 5 needle-leaf and 12 broad-leaf species were germinated under four conditions: 24°C + 400 μmol CO$_2$ mol air$^{-1}$, 24°C + 750 μmol CO$_2$ mol air$^{-1}$, 27°C + 400 μmol CO$_2$ mol air$^{-1}$, and 27°C + 750 μmol CO$_2$ mol air$^{-1}$. The elevated temperature and CO$_2$ affected germination percent (GP) of 7 tree species seeds. GPs of Pinus densiflora, P. thunbergii, Betula ermanii, and Maackia amurensis seeds were affected by the elevated temperature, while only that of P. jezoensis seed was influenced by the elevated CO$_2$. GPs of Malus baccata and Zelkova serrata seeds were influenced by both the elevated temperature and CO$_2$. In addition, the elevated temperature and CO$_2$ also affected mean germination time (MGT) of 12 tree species seeds. Particularly, MGTs of P. thunbergii and Rhododendron tschonoskii seeds were influenced by both factors. In conclusion, elevated temperature and CO$_2$ affected seed germination characteristics, which were reflected by significant differences among tree species. Specifically, these two factors exerted stronger influence on germination pattern such as MGT rather than seed germination percent.

Keywords: germination percent; mean germination time; needle-leaf; broad-leaf; seed germination characteristics
Despite the considerable number of studies on the effects of climate change on plants [16], there have been few attempts to investigate its effect on plant regeneration [17]. Several environmental factors such as temperature, salinity, light, and soil moisture simultaneously influence seed germination [9, 18-21]. Among several factors, temperature has been considered as the most important. The variation in the optimal temperature for seed germination depends on the considered species, and for the majority of species, seed germination occurs over a wide range of temperatures [22]. This variation in the optimal temperature and the germination percent between species constitutes some adaptive strategies to harsh environmental conditions. It has been shown that temperatures above the thermal optimum often provoke an inhibition of germination and irreversible damage [9, 23].

In addition, plant regeneration from seed is largely governed by germinability and speed of germination. These components have received considerably less attention in CO₂ research relative to studies of vegetative or reproductive output responses. Moreover, the limited literature has not been rigorously examined for generalizable patterns of responses and/or potential mechanisms. Marty and BassiriRad [24] presented a meta-analysis summarizing the results of studies that have addressed the parental and direct effects of enriched CO₂ on seed germination success and germination percent. There is little empirical evidence that enriched CO₂ can have a direct effect on a germinating seed, but similar to parental responses, this direct effect on germination is quite inconsistent [25-29].

A study on historical climate change period showed that woody plants migrate along with climatic zones, but its moving velocity is estimated to be 4–200 km per 100 years. Therefore, except for tree species with a high migration velocity, they will not be able to catch up with the migration of climatic zones and becomes at high risk of extinction due to climate change [30].

In Korea, summer is getting longer, while winter is getting shorter. This phenomenon unquestionably stems from climate change driven by global warming, and it affects seed germination and regeneration of woody plants. When seeds of woody plants germinate without enough dormancy time, they do not regenerate and finally die under unsuitable environmental conditions after germination.

Therefore, it is important to evaluate the effects of temperature and CO₂ on vegetation, ecosystems, and certain tree species in order to secure a scientific basis for establishing actions to address adaptation and mitigation of climate change [31, 32].

This is especially true considering that there are few studies in relation to seed germination of native tree species under the elevated temperature and CO₂ concentration in Korea. Therefore, we evaluated the effects on seed germination characteristics of tree species under different temperatures and CO₂ concentrations.

2 Materials and methods

2.1 Plant materials

Mature seeds of 17 forest tree species (5 needle-leaf and 12 broad-leaf tree species), which are major tree species in Korea, were collected from natural or artificial forests on October 2015 (Table 1) and stored in a refrigerator at -18°C before the experiments. The viability of seeds evaluated by tetrazolium (TZ) assay was as in Table 2. For breaking dormancy, the seeds were stratified at 4°C for 60 days or soaked in hot water (100°C) for 60 s followed by cold tap water for 1 day before imposing the temperature and CO₂ treatments (Table 2).

