A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Ecophysiological Mechanisms Underlying Seed Dormancy in Five Genea of Ranunculaceae Native to the Korean Peninsula

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ABSTRACT

The main aim of this study was to explore the diversity of seed germination and seedling emergence strategies in Korean temperate forest herbs and to study the environmental factors regulating germination. Embryo morphology and seed dormancy of two Berberidaceae species (*Leontice microrhyncha* and *Jeffersonia dubia*), eight Ranunculaceae species (*Adonis amurensis*, *Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*), three Melanthiaceae species (*Heloniopsis koreana*, *H. tubiflora*, and *Trillium tschonoskii*), and one Liliaceae species (*Erythronium japonicum*) were studied, and the types of seed dormancy were classified, and its regulation by phytohormones was discussed. All seeds of 14 species we studied had underdeveloped embryos which occupied about 7-20% of the full seed length at maturity. Most seeds of *A. buergeriana* and *P. tonkangensis* had morphological dormancy (MD), whereas the seeds of *L.*

microrhyncha, *J. dubia*, *A. amurensis*, *R. crucilobus*, *R. franchetii*, *E. japonicum*, *T. tschonoskii*, *T. coreanum* had morphophysiological dormancy (MPD). On the other hand, the seeds of *H. koreana*, *H. tubiflora*, *T. rochenbrunianum*, and *T. uchiyamai* had both MD and MPD, indicating that there was a different level of dormancy (MD and MPD) within the same seed population examined. Among the 14 species, the types of MPD were classified in seeds of Ranunculaceae. All six species, which had MPD, emerged seedlings mainly in spring after dormancy was broken. However, different dormancy mechanisms to attain this pattern of spring emergence were observed regardless of the time of seed dispersal. Seeds of *A. amurensis* had a special type of deep simple epicotyl MPD. Embryos grew at relatively warm temperatures in autumn with split pericarp, and seeds germinated after warm followed by cold temperature sequences. GA did not overcome the dormancy. Seeds of *R. crucilobus* had deep complex MPD. Embryo growth occurred at cold temperature (5^{o}C). Although germination occurred at cold temperature, it was more promoted at warm temperatures after cold stratification. GA did not overcome the dormancy. On the other hand, seeds of *R. franchetii* had deep simple epicotyl MPD. Embryos in the seeds grew at relatively warm temperature in early autumn, and germination occurred in late autumn. Germinated seeds required cold stratification for seedling emergence. In three *Thalictrum* species, seeds had non-deep simple MPD. Although embryo growth occurred at low temperatures, it was more promoted when the seeds were transferred from low to high temperatures. Seeds required only a cold stratification to break dormancy, and GA substituted for cold stratification. We collected data of seed dormancy types in Ranunculaceae from available

information and compared with congeners in different countries or continents. From the data, we found that there was a wide variety of dormancy types in five genera in Ranunculaceae. However, there were same types of MPD in eastern Asian - North American congeners in the genus *Thalictrum* with an Arcto-Tertiary distribution pattern. This indicates that identical types of dormancy are evidence that this type of dormancy is at least as old as the Tertiary. MD and MPD were regulated by phytohormones (ABA and GAs). In *A. buergeriana* seeds (MD), ABA content and sensitivity decreased rapidly, and GA content and sensitivity increased rapidly after burial. On the other hand, in *A. amurensis* seeds (MPD), ABA content decreased drastically after burial, but GA content did not increase before the seeds experienced temperature changes from high temperatures in summer to medium temperatures in autumn in the natural environment. When underdeveloped embryos grew rapidly in autumn, ABA was non-detectable and GA content increased. After embryo maturation, ABA content increased and GA content decreased at the same time, and thus, the seeds remained ungerminated during cold season in winter. When the seeds started to germinate after cold period in winter, GA content increased rapidly. GA_4 played a key role in stimulating embryo growth and germination in both MD and MPD. The changes of GA/ABA ratio were similar to the changes of embryo growth and germination in the buried seeds. These results indicate that MD or MPD in the basal angiosperm taxa also could be controlled by a hormone balance model.

Keywords: Berberidaceae, ecophysiology, Liliaceae, MD, Melanthiaceae, MPD, phytohormones, Ranunculaceae, seed dormancy, underdeveloped embryo

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GENERAL INTRODUCTION

Timing of seedling establishment is the crucial event in the plant life cycle, and determines its future success (Fenner and Tompson, 2005; Harper, 1977). The timing of seedling emergence depends on several variables, such as the plant life cycle, habitat preference, yearly temperature cycle in the native area, and geographical distribution (Baskin and Baskin, 1988; Nikolaeva, 1999; Vandelook and van Assche, 2008).

Seed dormancy is considered the most important plant mechanism for cueing germination and seedling emergence (Fenner and Tompson, 2005). Seeds of many wild plants in temperate regions of the world do not germinate and may require exposure to summer and/or winter temperatures to break dormancy (Baskin and Baskin, 1988, 2004b). Thus, the timing of seedling establishment is mainly regulated by dormancy breaking and germination requirements of the seeds.

Ripe seeds of several angiosperm taxa have an underdeveloped embryo at the time of seed dispersal, meaning that it must grow within the seed before germination can occur (Martin, 1946; Nikolaeva, 1977). These seeds are termed morphologically dormant (MD) or morphophysiologically dormant (MPD), where there is an additional physiological block preventing germination (Nikolaeva, 1977). So far, nine different types (levels) of MPD have been defined, based on temperature requirements for embryo growth, breaking of physiological dormancy (PD), and the ability of gibberellic acid to overcome dormancy (Baskin and Baskin, 1998, 2004a, 2014). A classification system of Baskin and Baskin (1998, 2004a) has been widely accepted in the area of seed research by horticulturists

(Geneve, 2003; Han et al., 2010; Heather et al., 2010; Niimi et al., 2006; Pérez, 2009), botanists and ecologists (Chien et al., 2011; Hao et al., 2014; Mattana et al., 2014; Vandelook and Van Assche, 2009; Walck et al., 2012), and molecular physiologists (Finch-Savage and Leubner-Metzger, 2006; Graeber et al., 2012; Leubner-Metzger, 2012; Linkies et al., 2010). Therefore, seed scientists have recently been conducting ecophysiological research of seed dormancy based on the classification system.

An extensive study of the requirements for dormancy breaking and germination of North American herbs revealed that MPD is the dominant seed dormancy type in perennial herbs of mesic deciduous forest (Baskin and Baskin, 1988, 1998). Later studies on seed dormancy of plants native in Europe and Japan showed that seeds of species with MPD are frequent in temperate mesic deciduous forests in general (Ali et al., 2007; Kondo et al., 2002, 2011; Vandelook and Van Assche, 2008, 2009). Over the past several decades, extensive seed dormancy studies have been conducted in herbaceous perennial species native to Korea. In particular, physiological seed dormancy (PD) breaking by cold stratification, GA treatment, and after-ripening, and physical dormancy (PY) breaking by scarification have been reported in many species (Kang et al., 2010; Kim et al., 2010; La and Jeong, 2008; Park et al., 2012; Yeam et al., 1985). Prior to this study, however, relatively few data has been collected on the ecophysiology of MPD in seeds with underdeveloped embryos of species that occur in temperate zone in the Korean peninsula. And none of the studies have been conducted on classification of the types of morphological seed dormancy.

It has been known that seed dormancy is regulated by phytohormones such as

abscisic acid (ABA), gibberellin (GA), cytokinins, ethylene, brassinosteroids (BR), auxins, and other signaling molecules such as nitrate and karrikins (a group of plant growth regulators of the butenolide class found in the smoke of burning plant material) (Cross et al., 2013; Kucera et al., 2005; Miransari and Smith, 2014). Among them, the control of seed dormancy and germination by key hormones GA and ABA is well known (Bewley, 1997; Cadman et al., 2006; Koornneef et al., 2002). However, most of studies have discussed hormonal regulating mechanism of seed dormancy of *Arabidopsis thaliana*, *Sorghum bicolor*, *Nicotiana* spp., *Hordeum vulgare*, and other cultivated species. Seeds of these species have only physiological dormancy (PD). Thus, there has been few researches on hormonal regulating mechanism in seeds of many species with MD or MPD in the basal angiosperm families. MPD is thought to be the ancestral seed dormancy type in angiosperm and gymnosperm (Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006; Forbis et al., 2002).

One method for better understanding of ecophysiology of seed dormancy is to identify particular families of plants in which very little information currently is available. A merit in focusing one's effort at the family level is that the physiological, ecological, biogeographical, and evolutionary relationships of the diversity of seed dormancy mechanisms among the species in the family can be determined (Hidayati, 2000). The aim of this dissertation research was to expend our understanding of germination ecophysiology of Ranunculaceae native to the Korean Peninsula by using comparative approach to fully characterize dormancy breaking and germination requirements of eight species in five genera of this family, and thus to identify the types of dormancy represented among them. From

the results, we wanted to know how the seed dormancy is controlled in the natural environment.

We divided four chapters into two parts for this dissertation (Fig. 1): Part I (chapter I and II) for determination of natural variations on phenology of dormancy break and its control mechanisms by phytohormones (GAs and ABA); Part II (chapter III and IV) for determination of embryo morphology, requirements for dormancy break, and finally for classification of the types of MPD from the results.

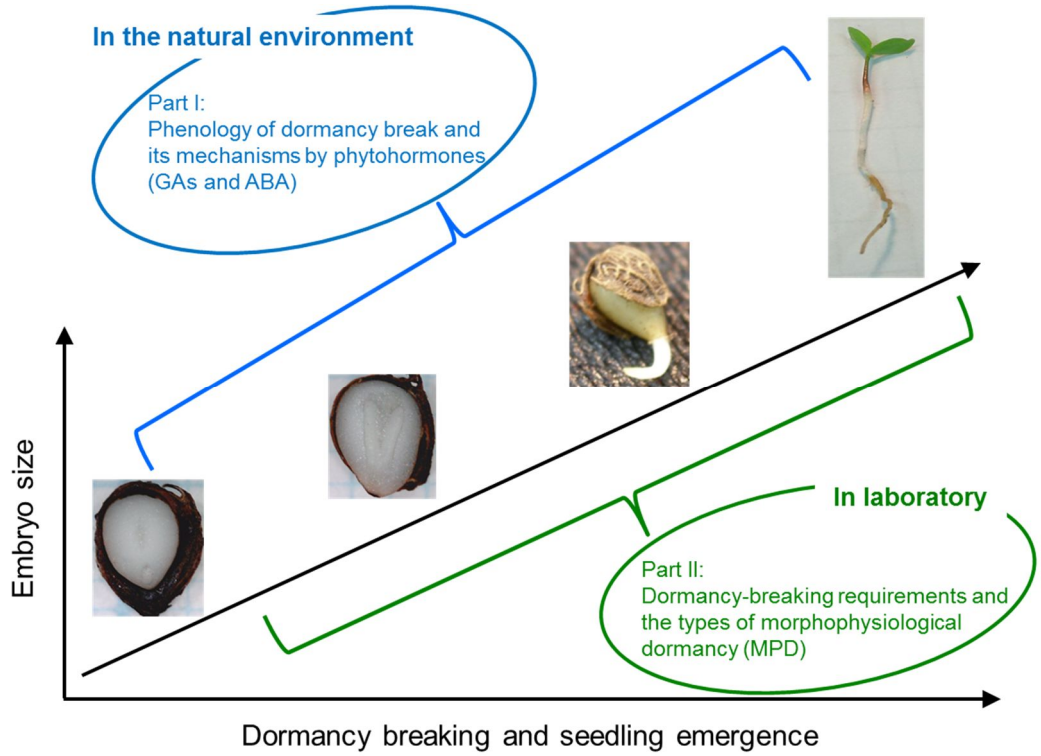

Fig 1. A simplified diagram of this dissertation research. In part I, we studied dormancy-breaking phenology and its regulation by phytohormones (GAs and ABA) in the natural field conditions. On the other hand, in part II, dormancy breaking-requirements in controlled laboratory conditions were determined, and the types of morphophysiological dormancy (MPD) were classified from the results.

LITERATURE REVIEW

Seed Dormancy

There are two methods for seeds to bridge the periods between seed dispersal and germination in a viable and ungerminated state in the soil (Bewley and Black, 1994; Nikolaeva, 1977). First, seeds can be prevented from germinating when the environmental circumstances are not suitable. Germination requires certain chemical and physical conditions, such as a sufficient availability of water, light, and suitable temperatures. Seeds that do not germinate because one of these factors is lacking are said to be in a state of "quiescence" (Baskin and Baskin, 2004a; Murdoch and Ellis, 2000). Secondly, all environmental conditions needed for germination may be available, but seeds still do not germinate. In this case, most seed scientists use the term 'dormancy' for these seeds that fail to germinate in conditions that are normally regarded as suitable for germination (Bewley and Black, 1994; Baskin and Baskin, 1998, 2004a).

Propagators of cultivated plants have long recognized that germination was influenced by climatic factors. The first recorded discussion of seed dormancy was written by Greek philosopher Theoprastus in about 300 B.C. (Evenari, 1981). He recognized that germination in some seeds increased (dormancy relaease).

Classification System for Seed Dormancy

History of Development of A Dormancy Classification System

With recognition that dormancy break was correlated with seasonal time of the year (in particular, cold in winter season), seed germination ecologists paid

increased attention to the work of seeds physiologists who had been researching the specific dormancy breaking requirements of seeds in the laboratory or greenhouse for several decades (Baskin and Baskin, 2008a). For example, Barton (1930) who was a researcher in the Boyce Thompson Institute for Plant Research was studying the effects of cold stratification on dormancy break in the early 1930s. The subsequent “marriage” of ecology and physiology in studies of seed dormancy turned out to be a very fruitful approach (Baskin and Baskin, 2008a).