2.2 Experimental design

The seed trial was carried out in four walk-in chambers (3 × 3 × 1.8 m) that allowed controllable conditions corresponding to each treatment at the Department of Forest Genetic Resources in the National Institute of Forest Science (NIFoS), Suwon City, Gyeonggido, Korea.

The temperature and CO₂ concentration in each chamber varied depending on the treatment: T1 as a control (24°C, ambient air CO₂), T2 (27°C, ambient air CO₂), T3 (24°C, enriched air CO₂), T4 (27°C, enriched air CO₂). The temperature and CO₂ concentration employed in the experiment were based on the annual mean temperature (24°C) during the growing season of May to September and annual mean CO₂ level (400 μmol CO₂ mol air⁻¹) in Korea, and the projected temperature and CO₂ level (27°C, 750 μmol CO₂ mol air⁻¹) at the end of the 21st century (2071–2100) according to the IPCC scenario [33]. The air in the chambers was circulated through charcoal filters and CO₂ was mixed into the air stream. During the whole experimental period, the relative humidity was maintained at 68 ± 1% and illumination at a photon flux density of 400 μmol m⁻² s⁻¹ during a 16-h photoperiod.
Table 1. Seed collection sites and annual mean (minimum, maximum) temperature of 17 tree species

| Scientific name                              | Abbreviation | Seed collection sites | Annual mean temperature (°C) 1981-2010 | Annual mean temperature (°C) 2015 | Remarks       |
|----------------------------------------------|--------------|-----------------------|----------------------------------------|----------------------------------|---------------|
| Chamaecyparis pisifera (Siebold & Zucc.) Endl. | Cpi          | Yeosu                 | 14.0 (11.3, 18.0)                      | 15.3 (12.5, 18.6)                | Introduced    |
| Larix kaempferi (Lamb.) Carriere             | Lka          | Chungju               | 11.2 (5.9, 17.7)                       | 12.9 (7.5, 19.0)                 | Introduced    |
| Picea jezoensis (Siebold & Zucc.) Carriere   | Pje          | Mt. Jiri              | Not collected                          | 8.5 (0.7, 14.0)                 | Native        |
| Pinus densiflora Siebold & Zucc.             | Pde          | Suwon                 | 12.0 (7.5, 17.2)                       | 13.6 (9.0, 19.1)                | Native        |
| Pinus thunbergii Parl.                       | Pth          | Yeosu                 | 14.0 (11.3, 18.0)                      | 15.3 (12.5, 18.6)                | Native        |
| Albizia kalkora Prain                        | Aka          | Mokpo                 | 13.9 (10.3-18.6)                       | 14.8 (11.5, 18.8)                | Native        |
| Betula ermanii Cham.                         | Ber          | Mt. Jiri              | Not collected                          | 8.5 (0.7, 14.0)                 | Native        |
| Corylopsis gotoana var. coreana (Uyeki) T.Yamaz. | Cgoc        | Suwon                 | 12.0 (7.5, 17.2)                       | 13.6 (9.0, 19.1)                | Native        |
| Fraxinus rhynchophylla Hance                 | Frh          | Suwon                 | 12.0 (7.5, 17.2)                       | 13.6 (9.0, 19.1)                | Native        |
| Maackia amurensis Rupr.                      | Mam          | Chungju               | 11.2 (5.9, 17.7)                       | 12.9 (7.5, 19.0)                | Native        |
| Malus baccata (L.) Borkh.                    | Mba          | Suwon                 | 12.0 (7.5, 17.2)                       | 13.6 (9.0, 19.1)                | Native        |
| Prunus padus L.                              | Ppa          | Chungju               | 11.2 (5.9, 17.7)                       | 12.9 (7.5, 19.0)                | Native        |
| Rhododendron mucronulatum Turcz.             | Rmu          | Suwon                 | 12.0 (7.5, 17.2)                       | 13.6 (9.0, 19.1)                | Native        |
| Rhododendron mucronulatum var. ciliatum Nakai| Rmuc         | Mt. Jiri              | Not collected                          | 8.5 (0.7, 14.0)                 | Native        |
| Rhododendron schlippenbachii Maxim.          | Rsc          | Suwon                 | 12.0 (7.5, 17.2)                       | 13.6 (9.0, 19.1)                | Native        |
| Rhododendron tschonoskii Maxim.             | Rs            | Mt. Jiri              | Not collected                          | 8.5 (0.7, 14.0)                 | Native        |
| Zelkova serrata (Thunb.) Makino              | Zse          | Imsil                 | 11.2 (5.5, 18.0)                       | 12.5 (7.0, 19.1)                | Native        |

Table 2. Initial viability, dormancy type, pretreatment method and substrates of 17 tree species seeds

| Scientific name                              | Viability (%) | Dormancy | Pretreatment | Substrate | References |
|----------------------------------------------|---------------|----------|--------------|-----------|------------|
| Chamaecyparis pisifera (Siebold & Zucc.) Endl. | 20            | No       | NT           | petri dish | [52]       |
| Larix kaempferi (Lamb.) Carriere             | 86            | Shallow  | CS           | petri dish | [52]       |
| Picea jezoensis (Siebold & Zucc.) Carriere   | 55            | Shallow  | CS           | petri dish | [52]       |
| Pinus densiflora Siebold & Zucc.             | 70            | No       | NT           | petri dish | [52]       |
| Pinus thunbergii Parl.                       | 100           | No       | NT           | petri dish | [52]       |
| Albizia kalkora Prain                        | 30            | Hard seedcoat | SWC           | soil       | [63]       |
| Betula ermanii Cham.                         | 49            | Shallow  | CS           | petri dish | [52]       |
| Corylopsis gotoana var. coreana (Uyeki) T.Yamaz. | 66          | Shallow  | CS           | soil       | [64]       |
| Fraxinus rhynchophylla Hance                 | 57            | Embryo/Hard seedcoat | CS           | soil       | [52]       |
| Maackia amurensis Rupr.                      | 25            | No       | NT           | soil       | [65]       |
| Malus baccata (L.) Borkh.                    | 69            | Embryo   | CS           | soil       | [52]       |
| Prunus padus L.                              | 46            | Embryo/Hard seedcoat | CS           | soil       | [52]       |
| Rhododendron mucronulatum Turcz.             | 61            | No       | NT           | petri dish | [52]       |
| Rhododendron mucronulatum var. ciliatum Nakai| 81            | No       | NT           | petri dish | [52]       |
| Rhododendron schlippenbachii Maxim.          | 75            | No       | NT           | petri dish | [52]       |
| Rhododendron tschonoskii Maxim.             | 65            | No       | NT           | petri dish | [52]       |
| Zelkova serrata (Thunb.) Makino              | 50            | Embryo   | CS           | soil       | [66]       |

NT: non-pretreatment, CS: cold stratification at 4°C for 60 days, SWC: soaking in hot water (100°C) for 60 s and then cold tap water for 1 day, Soil = peat moss : perlite = 1 : 1
2.3 Seed germination test

After pretreatments and stratifications, the seeds of the different treatments were left to germinate in the light/dark (16 h/8 h a day) in a sand pot (1 cm depth from the soil surface) or in a 90 mm Petri dish with five replications. Each containing 50 seeds was evaluated for germination. The sand pots and Petri dishes were constantly rearranged to avoid positional bias once a week and the four chambers were switched once a month to reduce chamber effects on seed germination.

Seeds in the Petri dish were considered to have germinated once the radicle had protruded by 1 mm, and seeds in the soil pot were considered to have germinated when new stem tips were observed on the soil surface every day for 60 days after sowing.