One problem with discussing seed dormancy is that there is no single recognized terminology to describe the many different types of seed dormancy (Geneve, 2003). Crocker (1916) described seven types of dormancy based on the treatments used to overcome the dormancy. This was one of the first classification systems, in which he documented the kinds of dormancy with regard to their cause: 1) underdevelopment of the embryo, 2) water-impermeable seed or fruit coat, 3) mechanical resistance of seed covering layers, 4) low gas permeability of seed covers, 5) metabolic (physiological) block in the embryo, 6) combined dormancy, and 7) secondary dormancy.

Subsequently, Nikolaeva (1977) in Russia defined dormancy based primarily upon physiological controls. Using her knowledge of the literature and her enormous experience with seed dormancy, Nikolaeva devised the most logical and detailed the classification system of seed dormancy yet developed (Baskin and Baskin, 2008a), which she subsequently revised slightly (Nikolaeva, 1977, 2001). The system is based on 1) seed internal structure (states of embryo development), 2) role of seed covering layers (impermeability to water, mechanical restriction to radicle protrusion, and presence of chemical inhibitors), 3) physiological

requirements for dormancy break (cold and/or warm stratification), and 4) effects of plant growth regulators on breaking physiological dormancy. In her system of dormancy classification, Nikolaeva used both a name and a letter/number formula to the seven types and various subtypes of dormancy she recognized: three types of exogenous (physical, chemical and mechanical), three types of endogenous (physiological, morphological and morphophysiological), and many types of combinational (exogenous + endogenous) dormancy. For example, non-deep simple morphophysiological dormancy (MPD) was represented by the formula C1bB-C1b. These seeds need a period of warm (C1b) temperatures to break physiological dormancy (PD) and for the growth of an underdeveloped embryo (B). After MPD (C1bB) is broken, the seeds, now with a fully developed embryo, germinate at warm temperature regimes (-C1b) (Baskin and Baskin, 2008a; Nikolaeva, 1977, 2001).

After collecting available evidence, Baskin and Baskin (1998) proposed that chemical and mechanical dormancy be recognized only as a part of physiological dormancy, and Nikolaeva (2004) agreed with this opinion. Later, Baskin and Baskin (2004a, 2008b) documented a modified version of Nikolaeva's classification system that included three hierarchical layers of classification: class, level and type. A closer look at this classification system can be helpful to explain horticultural, ecophysiological, biogeographical, and evolutionary concept of seed dormancy.

Lang (1987) proposed the terms eco (now environmental secondary dormancy), para (exogenous dormancy) and endo-dormancy (exogenous dormancy) to simplify the terminology. This system has currently been used in American

Society for Horticultural Science journals. But, this terminology is not sufficient to fully describe the diversity of seed dormancy found in seeds (Geneve, 2003). Because, in particular, there are many seeds with underdeveloped embryos at dispersal, in which they show various patterns of dormancy breaking in nature and have different dormancy breaking requirements. Thus, Lang's terminology is not adequate to explain the seed dormancy physiology.

Therefore, the most recent classification system by Baskin and Baskin (1998, 2004a, 2014) has been thought to fits well both the ecological and horticultural aspects of seed dormancy and widely used and accepted by most of the scientists working in the field of seed biology (Adams et al., 2005; Chien et al., 2011; Finch-Savage and Leubner-Metzger, 2006; Geneve, 2003; Graeber et al., 2012; Han et al., 2010; Heather et al., 2010; Jayasuriya et al., 2008; Jones and Kaye, 2014; Linkies et al., 2010; Mattana et al., 2014; Niimi et al., 2006; Pérez, 2009; Vandelook and Van Assche, 2009; Walck et al., 2012).

The Five Classes of Seed Dormancy

A comprehensive and experimentally useful classification system for seed dormancy has recently been proposed by Baskin and Baskin (2004a) (Finch-Savage and Leubner-Metzger, 2006). According to Baskin and Baskin (2004a), seed dormancy can be categorized into five classes [physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational dormancy (PY + PD)]. In temperate regions, many perennial herbs disperse seeds with underdeveloped embryos that must elongate to a critical length prior to radicle emergence (Baskin and Baskin, 1998).

Physiological Dormancy (PD). PD means there is a 'physiological inhibiting mechanism' in the embryo that prevents it from generating growth potential to overcome the mechanical restriction of the seed coat and/or other covering layers (Baskin and Baskin, 2004a). Dormancy break occurs at cool (about 0-10^{o}C) wet, warm (≥15^{o}C) wet, or warm dry conditions (after-ripening). PD can be subdivided into three levels: non-deep, intermediate and deep PD (Baskin and Baskin, 2004a; Nikolaeva, 1977).

Embryos cut out from seeds with non-deep PD usually grow and form normal seedlings. This type of dormancy can be broken by relatively short periods (days or up to several months) of stratification at either low (< 10°C), or relatively high temperatures (> 15°C). Non-deep PD can also be come out of dormancy during short period of dry storage (after-ripening) at room temperatures (Geneve, 2003). Addition of gibberellic acid (GA) generally breaks non-deep PD and can therefore substitute a stratification requirement. Once PD is broken, additional environmental conditions, such as light, nitrate or daily temperature fluctuations, may be required to initiate germination (Vandelook, 2009).

Seeds with intermediate or deep PD require much longer periods (> 3 months) of cold stratification for dormancy break (Geneve, 2003). Excised embryos will grow and produce normal seedlings in seeds with intermediate PD and in general GA can substitute for cold stratification. Embryos excised from seeds with deep PD, on the other hand, do not grow or they produce abnormal seedlings and GA cannot overcome dormancy (Baskin and Baskin, 1998).

Extensive dormancy study in seeds with PD has been conducted over past several decades in Korean native species, such as *Primula sieboldi* (Song and Lee,

2002), *Scrophularia takesimensis* (Kang et al., 2009), *Orostachys* 'Jirisan' (Kang et al., 2010), and *Aster koraiensis* (Kim et al., 2010). Seed dormancy of most of the species was broken by several weeks of cold stratification, dry after-ripening, or GA treatment. For example, final germination percentage in seeds of *Aster koraiensis* dramatically increased from 24% in after-ripened seeds for 3 months to 70% after 11 months of dry storage (Kim et al., 2010).

PD is the most abundant type and is found in seeds of gymnosperms and all major angiosperm clades (Baskin and Baskin, 1998, 2004b; Finch-Savage and Leubner-Metzger, 2006; Martin, 1946). PD is also the most common type of dormancy in all vegetation zones on earth except in the matorral (a type of vegetation dominated primarily by small-leaved, evergreen shrubs that occurs in a climate with cool, moist winters and hot, dry summers, i.e., Mediterranean-type climate) (Baskin and Baskin, 2003a, 2004b).

Morphological Dormancy (MD). Ripe seeds of many angiosperm taxa contains an embryo that is not yet fully developed (Martin, 1946). This underdeveloped embryo must grow inside the seeds before germination can occur, thereby delaying germination (Baskin and Baskin, 1998). The time-lag coinciding with this period of embryo elongation is considered as a type of dormancy. Since germination is delayed due to morphological characteristics of the embryo, the term morphological dormancy was applied (Baskin and Baskin, 1998; Nikolaeva, 1969). A stimulating effect of light on germination has been observed in a number of species with MD seeds (Bulowa and Ozeri, 1975; Pressman et al., 1977; Baskin and Baskin; 1990). But, seed in some other species such as *Aquilegia buergeriana* and *Pulsatilla tongkangensis* (Korean native species) can germinate under dark

conditions (Lee et al., unpublished data). Baskin and Baskin (2004a) proposed that a fresh seed has MD if the seed with the underdeveloped or undifferentiated embryo germinates within about 30 days without any dormancy-breaking pretreatments at suitable environmental circumstances. Among the 5,250 species for which germination data have been compiled, MD is not very common in any vegetation region on earth (Baskin and Baskin, 1998, 2004b).

Morphophysiological Dormancy (MPD). Morphophysiological dormancy (MPD) occurs in seeds with small, underdeveloped or undifferentiated embryos that also have PD. Unlike seeds with MD, seeds with MPD need more than about 30 days (up to several years) to germinate under favourable circumstances (Baskin and Baskin, 2004a). This dormancy class will be discussed in detail below.

Physical Dormancy (PY). One of major dormancy mechanisms is physical dormancy (PY), which refers to dormancy caused by a seed (or fruit) coat impermeable to water. If seed mass in freshly matured seeds does not increase, then the seed coat is impermeable to water. On the other hand, if seed mass increases ≥ 20% based on air-dry seed or fruit mass, then one can assume that the seed (or fruit) coat is permeable to water (Baskin an Baskin, 2003b). Since imbibition is an essential prerequisite for germination, the seed coat has to become water permeable before germination can occur. Often seeds with PY are also referred to as hard seeds (or hard fruit) coats. Impermeability of the seed or fruit coat is usually caused by a palisade cell layers of lignified cells (Arachchige, 2011; Baskin et al., 2000; Jayasuriya, 2008).

PY acquisition occurs at various seed moisture levels, usually from 15 % to 2.5 % based on fresh mass (Baskin and Baskin, 1998). During dormancy break, an

opening or slit of impermeable sites is formed in the seed or fruit coat through the water impermeable layers at a specialized morpho-anatomical site (water gap), thus allowing water to pass through the seeds (Baskin and Baskin, 2000). The water gap occurs in the seed coat at a place with species dependent specialized anatomy and morphology (Baskin et al., 2000). It is a important structure in the life cycle of plants with physical dormancy since it acts as an environmental signal detector for seed dormancy break and germination (Baskin et al., 2000; Baskin, 2003; Jayasuriya, 2008). Thus, the water gap opens in response to environmental circumstances that conditions are favorable for seedling establishment. These cues include natural environmental temperature fluctuations in seeds of *Trifolium subterraneum* (Fabaceae) (Taylor, 1981), wet heat in seeds of *Geranium solanderi* (Geraniaceae), *Dichondra repens* (Convolvulaceae) (Warcup, 1980), *Ornithopus compressus* (Fabaceae) (Revell et al., 1999) and several Rhamnaceae species (Turner et al., 2005), dry storage at ambient laboratory temperatures in seeds of several Geraniaceae species (Meisert, 2002), and *Sida spinosa* (Malvaceae) (Baskin and Baskin, 1984), dry heat in seeds of *Dodonaea viscosa* (Sapindaceae) (Baskin et al., 2004), and fire in seeds of *Alyogyne hakeifolia* and *A. huegelii* (Malvaceae) (Baker et al, 2005), and *Jacquemontia curtisii* (Convolvulaceae) (Spier and Snyder, 1998).

Detecting the environmental cues is very important for seeds with PY in nature because they can not return to the dormant stage once PY is broken (Baskin, 2003). Therefore, the function of the water gap is critical in regulating germination to favorable periods for seed germination and subsequent seedling establishment in nature (Baskin, 2003; Baskin et al., 2000). Sixteen angiosperm

families, but no gymnosperm family, are known to have seeds with PY (Baskin et al., 2000; Baskin et al., 2006). However, not all species of most of these 16 families have impermeable seed or fruit coats. For example, many tropical taxa in the Fabaceae have a water permeable seed or fruit coat (Baskin and Baskin, 2004b). From a world viewpoint, PY is the second (next to PD) major class of seed dormancy (Baskin and Baskin, 1998, 2003).

Combinational Dormancy (CD, PY + PD). Combinational dormancy is evident in seeds with water-impermeable seed or fruit coats (PY) combined with physiological dormancy (PD) (Baskin and Baskin, 1998, 2004a). For example, in seeds of redroot (*Ceanothus* spp.), redbud (*Cercis* spp.), golden raintree (*Koelreuteria* spp.), PY must be broken before PD can be broken by moist cold stratification (Baskin and Baskin, 1998). Therefore, for promoting germination in seeds with CD, both scarification and cold stratification are needed in propagation strategies.

Morphophysiological Dormancy (MPD)

Morphophysiological dormancy (MPD) is a combination of MD and PD. If the seeds with underdeveloped embryos require more than about 30 days and a dormancy-breaking treatment, such as warm and/or cold stratification, to germinate, they are classified as MPD (Baskin and Baskin, 1998; Nikolaeva, 1977). Before seeds with MPD can germinate, the embryo must grow to a critical length inside the seeds and PD must be broken. Breaking of PD and MD can occur at the same time, but sometimes PD break proceeds embryo elongation and in a few cases an additional PD has to be broken after the embryo has grown to its

full size (Baskin and Baskin, 1998).

Nine levels and extra one level (a specialized type of deep simple epicotyl) of MPD have been proposed related to the requirements for breaking seed dormancy, the temperature requirements for embryo elongation and response to gibberellic acid (GA_3) (Table 1). Based on temperatures at the time of embryo growth, seeds with MPD have been divided into two categories: simple, embryo grows at relatively high (≥15°C); complex, embryo grows during cold (0-10°C) stratification (Baskin and Baskin, 1998, 2004a).

Each type of MPD (simple or complex) can be subdivided into non-deep, intermediate, and deep MPD depending on the physiological states of seeds (Baskin and Baskin, 1998, 2004; Nikolaeva 1977). Those with horticultural interest include simple and epicotyl MPD (Geneve, 2003).

For example, seeds with deep simple MPD usually require warm (≥15°C) followed by cold (0-10°C) stratification during which time the embryos grow during the warm temperature conditions and then breaks PD during the chilling conditions.

In some species, cultivated and wild plants differ with respect to MPD. Seeds of *Anemone coronaria* L., 'de Caen' showed only MD, in which the seeds germinate well at warm temperatures, while wild populations of *Anemone* showed MPD (Ali et al., 2007; Horovitz et al., 1975; Mondoni et al., 2008, 2009).

An interesting species with MPD is *Thalictrum mirabile*, a North Amerian perennial herb (Walck et al., 1999). This species requires 8 weeks of chilling at 1°C to relieve endogenous physiological dormancy followed by warm temperatures to satisfy morphological dormancy prior to germination. This

sequence is a reversal of the more common warm followed by cold temperature to relieve MPD.