Germination percent (GP) was determined using the following equation: \( GP = \frac{SG}{TS} \times 100 \), where \( GP \) = percent germination, \( SG \) = seeds germinated, and \( TS \) = total seeds planted. Mean germination time (MGT) was calculated using the following formula: \( MGT = \frac{\sum (n \times d)}{N} \), where \( n \) = number of seeds germinated on each day, \( d \) = number of days from the beginning of test, and \( N \) = total number of seeds germinated at the termination of the experiment.

2.4 Statistical analysis

All data were statistically analyzed using analysis of variance of a completely randomized design. Means were compared using the Duncan multiple range test (DMRT) at the 5% level. Statistical analyses were conducted using SAS System for Windows, Version 8.01 (SAS Institute, USA).

3 Results

3.1 Germination Percent (GP)

The higher temperature and enriched CO\(_2\) had a positive or negative influence on GP of 17 tree seeds (Table 3, Figure 1). GP of two needle-leaf seeds (\( P. \) densiflora and \( P. \) thunbergii) and two broad-leaf seeds (\( B. \) ermanii and \( M. \) amurensis) were significantly affected by the higher temperature, and only the \( P. \) jezoensis seed was significantly changed by the enriched CO\(_2\) (p < 0.05). The changes of GP in response to the elevated temperature and CO\(_2\) were observed in two broad-leaf seeds (\( M. \) baccata and \( Z. \) serrata) of the 17 tree species.

| Scientific name | Temperature | \( CO_2 \) | Temperature \( \times CO_2 \) |
|-----------------|-------------|-------------|-----------------------------|
| Chamaecyparis pisifera (Siebold & Zucc.) Endl. | 0.15 n.s. | 2.02 n.s. | 0.02 n.s. |
| Larix kaempferi (Lamb.) Carriere | 0.21 n.s. | 1.24 n.s. | 0.52 n.s. |
| Picea jezoensis (Siebold & Zucc.) Carriere | 3.70 n.s. | 5.78* | 4.08 n.s. |
| Pinus densiflora Siebold & Zucc. | 5.20* | 4.11 n.s. | 0.58 n.s. |
| Pinus thunbergii Parl. | 26.53*** | 0.89 n.s. | 2.32 n.s. |
| Albizia kalkora Prain | 0.20 n.s. | 3.59 n.s. | 1.83 n.s. |
| Betula ermanii Cham. | 7.67* | 0.28 n.s. | 2.94 n.s. |
| Corylopsis gotoana var. coreana (Uyeki) T. Yamaz. | 1.62 n.s. | 2.63 n.s. | 1.34 n.s. |
| Fraxinus rhynchophylla Hance | 4.63 n.s. | 1.06 n.s. | 0.71 n.s. |
| Maackia amurensis Rupr. | 5.24* | 0.03 n.s. | 1.52 n.s. |
| Malus baccata (L.) Borkh. | 13.11** | 12.46** | 1.25 n.s. |
| Prunus padus L. | 2.05 n.s. | 2.05 n.s. | 4.82* |
| Rhododendron mucronulatum Turcz. | 2.23 n.s. | 0.10 n.s. | 0.92 n.s. |
| Rhododendron mucronulatum var. ciliatum Nakai | 0.35 n.s. | 0.04 n.s. | 0.04 n.s. |
| Rhododendron schlippenbachii Maxim. | 0.17 n.s. | 0.01 n.s. | 0.52 n.s. |
| Rhododendron tschonoskii Maxim. | 0.04 n.s. | 2.61 n.s. | 0.83 n.s. |
| Zelkova serrata (Thunb.) Makino | 47.06*** | 11.76** | 8.30* |

*, p < 0.05; **, p < 0.01; ***, p < 0.001; n.s., non-significance
Elevated temperature and CO₂ exerted stronger influence on germination pattern

Effects of the elevated temperature and CO₂ are described in Figure 2. Germination of *Chamaecyparis pisifera* and *Larix kaempferi* seeds was not affected at all by increased temperature and CO₂ concentration. GP of *P. jezoensis* seed was the lowest under the higher temperature and the enriched CO₂ (27°C + 750 μmol CO₂ mol air⁻¹) (p < 0.05) conditions. It was not affected by the elevated CO₂ concentration under the ambient temperature (24°C) but was significantly lower under enriched CO₂ and higher temperature (27°C). GP of *P. densiflora* seed was the lowest when both temperature and CO₂ were elevated (27°C + 750 μmol CO₂ mol air⁻¹). The enriched CO₂ influenced the reduction of GP, which was significantly higher at the elevated temperature (27°C) than at the ambient temperature (24°C) (p < 0.05). At the ambient CO₂, the higher temperature (27°C) induced the lower GP, and the effect of temperature change was more clearly evident under the enhanced CO₂. GP of *P. thunbergii*
seed was higher at the ambient temperature (24°C) than at the higher temperature (27°C), and there was no effect under the different CO₂ concentrations.

Germination pattern of broad-leaf seeds was significantly different from those of needle-leaf seeds (p < 0.05) (Figure 3). GP of *B. ermanii* seed was higher under the higher temperature (27°C), whereas there was no effect by the enhanced CO₂. The temperature effect on seed germination was clearer under the ambient CO₂ (400 μmol CO₂ mol air⁻¹) than the enriched CO₂ (750 μmol CO₂ mol air⁻¹). GP of *M. baccata* seed was influenced by the change of both temperature and CO₂ concentration (p < 0.05). Particularly, it was lower under the higher temperature (27°C) and was observed to be more significant under the elevated CO₂ (750 μmol CO₂ mol air⁻¹). The GP of *Z. serrata* seed was highly sensitive to the changes of both temperature and CO₂ concentration, and was significantly lower under the conditions of higher temperature and CO₂ concentration. In particular, the effect of CO₂ concentration was stronger at the ambient temperature (24°C) than at the elevated temperature (27°C).

Figure 2. Seed germination percent (top) and mean germination time (bottom) of five needle-leaf tree species under the elevated temperature and CO₂ concentration. All the values are mean of five replicates ± standard deviation (SD); the same letters are not significantly different at the 5% probability level by the Duncan’s multiple range tests.
Elevated temperature and CO$_2$ exerted stronger influence on germination pattern

3.2 Mean Germination Time (MGT)

MGTs of three needle-leaf tree seeds (C. pisifera, P. densiflora and P. thunbergii) were significantly influenced by the change of temperature ($p < 0.05$). In particular, MGT of P. thunbergii seed was affected by the changes of both temperature and CO$_2$ concentration (Table 4, Figure 1).

MGT of C. pisifera seed was lower under the higher temperature (27°C) regardless of CO$_2$ concentration (Figure 2). In contrast, MGT of L. kaempferi seed was the highest under the higher temperature and enriched CO$_2$ (27°C + 750 μmol CO$_2$ mol air$^{-1}$) conditions, and that of P. densiflora seed was higher under the higher temperature (27°C) regardless of CO$_2$ concentration (Figure 2). In addition, MGT of P. thunbergii seed was the lowest under the ambient temperature and CO$_2$ (24°C + 400 μmol CO$_2$ mol air$^{-1}$) and the highest under the higher temperature and enriched CO$_2$ (27°C + 750 μmol CO$_2$ mol air$^{-1}$) conditions.

In the case of broad-leaf tree species, MGTs of Corylopsis gotoana var. coreana, Maackia amurensis and Prunus padus seeds were affected by the alteration of temperature, while those of Fraxinus rhynchophylla, Malus baccata, Rhododendron mucronulatum, R. mucronulatum var. ciliatum and R. schlippenbachii seed were influenced by the change of CO$_2$ concentration (Table 4). On the other hand, MGT of R. tschonoskii seed was significantly affected by the change of both temperature and CO$_2$. 