Table 1. Nine types of morphophysiological dormancy (MPD) and temperature or temperature sequence required to break them (from Baskin and Baskin, 1998, 2004, 2014). This table was slightly modified from Baskin and Baskin (1998, 2004).

Type of MPD[y]	Temperature required[z]		GA overcomes dormancy	Example
	To break seed dormancy	At time of embryo growth		
Non-deep simple	W or C	W	+[x]	*Thalictrum mirabile*
Intermediate simple	W + C	W	+	*Aralia mandshuria*
Deep simple	W + C	W	+/-	*Jeffersonia diphylla*
Non-deep simple epicotyl	W	W	?	*Viburnum odoratissimum*
Deep simple epicotyl	W + C	W	+/-	*Asarum canadense*
Deep simple double	C + W + C	W	?	*Trillium camschatcense*
Non-deep complex	W + C	C	+	*Merendera montana*
Intermediate complex	C	C	+	*Sambucus* spp.
Deep complex	C	C	-	*Aconitum napellus*
Specialized type of deep simple epicotyl	W + C	W	?	*Hydrastis canadensis*

[z]W, warm (≥ 15°C) stratification; C, cold (0-10°C) stratification.

[y]MPD, morphophysiological dormancy.

[x]+, yes; +/-, yes/no (GA substitutes warm, but not for cold); -, no.

MPD is more common in temperate broadleaved evergreen forests and in temperate deciduous forests than in any other vegetation region on earth (Baskin and Baskin, 1998, 2003, 2004b). Seeds of many wild plants such as *Arisaema* spp., *Delphinium* spp., *Erythronium* spp., *Jeffersonia diphylla*, *Trillium* spp. in temperate deciduous forests have MPD (Baskin and Baskin, 2004b). Thus, this kind of seed dormancy has long been of interest to horticulturists and ecologists (Baskin and Baskin, 2004b; Gneneve, 2003).

Among nine types of MPD, two types of MPD have been known to have epicotyl dormancy (Baskin and Baskin, 2008, 2014). Seeds with 'epicotyl dormancy', in general, are dispersed in spring and germinate in autumn before they are subjected to winter chilling. In seeds with 'deep simple epicotyl MPD', the shoots of the seeds, however, do not emerge until the following spring. Therefore, the seeds need cold stratification in winter after radicle emergence in the natural environment. This pattern indicates that there is preventing mechanism for immediate growth of shoots or leaves (Baskin and Baskin, 1998). Epicotyl dormancy is usually broken during a cold stratification in radicle emerged seeds (Chien et al., 2011; Hao et al., 2014; Kondo et al., 2002). Therefore, only epicotyl needs cold stratification in the seeds. This group includes *Erythronium japonicum* in Liliaceae (Kondo et al., 2002), *Vibrunum* spp. in Adoxaceae (Chien et al., 2011), *Paeonia ludlowii* in Paeoniaceae (Hao et al., 2014), *Narcissus hispanicus* in Amaryllidaceae (Copete et al., 2011).

Epicotyl dormancy mostly occurs in seeds with deep simple MPD, but it also occurs in seeds with non-deep simple MPD (Baskin et al., 2008; Dhyani et al., 2013). In seed with non-deep simple epicotyl MPD, following warm stratification,

the root emerges at relatively warm temperatures but shoot emergence is delayed for 3-4 weeks or more. Cold stratification is not essential for dormancy break in this type (Baskin and Baskin, 2014).

One of the most complex dormancy mechanisms is found in seeds with 'deep simple double MPD' (Geneve, 2003). In this type, both the epicotyl and the radicle require chilling, but are released from the dormancy at different times. Seeds in this group require a chilling period to relieve radicle dormancy, followed by a warm period to allow the radicle to grow. After radicle emergence, a second cold period is needed to release the epicotyl from dormancy. Therefore, such seeds require at least two full growing seasons to complete germination and seedling emergence in the natural environment (Geneve, 2003). This group includes lily of the valley (*Convallaria majalis* L.) in Asparagaceae and *Trillium camschatcense* in Melanthiaceae (Geneve, 2003; Kondo et al., 2011).

One extra type of seed dormancy (a special type of deep simple epicotyl MPD) is found in seeds of *Hydrastis canadensis* (Ranunculaceae) (Baskin and Baskin, 1998). *Hydrastis canadensis* seeds are like those with deep simple MPD because embryo growth occurred in autumn before the seeds are subjected to winter chilling, but unlike those with deep simple MPD the seed or fruit coat splits open in autumn. *H. canadensis* seeds are some what like those with epicotyl dormancy because the root grows beyond the limits of the seed or fruit coat, but unlike those with epicotyl dormancy the root remains covered by endosperm until spring next year (Baskin and Baskin, 1998). Baskin and Baskin (1998, 2014) reported that perhaps, *H. hydrastis* represents a transitional stage between deep simple and deep simple epicotyl MPD.

From the literature, we can recognize that relatively few data has been collected on the ecophysiology of seed dormancy in seeds with underdeveloped embryos of species that occur in temperate zone in the Korean peninsula. And none of the studies have been conducted on classification of the types of MPD.

Control Mechanism of Seed Dormancy by Phytohormones

The plant hormones abscisic acid (ABA), gebberellins (GA), ethylene, brassinosteroids (BR), auxins, cytokinins and other signaling molecules such as nitrate and karrikinolides have profound effects on seed dormancy and germination at extremely low concentrations (Cross et al., 2013; Kucera et al., 2005; Miransari and Smith, 2014). Among them, the control of seed dormancy and germination by key hormones GA and ABA is well known (Bewley, 1997; Cadman et al., 2006; Koornneef et al., 2002). There is considerable evidence that ABA plays a key role in the induction and maintenance of seed dormancy, whereas gibberellins (GAs) are associated with dormancy release and germination (Cadman et al., 2006; Kucera et al. 2005). Dormant seeds treated with fluridone, which is a compound that inhibits carotenoid synthesis and, thus, ABA synthesis, often have similar germination pattern to non-dormant seeds, indicating that the continued synthesis of ABA is required for dormancy induction and maintenance in seeds of many species (Feurtado et al. 2007; Kucera et al. 2005; Yoshioka et al. 1998). Also, application of paclobutrazol, which is an inhibitor of GA biosynthesis, was found to prevent seed germination, indicating that a de novo biosynthesis of GAs is required during imbibition and, thus, dormancy break and germination (Karssen et al. 1989; Bradford and Nonogaki 2007). However, endogenous

changes in these phytohormones in buried seeds in the natural environment are still poorly understood (Garcia et al., 2012).

In addition to ABA and GA biosynthesis/content, it has recently been demonstrated that the GA/ABA hormone balance determines seed ability to germinate through dormant states that depends on synthetic and catabolic pathways of both hormones at the same time (Cadman et al., 2006). It appears to be the GA/ABA ratio, but not the absolute hormone contents, controls germination (Finch-Savage and Leubner-Metzger, 2006).

While dormancy maintenance also depends on low GA/ABA ratio, dormancy release and breaking are related to a net shift to increased GA biosynthesis and ABA degradation, resulting in high GA/ABA ratio (Ali-Rachedi et al., 2004; Cadman et al., 2006). In addition to these hormone contents and balance, the transition from the dormant to the nondormant state of many seeds is characterized by a decrease in ABA sensitivity and an increase in GA sensitivity (Chiwocha et al., 2005). However, most of these studies used seeds of *Arabidopsis thaliana*, *Sorghum bicolor*, *Nicotiana*, *Hordeum vulgare*, and so on. Seeds of these species have only physiological dormancy (PD). Thus, few studies have been conducted in seeds of many species with morphological (MD) or morphophysiological dormancy (MPD) in the basal angiosperm families.

Chien et al. (1998) reported that the strong seed dormancy of *Taxus mairei*, which has deep simple MPD, could be caused by a high ABA content and underdeveloped embryos in the seeds. They reported that warm stratification increased the growth of embryos and decreased ABA content, but the seeds still remained ungerminated, and then subsequent cold stratification induced the

response to GAs and initiated dormancy release and germination. In *Fraxinus excelsior*, which has deep simple MPD, seeds at maturity contained a high concentration of ABA and were dormant. When the seeds were warm stratified, underdeveloped embryos continued to grow and ABA concentrations decreased gradually. GA_3 was detected during the embryo growth. Subsequent cold stratification was required for germination and an increased GA_3 coincided with a decreased ABA level at the same time (Finch-Savage and Leubner-Metzger, 2006). It demonstrated that endogenous GA and ABA level is closely related to dormancy break and germination in seeds with MPD.

Hepher and Robert (1985) repoted that dormancy of *Trollius ledebouri* seeds, which has intermediate complex MPD, can be overcome by the application of GA_{4+7} or by testa removal. Frost-Christensen (1974) reported that seeds of *Eranthis hiemalis* have undeveloped embryos and the embryo grew rapidly when the seeds were moved to low temperatures (3-4^{o}C) after 3 weeks of incubation at room temperatures (20-25^{o}C). In the seeds, embryo growth without germination was promoted at room temperatures when the seeds were treated with GA. Embryo development is inhibited at low temperature by the specific inhibitor of GA biosynthesis, 2-chlorethyl choline chloride (CCC), but is recovered by the simultaneous addition of GA, indicating that one early effect of the cold stratification for embryo growth is to bring about a synthesis of GA.

Although hormonal regulation of seed dormancy and germination in seeds with fully developed embryos has been extensively studied, the control mechanism of MPD break by endogenous phytohormones such as ABA and GA has been poorly understood.

Ecological and Evolutionary Implications of Seed Dormancy

As one of the earliest traits expressed in the plant's life cycle, seed dormancy can be a critical determinant of colonization and successful establishment (Willis et al., 2014). The level and kind of seed dormancy strongly regulate the timing of germination in response to the seasons and plays an important role in plant evolution and adaptation to each geographical distributions (Baskin and Baskin, 2004a; Donohue et al., 2005; Forbis et al., 2002; Huang et al., 2010).

The classification system shows that a great diversity of morphological and physiological traits have evolved to control dormancy in response to different environmental circumstances (Baskin and Baskin, 1998, 2004a; Donohue, 2005; Vleeshouwers et al., 1995). Morphological dormancy is evident for evolution in seeds with embryos that are differentiated but very small compared with the full size of the entire seed (Forbis et al., 2002; Linkies et a;., 2010; Martin, 1946). Martin (1946) defined seed types with distinct embryo to endosperm ratios, arranged them in a seed phylogenetic tree and proposed evolutionary seed trends. The ratio of the length of embryo to seed (E : S ratio) describes the relative size of the embryo within the seed. A high E : S ratio (e.g. 0.9) indicates that the embryo fills up most of the seed volume, whereas a low E : S ratio (e.g. 0.1) indicates that the embryo is very small and the endosperm fills up most of the seed volume (Finch-Savage & Leubner-Metzger, 2006; Forbis et al., 2002; Martin, 1946). Seeds with low E : S ratios often need long times (several months or sometimes more than several years) for germination and seedling emergence, and the occurrence of abundant endosperm tissue are typical for MD or MPD class seeds.

Forbis et al. (2002) used methods of ancestral character state reconstruction

using E : S family means for 179 families calculated from a large dataset of 1,222 extant angiosperm species. Their analysis showed that the E : S ratios have increased in diverged angiosperms compared with ancestral angiosperms such as Papaveraceae, Magnoliaceae, Ranunculaceae, and Apiaceae. They proposed, based on these results, that a small embryo embedded in abundant endosperm, which is classified as MD, is the ancestral dormancy type of angiosperms. MD and MPD delays germination timing by the time the embryo needs to grow inside the seed before germination can occur. The dispersal of seeds with a small underdeveloped embryo that needs time to grow might have evolved as an ancient strategy to distribute germination times, since successful germination is highly dependent on environmental circumstances (Baskin and Baskin, 1998, 2004a; Finch-Savage & Leubner-Metzger, 2006; Forbis et al., 2002; Linkies et al., 2010). MD and MPD are typical not only for primitive angiosperm such as the Amborellaceae and Nymphaceae but also for primitive gymnosperms such as the Zamiaceae, Podocarpaceae and Taxaceae (Baskin an Baskin, 1998, 2007; Chen et al., 2013; Finch-Savage & Leubner-Metzger, 2006; Forbis et al., 2002; Martin, 1946).

More recently, Willis et al. (2014) reported evolutionary trends with different kind of seed dormancy using a data set comprising over 14,000 taxa in 318 families across the seed plants. They proposed that MPD could be the most likely ancestral state of seed plant, indicating that physiologically regulated seed dormancy was present at the origin of seed plant. And they found that physiological dormancy (PD) acted as an ‘evolutionary hub’ from which other dormancy class evolved, and that it was associated with higher rates of

phylogenetic diversification through higher speciation rates.

Within the MPD class of seed dormancy, evolutionary trends have been viewed in many species which show eastern Asia-eastern North America disjunct distribution patterns. According to Baskin and Baskin (1995, 1998), the presence of the same type of MPD in disjunct species in the same genera (congeners) with an Arcto-Tertiary distribution pattern indicates that the type of MPD is at least as old as Tertiary in Cenozoic. On the other hand, if disjunct species have different types of MPD, the differences may have been present in the Tertiary or evolved since that time. Regardless of the time of origin, each type of MPD in a genus should be considered as the product of selective pressures in each habitat. Thus, if congeners have different types of MPD, one could be derived from the other, or both could be derived from ancestors with even another type of MPD. This concept has recently been reported in many plant species with Arcto-Tertiary distribution patterns such as *Sambucus* spp. (Hidayati et al., 2000) *Osmorhiza aristata* (Walck et al., 2002), *Aristolochia* spp. (Adams et al., 2005), and *Chaerophyllum temulum* (Vandelook et al., 2007).