Figure 3. Seed germination percent (top) and mean germination time (bottom) of twelve broad-leaf tree species under the elevated temperature and CO$_2$ concentration. All the values are mean of five replicates ± standard deviation (SD); the same letters are not significantly different at the 5% probability level by the Duncan’s multiple range tests.
concentration. Meanwhile, MGTs of *M. amurensis*, *M. baccata*, *R. schlippenbachii*, *R. tschonoskii* and *Z. serrata* seeds responded to the interaction between temperature and CO₂ concentration (Table 4).

MGT of *C. gotoana* var. *coreana* seed was the highest under ambient temperature and enriched CO₂ (24°C + 750 μmol CO₂ mol air⁻¹) and the lowest under the higher temperature (27°C) regardless of CO₂ concentration (Figure 3). MGT of *M. amurensis* seed was more sensitive to temperature change than CO₂ change, and it was the highest under higher temperature and ambient CO₂ (27°C + 400 μmol CO₂ mol air⁻¹). MGT of *M. baccata* seed was the highest under ambient temperature and enriched CO₂ (24°C + 750 μmol CO₂ mol air⁻¹) and that of *P. padus* seed was the lowest under the higher temperature and ambient CO₂ (27°C + 400 μmol CO₂ mol air⁻¹). *Rhododendron mucronulatum* seed was the lowest under the higher temperature and ambient CO₂ (27°C + 400 μmol CO₂ mol air⁻¹) conditions, but the highest under higher temperature and enriched CO₂ (27°C + 750 μmol CO₂ mol air⁻¹). MGTs of *R. schlippenbachii* and *R. tschonoskii* seeds were also the lowest in the higher temperature and the ambient CO₂ (27°C + 400 μmol CO₂ mol air⁻¹) conditions and the highest under higher temperature and enhanced CO₂ (27°C + 750 μmol CO₂ mol air⁻¹). Lastly, the MGT of *Z. serrata* seed was the lowest under the higher temperature and enriched CO₂ (27°C + 750 μmol CO₂ mol air⁻¹) conditions.

### Discussion

Each individual species has a base and ceiling temperature that represents the extremes at which germination can occur. Below and above these extremes no germination can occur [34]. If climate change results in temperatures that exceed the ceiling for a species, then that species will not be able to germinate; thus, affecting its survivability.

In our study, seed germination of 6 species were affected by the higher ambient temperature (Figure 1), and GP of 5 tree seeds, except for *B. ermanii*, was

| Scientific name                          | Temperature | CO₂          | Temperature × CO₂ |
|------------------------------------------|-------------|--------------|-------------------|
| *Chamaecyparis pisifera* (Siebold & Zucc.) Endl. | 14.80**     | 0.06n.s.     | 2.78n.s.          |
| *Larix kaempferi* (Lamb.) Carriere       | 4.39n.s.    | 3.61n.s.     | 2.59n.s.          |
| *Picea jezoensis* (Siebold & Zucc.) Carriere | 0.09n.s.    | 0.41n.s.     | 0.30n.s.          |
| *Pinus densiflora* Siebold & Zucc.       | 10.28**     | 1.53n.s.     | 1.82n.s.          |
| *Pinus thunbergii* Parl.                 | 54.90***    | 19.84***     | 0.58n.s.          |
| *Albizia kalkora* Prain                  | 1.71n.s.    | 0.84n.s.     | 2.23n.s.          |
| *Betula ermanii* Cham.                   | 1.67n.s.    | 1.09n.s.     | 2.76n.s.          |
| *Corylopsis gotoana* var. *coreana* (Uyeki) T. Yamaz. | 69.18***    | 3.46n.s.     | 4.49n.s.          |
| *Fraxinus rhynchophylla* Hance            | 0.00n.s.    | 5.11*        | 0.16n.s.          |
| *Maackia amurensis* Rupr.                | 18.48***    | 1.54n.s.     | 6.27*             |
| *Malus baccata* (L.) Borkh.              | 0.54n.s.    | 8.83**       | 10.44**           |
| *Prunus padus* L.                        | 9.21*       | 1.07n.s.     | 1.48n.s.          |
| *Rhododendron mucronulatum* Turcz.       | 0.63n.s.    | 4.83*        | 3.00n.s.          |
| *Rhododendron mucronulatum* var. *ciliatum* Nakai | 0.40n.s.    | 5.85*        | 0.04n.s.          |
| *Rhododendron schlippenbachii* Maxim.    | 1.42n.s.    | 35.55***     | 8.62*             |
| *Rhododendron tschonoskii* Maxim.        | 7.84*       | 59.27***     | 9.92**            |
| *Zelkova serrata* (Thunb.) Makino        | 0.70n.s.    | 3.28n.s.     | 8.64**            |

*, p < 0.05; **, p < 0.01; ***, p < 0.001; n.s., non-significance
reduced at the higher temperature. In addition, elevated temperature affected MGTs of 7 species of 17 tree seeds. MGTs of P. densiflora, P. thunbergii, and M. amurensis seed were higher under the higher temperature, but MGTs of C. pisifera, C. gotoana var. coreana and P. padus seed were lower under the higher temperature.

Several researchers have shown that the optimal temperature for germination and seedling growth depends on the species [35, 36]. Generally, temperature plants germinate between 0°C and 35°C whereas tropical plants germinate between 10°C to 45°C. Within the species, optimal temperature varies significantly between the genotypes [37], and high temperature tolerance of seed contributes to the attributes of the species [38].

Our findings also showed a variety of species responses to the increase in temperature, as was the case in previous studies. This means that the optimum temperature range for seed germination in 17 species is different. This is due to the different seed shapes (seed size and weight) and physiological characteristics found in each species.

In our results, three species (P. densiflora, P. thunbergii and M. amurensis) seeds that showed lower GP had higher MGT at the higher temperature (Figure 2), which means delayed seed germination at the higher temperature. In particular, seeds of Pinus species were highly sensitive to higher temperature and lost their germinability due to decay under the higher temperature. In an accelerated aging test of Pinus seeds, high temperature accelerated pine seed aging [39]. This suggests that the failure to germinate was more likely a consequence of seed mortality rather than of delayed or aborted germination. It is generally known that the higher the temperature, exposure to any temperature beyond the optimum range for germination can negatively affect seed germination, as in the above three species [34].

Germination substrate can also affect seed germination. In our study, the seeds of M. baccata and Z. serrata germinated in sand decreased in GP at higher temperatures (Figure 3). Many studies have shown that seeds germinated in the soil have seen a decrease in germination percent at higher temperatures [40]. Several factors under high soil temperature are considered key to germination reduction: maximum temperature attained [41, 42], soil moisture and seed water content [43], seed structure, anatomy and morphology (e.g. size, seed coat) [44], and seed dormancy dynamics [41]. However, the relative importance of any individual factor is difficult to assess, and maximum temperature and heat duration are considered foremost to seed germination reduction [45]. Much of the literature assumes an inverse relationship between temperature and duration [46]. The long term exposure of the seeds at higher temperatures, as shown in our experiments, may be the main cause of the decline in seed germination.

As shown above, high temperature has a detrimental effect on seed germination causing thermos-inhibition [47], and temperature causing thermo-dormant changes varies with the genotype [48]. In general, high temperatures reinforce dormancy or may even induce it [49]. A drastic change in temperature will also have a significant effect on germination due to the temperature dependence of hormones and enzymes. If the temperature window is breached, then these enzymes may become inactive [50].

However, unlike the above results, high temperatures sometimes break internal dormancy and stimulate germination [51]. In our study, B. ermanii seeds have increased their GP at high temperature (Figure 3). This is also due to the different temperature ranges of optimum germination for Betula species. Birch seeds such as B. ermanii require relatively high temperatures to germinate. The optimal germination temperature range for birch seeds is 30°C for 8 hours and 20°C for 16 hours [52]. Further, several reasons have been given for the enhancement of seed germination by high temperature. Among these are fracturing of hard seed coats, stimulation of seed embryos, and desiccation of seed coats [53].