Study Species

In total, germination study was conducted with 14 native plant species in temperate zone in the Korean Peninsula. Although these 14 species are only a small subset of the total amount of species growing in Korea, the species selected cover different natural habitat. The species for this dissertation research include two Berberidaceae species (*Leontice microrhyncha* and *Jeffersonia dubia*), eight Ranunculaceae species (*Adonis amurensis*, *Aquilegia buergeriana* var. *oxysepala*,

Pulsatilla tongkangensis, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum* var. *grandisepalum*, *T. uchiyamai*, and *T. coreanum*, three Melanthiaceae species (*Trillium tschonoskii*, *Heloniopsis koreana*, and *H. tubiflora*), and one Liliaceae species (*Erythronium japonicum*). All study species are perennial herbs and flowers in spring except three *Thalictrum* species which flower in summer in Korea. The four plant families has been known that seeds have underdeveloped embryos at maturity (Martin, 1946). Among the species, seed dormancy types of Ranunculaceae were classified in detail and compared with seeds in plants which grow in different countries or continents.

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PART I. Phenology of Dormancy Break and Its Mechanisms by Phytohormones in Seeds of Ranunculaceae Species Native to the Korean Peninsula

CHAPTER I. Phenology of Embryo Growth, Seed Germination, and Seedling Emergence of Five Genera of Ranunculaceae Native to the Korean Peninsula

CHAPTER II. Differences of Dormancy Habit between Morphological (MD) and Morphophysiological dormancy (MPD) in Seeds with Underdeveloped Embryos by Phytohormones

CHAPTER I

Phenology of Embryo Growth, Seed Germination, and Seedling Emergence of Five Genera of Ranunculaceae Native to the Korean Peninsula

ABSTRACT

Phenology of Dormancy break in seeds of five genera of Ranunculaceae was investigated. Ranunculaceae has been described as having seeds with small, underdeveloped embryos at the time of dispersal, and morphological (MD) or morphophysiological dormancy (MPD). However, there are few reports on embryo growth, morphology, and seed germination in this family native to the Korean Peninsula. Seeds of *Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Adonis amurensis*, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum* were collected and sown on field in the natural environment. After sowing, the seeds were exhumed every 1 or 2 weeks and embryo growth, germination, and seedling emergence were observed. In *A. buergeriana* and *P. tonkangensis*, embryo growth, germination, and seedling emergence were completed within 30 days, whereas those in the other six species were delayed for several months. In seeds of *A. amurensis*, embryos grew in autumn after warm period in summer, whereas germination and seedling emergence occurred in late winter and early spring next year, respectively. In *R. crucilobus*, embryo growth, germination, and seedling emergence were occurred

at cold temperatures from late winter to early spring next year, whereas in *R. franchetii*, both embryo growth and germination occurred in autumn before cold season in winter and seedling emerged late winter next year, indicating time lag between germination and seedling emergence. In three *Thalictrum* species, embryo growth, germination, and seedling emergence showed similar patterns, in which those were occurred from March to April next year. Therefore, all species in this study had underdeveloped embryos at maturity and showed MD or MPD. These results indicate that there are different dormancy-breaking mechanisms depending on life cycle of the species in the natural environment, even in the same genus. The mechanisms enabled the species produce seedlings at the beginning of growing season.

Keywords: morphophysiological dormancy, native plants, phenology, Ranunculaceae, seed dormancy, underdeveloped embryos

INTRODUCTION

The most important environmental factors controlling the timing of seed germination are temperature, light, and water (Baskin and Baskin, 1988). However, in a mesic temperate region, temperature frequently is the critical factor and the timing of seed germination in the majority of species is controlled through seasonal temperature changes in nature (Baskin and Baskin, 1988; Baskin and Baskin, 1998).

Timing of seed germination is a crucial event in a plant's life cycle to ensure that seeds germinate at the most favorable conditions for seedling establishment (Harper, 1977). Phenology is plant trait changes through seasonal changes in local environment. The phenology is related to reproductive success, survival and growth, and plant species distribution (Chuine, 2010). Therefore, the phonological observation of seed dormancy traits such as embryo morphology, germination, and cotyledon emergence is important to determine how dormancy pattern is controlled in the natural environment.

Many species in various plant families including Amaryllidaceae, Apiaceae, Berberidaceae, Caprifoliaceae, Hyacinthaceae, Liliaceae, Melanthiaceae, and Ranunculaceae occur in temperate deciduous forest and their seeds have small underdeveloped embryos at dispersal, meaning that they must grow within the seed before germination occur (Baskin and Baskin, 1998; Mamut et al., 2014; Vandelook and van Assche, 2008). If seeds with the underdeveloped embryos germinate in 30 days, the seeds have morphological dormancy (MD) and require no dormancy-breaking pretreatment for germination. If the underdeveloped

embryos are dormant at dispersal, the seeds have morphophysiological dormancy (MPD), which can be broken by warm (≥ 15°C) and/or cold (0-10°C) stratification before radicles protrude from the seeds (Baskin and Baskin, 1998; Nikolaeva, 1977).

One of the best represented plant families in the Korean peninsula is Ranunculaceae. However, little is known about the germination ecophysiology of species of Ranunculaceae that occur in temperate zone in Korea. In the preliminary observations, we found that seeds in the species of Ranunculaceae native to Korea had small embryos compared with the amount of endosperm. This indicates that the seeds might have MD or MPD. Therefore, the aim of our research was to determine whether seeds in eight Ranunculaceae species (*Aquilegia buergeriana*, *Pulsatilla tonkangenesis*, *Adonis amurensis*, two *Ranunculus* species, and three *Thalictrum* species) have MD or MPD and, if so, how the seed dormancy is controlled in the natural environment.

MATERIALS AND METHODS

Study Species

We selected eight native plant species of Ranunculaceae including *Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Adonis amurensis*, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*. Among them, *P. tonkangensis*, *R. crucilobus*, *T. rochenbrunianum*, and *T. uchiyamai* are endemic species, and *T. coreanum* is a rare and endangered species in Korea (Korea National Arboretum, 2008).

Seed Collection

Mature fruits were collected on 12 May and 24 May in 2010 and on 22 May 2011 from *A. amurensis* and on 18 Jun. 2011 from *A. buergeriana* from plants growing in the Hantaek Botanical Garden (37°09´N, 127°40´E), Yongin, Korea. *P. tonkangensis* seeds were collected on 7-18 May 2012 from plants growing within the glass house in the Yeongwol Agricultural Technology Center, Korea (37°18´N, 128°46´E).

Mature fruits were collected on 12-24 Sep. 2011 from *T. rochenbrunianum* and on 24-30 Sep. 2011 from *T. uchiyama*, and on 30 Aug. from *T. coreanum*, respectively, from plants growing in the Hantaek Botanical Garden.

Mature fruits of *R. crucilobus* were collected on 30 May and 1 Jun. 2012 from plants growing in the Hantaek Botanical Garden and those of *R. franchetii* were collected on 29 May 2013 from plants growing in a natural population (37°69´N, 128°76´E) in Daegwallyeong, Gangwon Province, Korea.

Fruits or seeds were allowed to dry in laboratory conditions (20-25°C, 8-11 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light) for 1-2 weeks, then packed in sealed plastic bags and stored dry at 5°C until the beginning of the experiment. Preliminary experiments indicated that dry storage at 5°C for about several months did not affect seed dormancy status.

Phenology of Embryo Growth, Germination, and Seedling Emergence

Seeds of *A. buergeriana* and *P. tonkangensis* were sown on 4 Jul. 2011 and on 1 Aug. 2012, respectively. Seeds of *A. amurensis* were sown on 17 Jun. 2010 and 29 Jun. 2011. Seeds of *T. rochenbrunianum*, *T. uchiyama*, and *T. coreanum* were sown on 28 Sep. 2011, 28 Sep. 2011, and 7 Oct. 2012, respectively. Seeds of *R. crucilobus* were sown on 16 Aug. 2012 and 23 Jul. 2013, and those of *R. franchetii* seeds sown on 23 Jul. 2013.

Embryo growth

Embryo growth phenology was monitored in seeds buried under natural conditions from 2010 to 2014. About 400 seeds were placed in fine-mesh polyester bags filled with potting soil and buried in trays filled with the same potting soil. Trays were placed at ground level in an experimental garden in the campus of Seoul National University, Seoul, Korea. Every 1 or 2 weeks, a bag was exhumed and 10-20 seeds were selected randomly for embryo growth measurement. Seeds were cut into thin sections using a razor blade, and the length of seeds and embryos was measured under a dissecting microscope fitted with an ocular micrometer (KSZ-1B, Samwon Scientific Co., Ltd., Seoul, Korea). The

ratio of embryo length to seed length (E:S ratio) was calculated to correct for a positive correlation between seed length and embryo length (Vandelook et al., 2007).

Germination

Three replicates of 30 seeds were sown on 10 cm plastic pots filled with potting soil and buried in trays filled with the same potting soil. Trays were placed at ground level in an experimental garden in the campus of Seoul National University, Seoul, Korea. Seeds with an emerged radicle were counted and removed every 1 or 2 weeks. A seed was considered as 'germinated' when radicle protrusion occurred and reached at least 1 mm. Intact seeds that had not germinated were buried again on the field.

Seedling Emergence

Timing of seedling emergence was monitored by sowing three replicates of 30 seeds at the depth of 3 cm in plastic pots filled with potting soil and buried in the trays described above. The trays were placed in a shady site in the garden. Emerged seedlings were counted and removed every 1 or 2 weeks. The pots were covered with a net to prevent disturbance by birds. The soil temperature at a depth of 3 cm was recorded every 30 min with a thermo-data logger (Watch Dog Model 450 for 2010-2012 experiment and Model 1000 for 2013-2014 experiment; Spectrum Technologies, Inc., Plainfield, IL, USA), and weekly maximum and minimum temperatures were calculated.

Embryo Morphology

After measuring the length of seeds and embryos using the dissecting microscope, the sections of the seeds were viewed at 60 to 120× magnification and photographed with a Miview USB digital microscope (MV 1302U, CosView Technologies Co., Ltd., Shenzhen, China). Images were used to measure the area of both the embryo and endosperm from samples of up to 20 seeds to quantify the embryo development during the field experiment.

RESULTS

Aquilegia buergeriana

The E:S ratio of freshly matured seeds of *A. buergeriana* was 0.13 ± 0.01 (Figs. I-3B and I-4A). Both embryo growth and germination were completed within 30 days in summer when soil temperatures were high. Emerged seedlings were first observed from 26.7% of the seeds at 2 weeks after sowing. By the end of July, seedling had emerged from 78.3% of the seeds.

Pulsatilla tonkangensis

The E:S ratio of freshly harvested seeds of *P. tonkangensis* was 0.20 ± 0.01 (Figs. I-5B and Fig. I-6A). Embryos grew to their full length within 2 weeks. Both germination and seedling emergence were completed within 4 weeks in summer when mean daily maximum and minimum temperatures were 24.7 and 21.6^{o}C, respectively.

Adonis amurensis

The initial E:S ratio of freshly matured seeds of *A. amurensis* was 0.08 ± 0.01 in 2010 (Figs. I-1 and I-2). The E:S ratio in seeds of 2010 and 2011 was similar (Figs. I-3C and I-4B). Embryos grew only a little in summer of 2010, when mean weekly maximum and minimum soil temperatures were about 26 and 22^{o}C, respectively. However, between 23 Sep. and 18 Nov. 2010, during which time mean weekly maximum and minimum soil temperatures were 16.3 and 13.0^{o}C, respectively, embryos grew rapidly (Figs. I-1 and I-2). Embryos had reached to

the critical E:S ratio (0.80 ± 0.05) required for germination between 18 Nov. and 16 Dec. 2010 during which time mean weekly maximum and minimum soil temperatures were 4.7 and 3.0°C, respectively. From 17 Nov. 2010, split seed coat was observed in most of the seeds in which the embryo was fully elongated (Fig. I-2). However, radicles did not emerge immediately, and afterwards seeds with a split seed coat had been under the winter cold and snow for several months.

Germination began between 10 and 24 Feb. 2011. On 24 Feb., 49.3 ± 3.57% of seeds had germinated (Figs. I-1). Three weeks later, on 17 Mar. 2011, 92.8 ± 3.65% of seeds had already germinated. Phenology of embryo growth and germination was similar in both 2010 (Fig. I-1) and 2011 (Fig. I-3C) studies.

The first newly emerged seedlings of *A. amurensis* were observed on 4 Apr. 2012 and the last ones on 16 Apr.; during this time, mean weekly maximum and minimum soil temperatures were 16.2 and 5.4°C, respectively. During this 2 week period, 91.7 ± 8.3% of the seeds produced emergent seedlings; no additional seedlings emerged thereafter (Fig. I-3C).

Ranunculus crucilobus

The initial E:S ratio of freshly matured seeds of *R. crucilobus* was 0.16 ± 0.01 in 2012 (Fig. I-5C), and the ratio in seeds buried in the field, until 9 Dec. 2012, was only 0.30 ± 0.04 (Fig. I-5C). After cold period in winter, embryos had reached to the critical E:S ratio (0.83 ± 0.04) required for germination between 3 and 10 Mar. 2013 during which time mean weekly maximum and minimum soil temperatures were 8.2 and 0.5°C, respectively.

Germination began between 3 and 10 Mar. 2013. On 10 Mar., 5.6 ± 5.56% of

seeds had germinated (Fig. I-5C). Three weeks later, on 31 Mar. 2013, 90.9 ± 2.98% of seeds had already germinated.

The first newly emerged seedling of *R. crucilobus* was observed on 31 Mar. 2013 and the last ones on 14 Apr.; during this time, mean weekly maximum and minimum soil temperatures were 15.2 and 3.6°C, respectively. During this 2 week period, 91.7 ± 4.41% of the seeds produced emergent seedlings; no additional seedlings emerged thereafter (Fig. I-5C). Phenology of embryo growth, germination, and seedling emergence was similar in both 2012 (Fig. I-5C) and 2013 (Fig. I-7B) studies.

Ranunculus franchetii

The mean maximum and minimum soil temperatures were 26.4 and 22.9 from 23 Jul. to 14 Sep. and 18.8 and 13.5 from 15 Sep. to 14 Nov. 2013 (Fig. I-7A). The initial E:S ratio of freshly matured seeds on 23 Jul. was 0.16 ± 0.01 (Fig. I-7C). Embryos grew little until they started slow growth in September. From the mid-September, when temperature was medium, embryos grew rapidly and reached a mean of 0.87 E:S ratio on 14 Nov., by which time 83.3% of seeds had germinated (Fig. I-7C).

Seeds sown on 23 Jul. 2013 did not germinate immediately. After the high temperatures of summer and medium temperatures of autumn, germination was first observed on 31 Oct. 2013 (Fig. I-7C). Many remaining seeds germinated rapidly from 31 Oct. to 14 Nov., with mean daily maximum and minimum soil temperatures of 9.7 and 6.8°C, respectively, and 83.3 ± 16.7% of seeds had germinated by 14 Nov. 2013.

Thalictrum rochenbrunianum

The initial E:S ratio of freshly matured seeds was 0.21(Figs. I-3D and I-4C). Embryos in seeds grew little from autumn to mid-winter. After low temperature period in winter, embryos grew rapidly after late February. The embryos had reached the critical E:S ratio of 0.71 required for germination on 14 Mar. 2012.

Germination began between 11 and 24 Mar. 2012. On 15 Apr. 2012, 91.0% of the seeds had already germinated (Figs. I-3D and I-4C).

The first newly emerged seedlings were observed between 31 Mar. and 8 Apr. 2012 (Figs. I-3D and I-4C). During this period, 81.3% of the seeds produced emergent seedlings in *T. rochenbrunianum*; no additional seedlings emerged thereafter.

Thalictrum uchiyamai

The initial E:S ratio of freshly matured seeds was 0.13 (Figs. I-3E and I-4D). Embryos in seeds grew little from autumn to mid-winter. After low temperature period in winter, embryos grew rapidly after late February. The embryos had reached the critical E:S ratio of 0.82 required for germination on 21 Mar. 2012 .

Germination began between 11 and 24 Mar. 2012 (Figs. I-3E and I-4D). 94.6% of seeds in *T. uchiyamai* had already germinated on 25 Mar. 2012.

The first newly emerged seedlings of the three species were observed between 31 Mar. and 8 Apr. 2012 (Figs. I-3E and I-4D). During this period, 77.1% of the seeds produced emergent seedlings in *T. uchiyamai*; no additional seedlings emerged thereafter.

Thalictrum coreanum

The initial E:S ratio of freshly matured seeds was 0.19 (Figs. I-5D and I-6C). Embryos in seeds grew little until mid-winter. After low temperature period in winter, embryos grew rapidly after mid-March in *T. coreanum*. The embryos had reached the critical E:S ratio of 0.88 required for germination on 31 Mar. 2013.

Germination began between 11 and 24 Mar. 2012 (Figs. I-5D and I-6C). On 7 Apr. 2013, 96.4% of seeds in *T. coreanum* had already germinated.

The first newly emerged seedlings of the three species were observed between 31 Mar. and 8 Apr. (Figs. I-5D and I-6C). During this period, 90% of the seeds produced emergent seedlings in *T. coreanum*; no additional seedlings emerged thereafter (Figs. I-5D and I-6C).

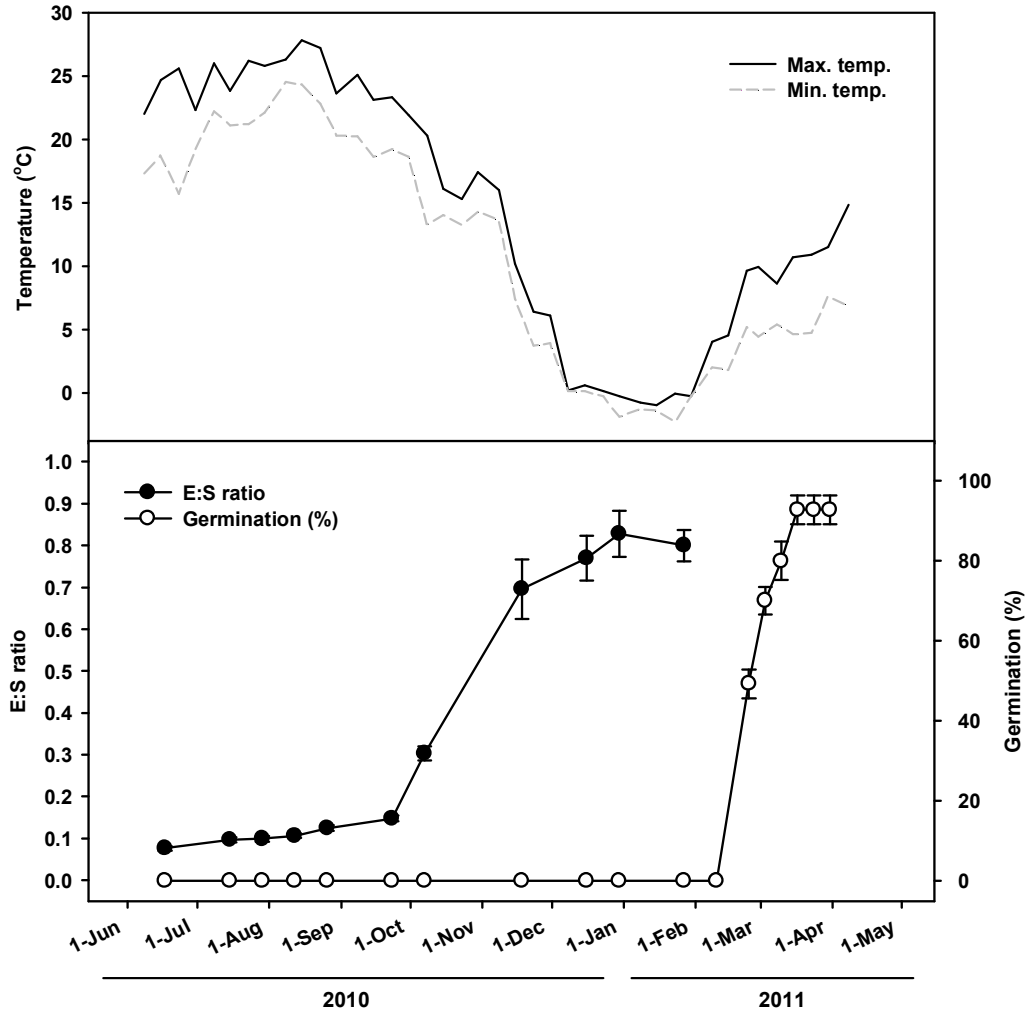

Fig. I-1. Mean weekly maximum and minimum soil temperatures and penology of embryo growth and germination of *Adonis amurensis* seeds buried at a depth of 3 cm in 2010. Vertical bars represent SE. The E:S ratio is the ratio of embryo length to seed length. The soil temperature at a depth of 3 cm was measured every 30 min with a thermo data logger and the mean weekly maximum and minimum soil temperatures were calculated.

Fig. I-2. Embryo growth and radicle emergence of *Adonis amurensis* seeds kept outdoor in Seoul, Korea in 2010. Split pericarp (dehiscence) was observed from Nov. 2010. Scale bars are 1 mm. Em, embryo; En, endosperm; Pe, pericarp; SC, seed coat.

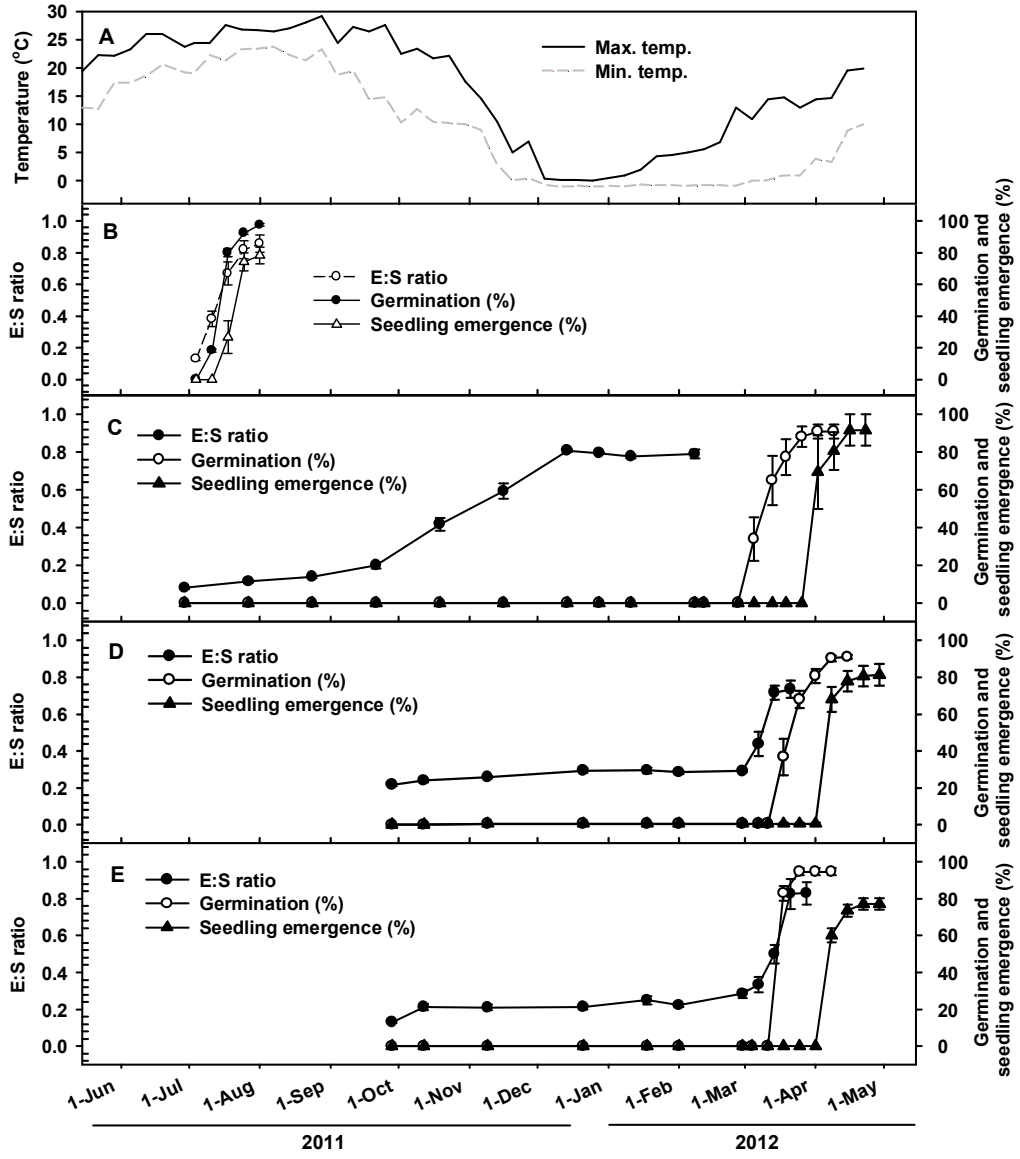

Fig. I-3. Mean weekly maximum and minimum soil temperatures (A) and penology of embryo growth and germination of *Aquilegia buergeriana* (B), *Adonis amurensis* (C), *Thalictrum rochenbrunianum* (D), and *T. uchiyamai* (E) seeds buried at a depth of 3 cm in 2011. Vertical bars represent SE. The E:S

ratio is the ratio of embryo length to seed length. The soil temperature at a depth of 3 cm was measured every 30 min with a thermo data logger and the mean weekly maximum and minimum soil temperatures were calculated.