High temperatures also increase the speed of germination [34]. This is because high temperatures can speed up the chemical reaction in seed germination [54]. In our results, the lower MGT of C. gotoana var. coreana and P. padus seeds with impermeable hard seed coat were more pronounced at the elevated temperature (Figure 3). However, germination speed can differ according to species, soil structures, sowing methods and especially temperature and soil moisture rations [54].

CO₂ has been shown to stimulate the germination of various seeds at relatively high concentration [55]. Doubling the CO₂ concentration resulted in an increase in the speed and final percent of germination, for Medicago sativa, Amaranthus hybridus and Chenopodium album [25].

Unlike the above results, in our study, an increase in CO₂ concentrations did not contribute positively to seed germination. CO₂ enrichment affected seed GPs of only three species (M. baccata, Z. serrata, and P. jezoensis) out of 17 tree species (Figure 1). Seed germination of the three species was significantly lower under CO₂ enrichment (Figure 2). As such, the effects of seed germination on CO₂ changes appear to vary from species to species. Corbineau and Côme [56] were suggested that CO₂ does not always have a beneficial effect on seed germination. Several reports mention that at high concentration it inhibits the
germination of some seeds. In addition, Omer and Horyath [57] suggested that increased \( \text{CO}_2 \) concentration does not significantly affect the seed germination of many plants, but could cause variations in seed germination patterns as a result of our study.

\( \text{CO}_2 \) enhancement significantly influenced the seed MGTs of 7 tree species, and seeds of 6 tree species, except \( F. \text{rhynchophylla} \), showed significantly higher MGT under \( \text{CO}_2 \) enhancement (Figure 3). Similar to the results of our study, previous studies have shown that elevated \( \text{CO}_2 \) concentration can have different influences on seed germination and emergence among different species [25]. In particular, our studies show that \( F. \text{rhynchophylla} \) seed has a positive effect of a reduction in the MGT from high \( \text{CO}_2 \) unlike other species, but it is not easy to find the cause. However, these species were germinated in the soil unlike other species, and they had different types of dormancy.

In addition, the effect of \( \text{CO}_2 \) concentration on seed germination may even differ among genotypes of the same species, and there is a strong interaction between genotype and treatment. This means that genetic variation for a selective response to changes in \( \text{CO}_2 \) concentration may be present in natural populations [26].

Although the germination response to \( \text{CO}_2 \) has been suggested to be generally positive because of an enhancement in ethylene production due to \( \text{CO}_2 \) [58], many studies have shown contradictory results, similar to our study results [59]. Summing up, the effect of \( \text{CO}_2 \) enrichment on seed germination is very different among different species, and their effects may be positive [60], neutral [61], or negative [26], with a strong dependence on the species studied [62]. No significant interaction was observed between \( \text{CO}_2 \) and temperature on the germination response [25], and this was also re-confirmed in our study.

In conclusion, increased temperature and \( \text{CO}_2 \) had a direct effect on seed germination characteristics, but the direct effect of the increase in \( \text{CO}_2 \) on seed germination was relatively minor. Also, two factors exerted stronger influences on germination pattern (such as MGT) rather than seed GP of woody plants. On the other hand, the germination effects of seeds from changes in temperature and \( \text{CO}_2 \) concentration varied greatly depending on the species. Various factors appear to be involved in the interspecific differences, including seed morphological structure, dormancy type, germination substrate, and optimum germination temperatures. These results indicate that if the increase in temperature has negative effects total germination and germination speed, persistence of individual species will be altered and the distribution of species could change. Similarly, if increasing temperatures have a differential positive effect on germination of some species, then these species will promote their fitness and also change their distribution. This has incredible ecological and economic effects for the local ecosystems and surrounding areas. However, more accurate and more data is needed to predict the species distribution changes due to temperature and \( \text{CO}_2 \) changes. In other words, additional research should be continued in order to more accurately interpret the effects of seed germination on species, considering various factors including natural conditions.

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