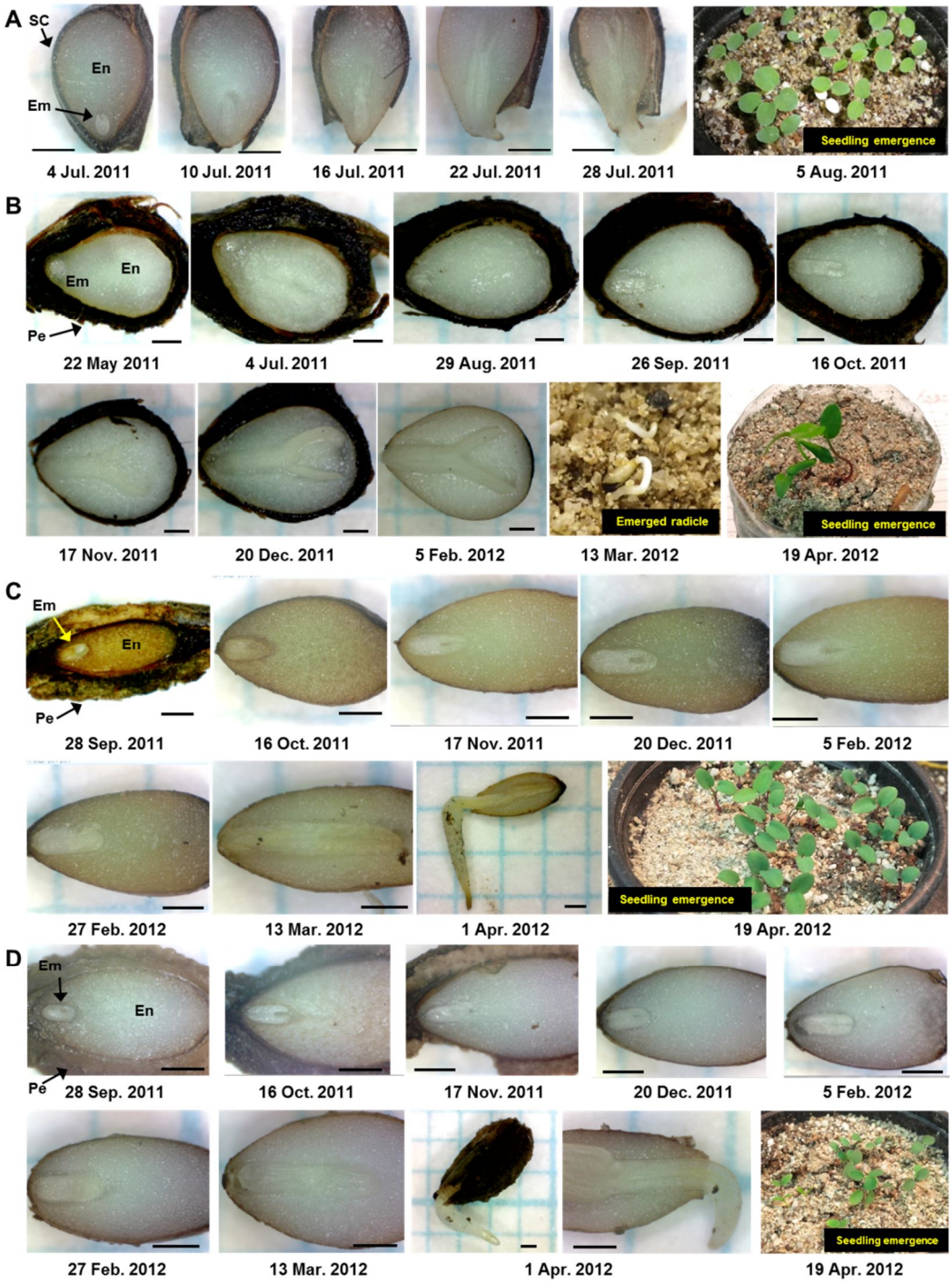
A
SC
En
Em
4 Jul. 2011
10 Jul. 2011
16 Jul. 2011
22 Jul. 2011
28 Jul. 2011
Seedling emergence
5 Aug. 2011
B
Em
En
Pe
22 May 2011
4 Jul. 2011
29 Aug. 2011
26 Sep. 2011
16 Oct. 2011
17 Nov. 2011
20 Dec. 2011
5 Feb. 2012
Emerged radicle
13 Mar. 2012
Seedling emergence
19 Apr. 2012
C
Em
En
Pe
28 Sep. 2011
16 Oct. 2011
17 Nov. 2011
20 Dec. 2011
5 Feb. 2012
27 Feb. 2012
13 Mar. 2012
1 Apr. 2012
Seedling emergence
19 Apr. 2012
D
Em
En
Pe
28 Sep. 2011
16 Oct. 2011
17 Nov. 2011
20 Dec. 2011
5 Feb. 2012
27 Feb. 2012
13 Mar. 2012
1 Apr. 2012
Seedling emergence
19 Apr. 2012

Fig. I-4. Embryo growth, radicle emergence, and seedling emergence in seeds of *Aaquilegia buergeriana* (A), *Adonis amurensis* (B), *Thalictrum rochenbrunianum* (C), and *T. uchiyamai* (D) kept outdoor in Seoul, Korea in 2011. Scale bars are 0.05 mm. Em, embryo; En, endosperm; Pe, pericarp; SC, seed coat.

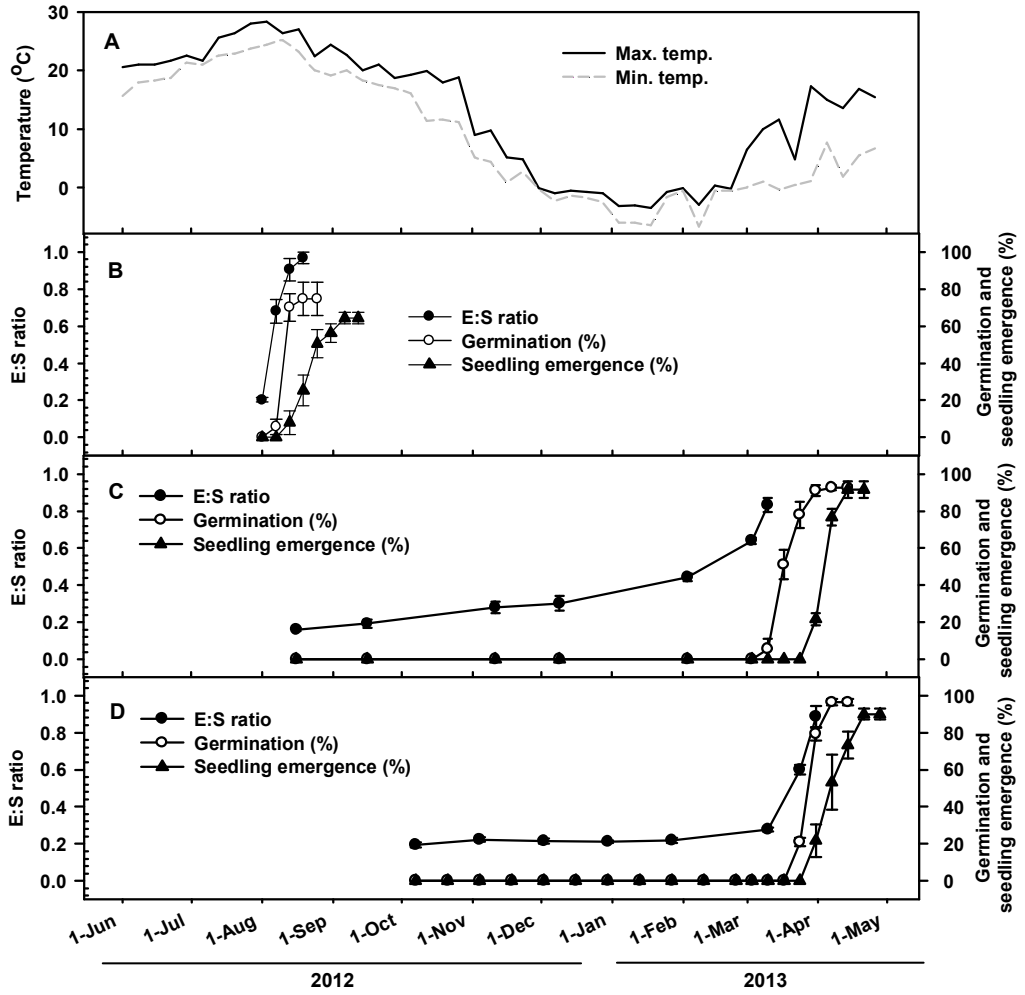

Fig. I-5. Mean daily maximum and minimum soil temperatures (A) and phenology of embryo growth and germination of *Pulsatilla tonkangensis* (B), *Ranunculus crucilobus* (C), and *Thalictrum coreanum* (D) seeds buried at a depth of 3 cm in 2012. Vertical bars represent SE. The E:S ratio is the ratio of embryo length to seed length. The soil temperature at a depth of 3 cm was measured every 30 min with a thermo data logger and the mean daily maximum and minimum soil temperatures were calculated.

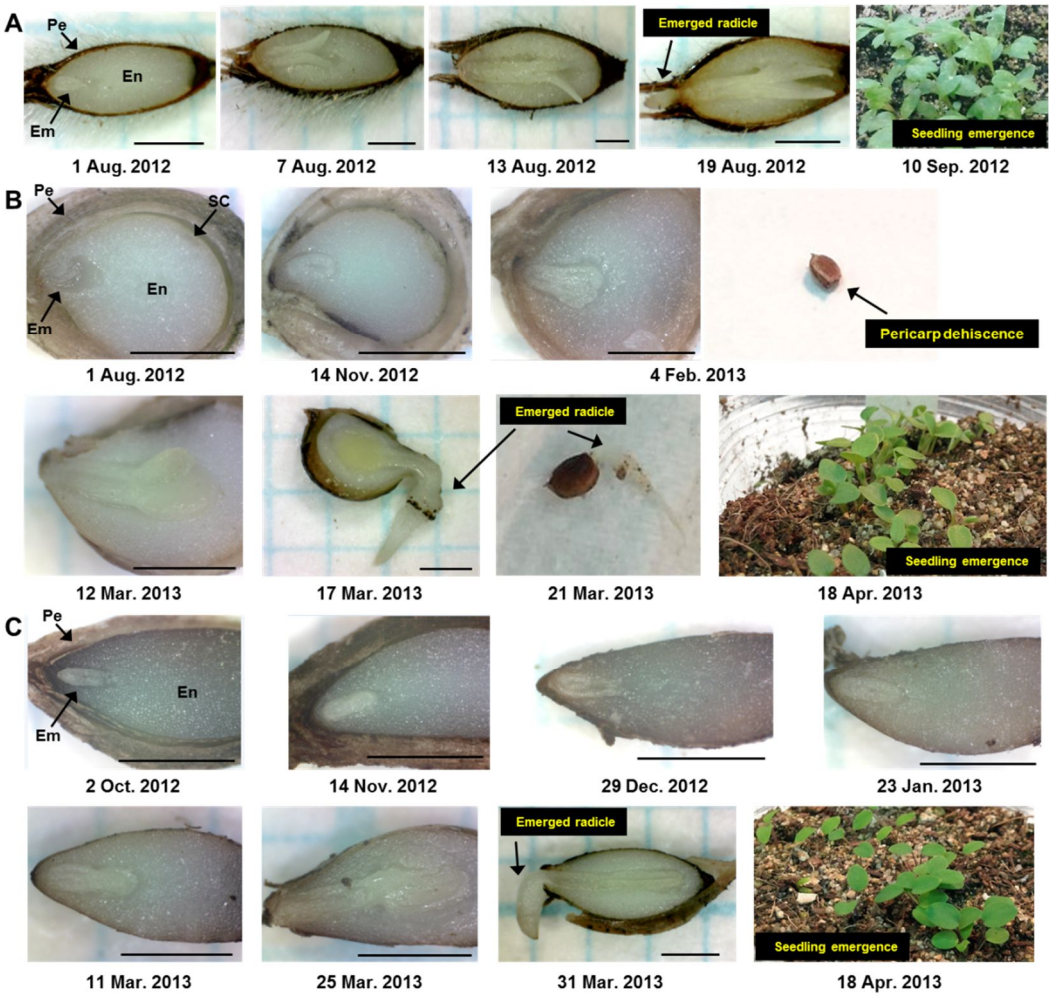

Fig. I-6. Embryo growth, radicle emergence, and seedling emergence in seeds of *Pulsatilla tonkangensis* (A), *Ranunculus crucilobus* (B), and *Thalictrum coreanum* (C) kept outdoor in Seoul, Korea in 2012. Scale bars are 1 mm. Em, embryo; En, endosperm; Pe, pericarp; SC, seed coat.

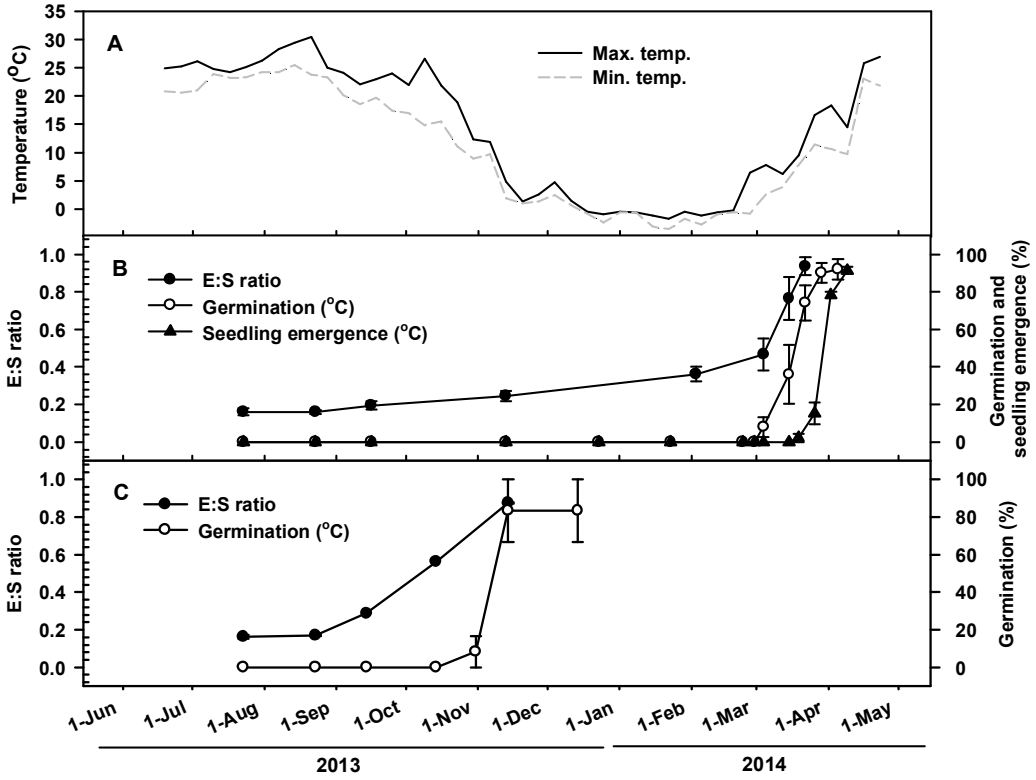

Fig. I-7. Mean daily maximum and minimum soil temperatures (A) and phenology of embryo growth, germination or seedling emergence of *Ranunculus crucilobus* (B) and *R. franchetii* (C) seeds buried at a depth of 3 cm in 2013. Vertical bars represent SE. The E:S ratio is the ratio of embryo length to seed length. The soil temperature at a depth of 3 cm was measured every 30 min with a thermo data logger and the mean daily maximum and minimum soil temperatures were calculated.

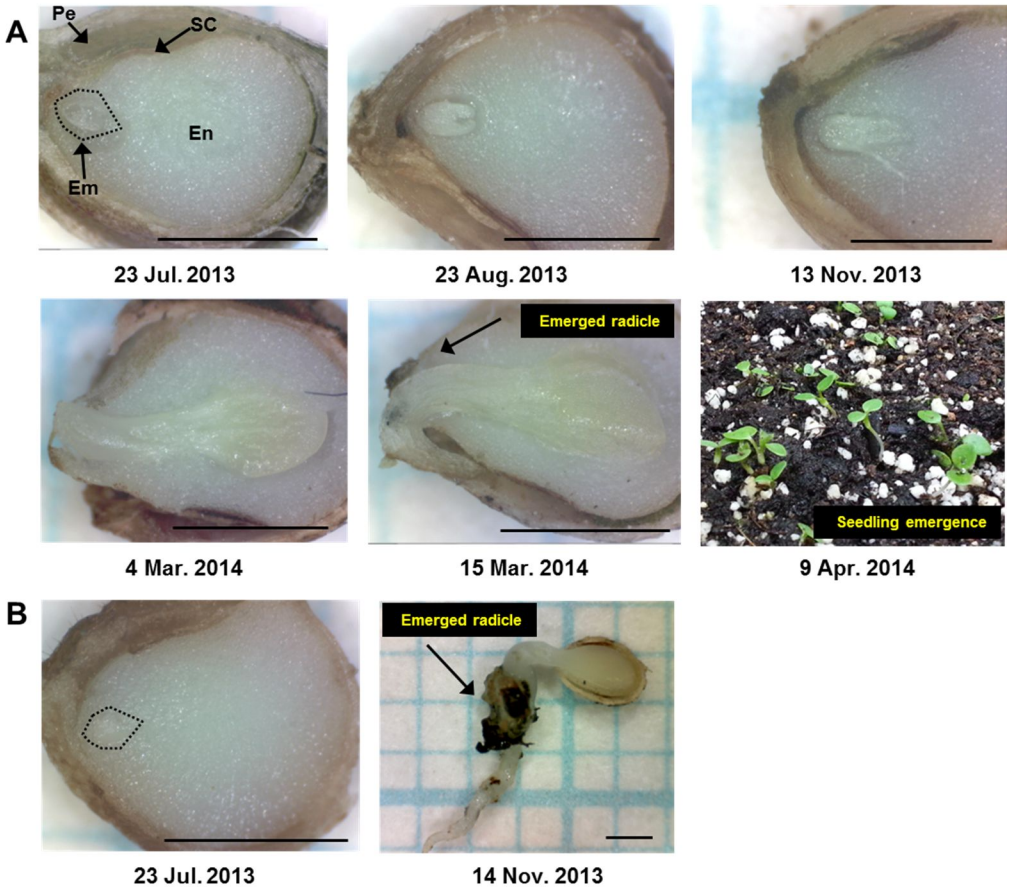

Fig. I-8. Embryo growth, radicle emergence, or seedling emergence in seeds of *Ranunculus crucilobus* (A) and *R. franchetii* (B) kept outdoor in Seoul, Korea in 2013. Scale bars are 1 mm. Em, embryo; En, endosperm; Pe, pericarp; SC, seed coat.

DISCUSSION

Seeds of eight Ranunculaceae species (*A. buergeriana*, *P. tonkangensis*, *A. amurensis*, *R. crucilobus*, *R. franchetii*, *T. rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*) in this study showed different dormancy habit in the natural environment. Although germination and seedling emergence of *A. amurensis*, two *Ranunculus* species and three *Thalictrum* species occurred during similar period from late winter to early spring, the time of embryo growth was clearly different. Embryos in seeds of *A. amurensis* and *R. frachetii* grew at medium temperature in autumn after high temperature in summer. On the other hand, embryos in seeds of three *Thalictrum* species grew in late winter when soil temperatures increased to about 10^{o}C. *R. crucilobus* grew consistently at relatively low temperatures in winter. It has been reported that seeds need warm followed by cold temperature sequence for breaking dormancy in many species whose embryo growth occurs in autumn (Baskin and Baskin, 1998; Kondo et al., 2002). However, *T. mirabile* seeds (native in North America) need only low temperatures for dormancy break, in which embryo growth occurs in early spring next year (Walck et al., 1999). This result indicates that temperature requirements for dormancy break might be different depending on the species in the natural environment.

Temperate forests are predictable in the sense that every year the forest canopy closes in spring and opens in autumn. Taking into account that winter is the severe season in the temperate regions, seedlings acquire the highest fitness by emerging in spring (Vandelook, 2009). Indeed most of the typical woodland herbs studied so far, maintain the spring emergence strategy, but they have evolved different

mechanisms resulting in spring emergence. The typical mechanism is found in seeds of which seed dormancy is broken by cold stratification in winter and whereby seedlings emerge shortly after radical emergence in spring next year. Very often seeds of these kind of species can germinate at low temperatures, enabling seedlings to emerge as soon as temperatures rise very early in the growing season.

Seeds of all eight species in this study had small embryos at maturity. To determine whether seeds have underdeveloped embryos at maturity, embryo growth data has to be demonstrated. For example, in *Drosera angelica*, seeds at dispersal have small embryos relative to the length of the seeds. However, embryos did not elongate in the seeds prior to emergence of the radicle; thus the seeds were considered to have fully matured embryos and only physiological dormancy (PD) (Baskin and Baskin, 2005). We found that embryos in seeds of the eight species grew to the critical length before radicle emerged. Thus, the embryos were underdeveloped.

In freshly harvested seeds of *A. buergeriana* and *P. tonkangensis*, germination ($\geq$ 70%) and seedling emergence ($\geq$ 65%) were completed within 30 days. On the other hand, seeds of the other six species (*A. amurensis*, two *Ranunculus* species, and three *Thalictrum* species) did not germinate for several months in the natural environment. If embryo growth and germination are completed at suitable temperatures within 30 days, without a dormancy-breaking treatment, seeds have MD (Baskin and Baskin, 1998; Nikolaeva, 1977). However, if seeds with underdeveloped embryos need a dormancy breaking pretreatments such as cold and/or warm stratification before they can germinate, they have both

morphological and physiological dormancy, which is known as morphophysiological dormancy (MPD). Thus, seeds of *A. buergeriana* and *P. tonkangensis* have MD and those of the other six species have MPD.

It has been reported that MD is not very common in any vegetation region on earth and MPD is more common in temperate deciduous forests than in any other vegetation region on earth (Baskin and Baskin, 1998; Baskin and Baskin, 2003). However, we found that two perennial species (*A. buergeriana* and *P. tonkanensis*) had MD in the Korean Peninsula. This indicates that as we investigate more about the biogeography of seed dormancy and germination, undoubtedly many additional species with MD will be added to the dormancy list.

Baskin and Baskin (2014) recognized nine types of MPD based on the temperature requirements for dormancy breaking, temperatures at the time of embryo growth, and responses to gibberellic acid. The nine types are non-deep simple, intermediate simple, deep simple, non-deep simple epicotyl, deep simple epicotyl, deep simple double, non-deep complex, intermediate complex, and deep complex MPD. In addition, they described one extra type (a special type of deep simple epicotyl MPD) for seeds of *Hydrastis canadensis*. Therefore, more detailed studies are needed to classify which types of MPD the study species have.

Two *Ranunculus* species (*R. crucilobus* and *R. franchetii*) showed different dormancy pattern in the natural environment, whereas three *Thalictrum* species (*T. rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*) had similar dormancy pattern in this study. Seeds of *R. crucilobus* germinated in early spring, whereas seeds of *R. franchetii* germinated in autumn in Korea. Interspecific variations in stratification temperatures for dormancy-break and germination within the same

genera were reported in *Lonicera fragrantissima* and *L. morrowii* (Hidayati et al., 2000), *Corylopsis coreana* and *C. sinensis* (Roh et al., 2008), and in *Muscari* spp. (Doussi and Thanos, 2002).

We found that germination of *R. franchetii* had already ended within November. But, cotyledons did not emerge immediately after radicle emergence. Germinated seeds in the field did not produce seedlings in laboratory conditions when the seeds were moved to room temperatures (data not shown). Many plants whose cotyledons do not emerge immediately after radicle emergence are known. This phenomenon is referred to as 'epicotyl dormancy' (Baskin and Baskin, 1998). Epicotyl is the embryonic leaves and shoot, and it contains the growing point (apical meristem) and the first two leaves (Mullen et al., 2008). Mesocotyl are called the first internode, and hypocotyl is the stem tissue between epicotyl and the radicle (Mullen et al., 2008). In *R. franchetii* seeds, although emerged radicles grew continuously at room temperatures, the emergence of leaves and shoot did not occurred in the seeds (data not shown). Therefore, *R. franchetii* seeds might have epicotyl dormancy. Similar results were found in *Erythronium japonicum* native in Japan (Kondo et al., 2002) and *Narcissus hispanicus* native in the Iberian Peninsula (Copete et al., 2011). Within classification system of MPD, there are two types of epicotyl MPD: these are deep simple epicotyl and non-deep simple epicotyl MPD. Thus, more detailed study is needed to clarify which typs of epicotyl MPD the seeds have.

The seeds coats of *A. amurensis* began to split from November, indicating that embryos in the seeds had fully elongated at that time. However, seeds did not germinate until the beginning of February next year. Seeds with a split seed coat

had been under the winter cold and snow for several months in the natural environment. Germination occurred in early spring next year. Baskin and Baskin (1998) reported a special type of deep simple epicotyl MPD for seeds of *Hydrastis canadensis* which has some characteristics of both deep simple MPD and of deep simple epicotyl MPD. Seeds with this special kind of MPD are characterized by embryo elongation and seed coat splitting in autumn and emergence of both the radicle and the seedlings in spring next year. In the kind of MPD, radicles grow continuously inside the seeds in autumn, but they did not protrude from the seeds before subjected to the winter chilling. This indicates that the seeds of *A. amurensis* might have a special type of deep simple epicotyl MPD. But temperature requirements (warm and/or cold sequence) for dormancy break have to be studied for the concrete classification.

It takes more than seven months to obtain seedlings in the study species except *A. buergeriana* and *P. tongkangensis*. Therefore, requirements for dormancy break, such as temperature and light, have to be determined for practical propagation strategies in these species.

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CHAPTER II

Dormancy Habit Differences between Morphological (MD) and Morphophysiological dormancy (MPD) in Seeds with Underdeveloped Embryos by Phytohormones

ABSTRACT

There have been few studies on hormonal regulation of dormancy in buried seeds with morphological (MD) and morphophysiological dormancy (MPD). The phenology of embryo growth and germination in seeds of *Aquilegia buergeriana* and *Adonis amurensis* (Ranunculaceae) was investigated in the natural environment, and the seasonal changes of endogenous phytohormone levels (ABA and GAs) were analyzed. *A. buergeriana* seeds had MD, and embryo growth and germination were completed within 30 days in the natural environment when soil temperatures were high. On the other hand, *A. amurensis* seeds had MPD, and the seeds experienced high in summer followed by cold temperatures in winter to break seed dormancy in the natural environment. In *A. buergeriana* seeds, ABA content and sensitivity decreased rapidly, and GA content and sensitivity increased rapidly after burial. On the other hand, in *A. amurensis* seeds, ABA content decreased drastically after burial but GA content did not increase before the seeds experienced temperature changes from high in summer to medium temperatures in autumn in the natural environment. When underdeveloped embryos grew rapidly, ABA was non-detectable and GA content increased. After embryo maturation in

autumn, ABA content increased and GA content decreased at the same time, and thus, the seeds remained ungerminated during cold season in winter. When the seeds started to germinate after cold period in winter, GA content increased rapidly. GA_4 played a key role in stimulating embryo growth and germination in the seeds with MD and MPD. The changes of GA/ABA ratio were similar to the changes of embryo growth and germination in the buried seeds. These results indicate that MD or MPD in the basal angiosperm taxa also is controlled by a hormone balance model.

Keywords: morphophysiological dormancy, native plants, phenology, plant hormones, seed dormancy, underdeveloped embryos

INTRODUCTION

The key role of gibberellin (GA) and abscisic acid (ABA) in seed dormancy and germination is well known (Bewley, 1997; Cadman et al., 2006; Koornneef et al., 2002). There is considerable evidence that abscisic acid (ABA) plays a key role in the induction and maintenance of seed dormancy, whereas gibberellins (GAs) are associated with dormancy release and germination (Cadman et al., 2006; Kucera et al. 2005). Dormant seeds treated with fluridone, which is a compound that inhibits carotenoid synthesis and, thus, ABA synthesis, often have similar germination pattern to non-dormant seeds, indicating that the continued synthesis of ABA is required for dormancy induction and maintenance in seeds of many species (Feurtado et al. 2007; Kucera et al. 2005; Yoshioka et al. 1998). Also, application of paclobutrazol, which is an inhibitor of GA biosynthesis, was found to prevent seed germination, indicating that a de novo biosynthesis of GAs is required during imbibition and, thus, dormancy break and germination (Karssen et al. 1989; Bradford and Nonogaki 2007); however, endogenous changes in these phytohormones in buried seeds are still poorly understood (Garcia et al., 2012)

In addition to ABA and GA biosynthesis/content, it has recently been demonstrated that ABA-gibberellic acid hormone balance mechanism controls cycling through dormant states that depends on synthetic and catabolic pathways of both hormones at the same time (Cadman et al., 2006). It appears to be the GA/ABA ratio, but not the hormone contents, controls germination (Finch-Savage and Leubner-Metzger, 2006).

While dormancy maintenance also depends on low GA/ABA ratio, dormancy

release and breaking are related to a net shift to increased GA biosynthesis and ABA degradation, resulting in high GA/ABA ratio (Ali-Rachedi et al., 2004; Cadman et al., 2006). In addition to these hormone contents and balance, the transition from the dormant to the nondormant state of many seeds is characterized by a decrease in ABA sensitivity and an increase in GA sensitivity (Chiwocha et al., 2005). However, most of these studies used seeds of *Arabidopsis thaliana*, *Sorghum bicolor*, *Nicotiana*, *Hordeum vulgare*, and so on. Seeds of these species have only physiological dormancy (PD). Thus, few studies have been conducted in seeds of many species with morphological (MD) or morphophysiological dormancy (MPD) in the basal angiosperm families.

If seeds with the underdeveloped embryos germinate in 30 days, the seeds have MD and require no dormancy-breaking pretreatment for germination. If the underdeveloped embryos are dormant at dispersal, the seeds have MPD, which can be broken with warm ($\geq 15^{o}C$) and/or cold ($0\sim10^{o}C$) stratification before radicles protrude from the seeds (Baskin and Baskin, 1998; Nikolaeva, 1977). In seeds of *Taxus* species which has deep simple MPD, Devillez (1976) reported that warm stratification induced after-ripening in underdeveloped embryos and overcame morphological dormancy, while cold stratification increased embryo growth and physiological changes. Chien et al. (1998) reported that the strong seed dormancy of *T. mairei* could be caused by a high ABA content (8888 pg per seed) and underdeveloped embryos in the seeds. They concluded that warm stratification increased the growth of embryos and decreased ABA content, but the seeds still remained ungerminated, and then subsequent cold stratification induced the response to GAs and initiated dormancy release and germination. In *Fraxinus*

excelsior, which has deep simple MPD, seeds at maturity contained a high concentration of ABA and were dormant. When the seeds were warm stratified, underdeveloped embryos continued to grow and ABA concentrations decreased gradually. GA_3 was detected during embryo growth. Subsequent cold stratification was required for germination and an increased GA_3 coincided with a decreased ABA level at the same time (Finch-Savage and Leubner-Metzger, 2006). It demonstrated that endogenous GA and ABA level is closely related to dormancy break and germination in seeds with MPD.

In this study, we selected two species (*Aquilegia buergeriana* and *Adonis amurensis*) in Ranunculaceae, a basal angiosperm family because the two species showed MD and MPD, respectively, in which *A. buergeriana* seeds germinated to more than 70% within 30 days whereas *A. amurensis* seeds required warm followed by cold temperature sequences (for more than 8 months) for germination. Therefore, we wanted to know how the different dormancy habit is controlled in buried seeds in the natural environment by a content and balance of, and sensitivity to phytohormones ABA and GA. We described the phenology of embryo growth and germination, response to exogenous PGRs combinations, and seasonal changes in endogenous ABA and GAs.

MATERIALS AND METHODS

Seed Collection

Mature fruits were collected on 6~16 May 2012 from *A. amurensis* and 18 Jun. 2012 from *A. buergeriana* growing in the Hantaek Botanical Garden (37°09′N, 127°40′E), Yongin, Korea. Fruits or seeds were allowed to dry in laboratory conditions (20~25°C, 8~11 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light) for 1~2 weeks, then packed in sealed plastic bags and stored dry at 5°C until the beginning of the experiment.

Phenology of Embryo Growth and Germination in Buried Seeds

Seeds of *A. amurensis* and *A. buergeriana* were sown on 15 Jul. 2012 and on 1 Aug. 2012.

Embryo growth

Embryo growth was monitored in buried seeds under natural conditions. About 400 seeds were placed in fine-mesh polyester bags filled with potting soil and buried in trays filled with the same potting soil. Trays were placed at ground level in an experimental garden in the campus of Seoul National University, Seoul, Korea. Every 6 days for *A. buergeriana* or every 1 month for *A. amurensis* a bag was exhumed and 10~20 seeds were selected randomly for embryo growth measurement. Seeds were cut into thin sections using a razor blade, and the length of seeds and embryos was measured under a dissecting microscope fitted with an ocular micrometer (KSZ-1B, Samwon Scientific Co., Ltd., Seoul, Korea). The ratio of embryo length to seed length (E:S ratio) was calculated to correct a

positive correlation between seed length and embryo length (Vandelook et al., 2007).

Germination

Three replicates of 20 seeds were sown on 10 cm plastic pots filled with potting soil and buried in trays filled with the same potting soil. Trays were placed at ground level in the experimental garden. Seeds with an emerged radicle were counted and removed every 6 days for *A. buergeriana* or every 1~2 weeks for *A. amurensis*. A seed was considered 'germinated' when radicle protrusion occurred and reached at least 1 mm. Intact seeds that had not germinated were buried again in the field.

Laboratory Experiment

Seeds were treated with 500 $mg \cdot L^{-1}$ benomyl for more than 3 h for fungal control before being used in laboratory experiment. Unless otherwise stated, each of the three replicates of 20 seeds was used. The seeds were placed on two sheets of filter paper (Whatman No. 2, GE Healthcare Co., Ltd., Buckinghamshire, UK) in 90 × 15 mm Petri dishes and moistened with distilled water. All dishes were wrapped with parafilm (Pechiney Plastic Packaging, Menasha, WI, USA) to restrict water loss during incubation. At all temperature regimes, a 12-h light/dark photoperiod was provided by a cool white fluorescent lamps that provided a photon flux density of approximately 30~40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the incubators (DS-13MCLP, Dasol Scientific Co., Ltd., Hwaseong, Korea). Radicle emergence was monitored to calculate percent germination. Seeds were considered 'germinated' when radicles emerged at least 1 mm.

Effect of Exogenous PGRs Treatment on Dormancy Break and Germination

To study the correlation between phytohormones (ABA and GA) and seasonal changes of seed dormancy in the natural environment, seeds of *A. amurensis* and *A. buergeriana* were soaked in 13 solutions with PGRs combinations: distilled water (control), GA_3, GA_{4+7}, ABA, fluridone (Flu), paclobutrazole (Pac), GA_3 + Flu, GA_3 + Pac, GA_3 + ABA, GA_{4+7} + Flu, GA_{4+7} + Pac, GA_{4+7} + ABA, ABA + Flu.

100 $mg \cdot L^{-1}$ GA_3, 100 $mg \cdot L^{-1}$ GA_{4+7}, 100 $mg \cdot L^{-1}$ ABA, 20 $mg \cdot L^{-1}$ Flu, and 50 $mg \cdot L^{-1}$ Pac were used for each treatment.

In *A. buergeriana*, seeds at harvest (before burial) were soaked in the solutions for 24 h at room temperature, and then incubated at 25/15°C for 32 days. In addition, seeds were buried on 1 Aug. 2012 and then exhumed at 6 days after burial. Exhumed seeds were also soaked in the solutions. At this time, embryos had already started to grow inside the seeds, but still ungerminated.

In *A. amurensis*, seeds at harvest (before burial) were soaked in the solutions for 48 h at room temperature, and then incubated at 15/6°C for 12 weeks. In addition, seeds were buried on 15 Jul. 2012, and then exhumed on 26 Sep. and 6 Nov. in 2012, and 4 Jan., 29 Jan. and 3 Mar. in 2013 after burial. After exhuming, the seeds were treated with the PGRs solutions.

During incubation, germinated seeds were counted and re-sterilized with 500 $mg \cdot L^{-1}$ benomyl. In addition, the number of seeds of *A. amurensis* with a split pericarp but no radicle protrusion was counted. A split pericarp in *A. amurensis* is an indicative of the embryo growth.

Endogenous Phytohormones (ABA and GAs) Analyses

To detect the endogenous hormones during dormancy break, *A. buergeriana* seeds collected in 2012 were buried on 1 Aug. 2012 in the experimental garden and then exhumed three times after burial: 1) seed at harvest, 2) seeds at 6 days after burial, and 3) seeds at 12 days after burial. The exhumed seeds at each time were used for the phytohormones analyses.

In addition, *A. amurensis* seeds collected in 2012 were buried on 15 Jul. 2012 in the experimental garden and then exhumed seven times after burial: at harvest, on 28 Aug., on 26 Sep., on 31 Oct., on 31 Dec. in 2012, and 29 Jan. and 28 Feb. in 2013. The exhumed seeds at each time were used for the phytohormones analyses. Each sample was stored at -80ºC until they were analyzed for GAs and ABA.

Before starting the experiments, all seeds were lyophilized and weighed. Extraction and purification was conducted according to Geng et al. (2007), Miao et al. (2011), and Zhang et al. (2012) with some modifications. Frozen sample was powdered in liquid nitrogen. Then 80% methanol containing 1 mM butylated hydroxytoluene (BHT) as an antioxidant was added to the fine powder and stored at 4ºC for 24 h in the dark. The supernatant was collected by centrifugation at 15,000 rpm for 15 min at 4ºC. The precipitate was re-extracted once more in the same way.

Each supernatant was well mixed with 0.2 g polyvinylpyrolidone (PVPP, Sigma Chemical Co., St. Louis, MO, UK) for 30 min under dark condition and collected through centrifugation. Then, the supernatants were put in flask (25 cm^3) and were evaporated off the methanol at 35ºC under reduced pressure with a rotary evaporator. The extracts were dissolved in 5% MeOH and filtered with Sep-

Pak C18 cartridges (Waters, Hichrom Ltd., Berkshire, UK). The solvent passed through Sep-Pak was discarded. The residue in Sep-Pak cartridges was eluted by 80% MeOH. The filtrates were re-filtered with Sep-Pak C18 cartridges. Then eluted solution was evaporated off the methanol at 35ºC under reduced pressure with a rotary evaporator and then dissolved with 80% methanol (2mL) and collected in 2mL vials. The extracts were filtered through 0.2μm membrane filter and then concentrated (200 μL) using Speed Vac (OPR-SVQ-70, Operon Co., Korea).

For phytohormones analyses, ten μL of each sample was analyzed using HPLC-ESI-MS/MS system in The National Instrumentation Center for Environmental Management (NICEM). A Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap instrument (Thermo Scientific, Waltham, MA, USA) equipped with Dionex Ultomate 3000 RSLCnano HPLC system was used. Mass spectromeric analyses were performed using a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap instrument mass spectrometer, with ESI interface. Ionization of analytes was carried out using electrospray ionization (ESI). The capillary temperature was maintained at 320ºC, the ion source voltage was set at 3.5 kV and the sheath and Aux gas was set at 30 and 5 units. The capillary voltage was set at 3.5kV. The average scan time was 0.01 min while the average time to change polarity was 0.02 min. The HCD energy was generally chosen in order to maintain about 20% abundance of the precursor ion.

GA_3 (48880, Sigma Chemical Co., Poole, UK), GA_4 (G7276, Sigma Chemical Co. UK), and ABA (A1049, Sigma Chemical Co. UK) were used as the standard for determining the hormones.

RESULTS

Phenology of Embryo Growth and Germination in Buried Seeds

Aquilegia buergeriana

Both embryo growth and germination were completed within 30 days in summer when temperatures were high (Fig. II-1). E:S ratio increased from 0.13 on 1 Aug. to 0.70 on 13 Aug. (12 days after burial) in 2012. After 12 and 18 days of burial, 56.7 and 65.8 % of the seeds had germinated.

Adonis amurensis

Embryos grew only a little in summer in 2012, when temperatures were relatively high (Fig. II-1). However, between late September and early November in 2012, during which time temperatures were medium (about 15^{o}C on average), embryos grew rapidly. Embryos had fully elongated to the critical length required for germination between middle November and early December in 2012 during which time mean temperatures were relatively low (4~6^{o}C). However, radicles did not emerge until the beginning of February next year (Fig. II-1).

Germination began between late February and early March in 2013. Thus, there was a time lag between the time of embryo growth and germination.

Effect of Exogenous PGRs Treatment on Dormancy Break and Germination

Aquilegia buergeriana

Freshly matured seeds (control) started to germinate after 8 days of treatment and most of the seeds germinated within 32 days, giving a maximum of about 80% germination (Fig. II-2A). GA_3 and GA_{4+7} promoted germination rate slightly but

not final germination percentage (Fig. II-2A). ABA and Flu delayed germination slightly, but the final germination percentages were similar to that of control. However, Pac, GA_3 + Pac, and GA_{4+7} + Pac (solutions including Pac) prevented germination, in which no seeds treated with Pac germinated in 32 days. GA_3 + Flu and GA_{4+7} + Flu, and ABA + Flu (solutions including Flu) promoted germination rate slightly but not final germination percentage significantly (Fig. II-2A). Seeds treated with GA_3 + ABA reduced germination, but GA_{4+7} + ABA had a similar germination rate compared to the control, indicating that GA_{4+7} was much more effective in stimulating germination than GA_3.

Buried seeds were exhumed at 6 days after burial and treated with the PGRs (Fig. II-2B). In the seeds after 6 days of burial, effect of the PGRs was similar to that in freshly matured seeds. In particular, GA_{4+7} and GA_{4+7} + Flu had similar effect on germination, indicating that ABA is not important on germination in the seeds.

Adonis amurensis

Intact fresh *A. amurensis* seeds incubated at 15/6°C for 12 weeks did not germinate and did not show a split pericarp (Fig. II-3). When the seeds were treated with any solutions including GA_3 or GA_{4+7}, pericarp splitting occurred, although the solutions included Pac or ABA. However, most of the seeds did not reach germination stage, in which seeds treated with GA_3 + Flu and GA_{4+7} + Flu germinated to only 6.7 and 4.4%, respectively (Fig. II-3). When GA_3 or GA_{4+7} were combined with ABA, promoting effect on pericarp splitting was reduced.

Seeds buried in July were exhumed on 26 Sep. and treated with the PGRs solutions, and then incubated at 15/6°C (Figs. II-4A and 4B). On 26 Sep., pericarp

splitting and germination were more promoted in the treated seeds than those in fresh seeds (Figs. II-4A and 4B). However, no control seeds showed pericarp splitting and germination. All PRGs solutions including GA_3 and GA_{4+7} promoted pericarp splitting and more than 50% seeds germinated in solutions of GA_3 + Flu and GA_{4+7} + Flu, indicating synergistic effect on germination. Flu promoted pericarp splitting, but the effect was higher in combinations with GA_3 or GA_{4+7} than in Flu alone. GA_3 + Pac, GA_{4+7} + Pac, and GA_{4+7} + ABA stimulated pericarp splitting or germination compared to the control, although they included Pac or ABA. But GA_{4+7} was more effective in stimulating germination than GA_3.

On 6 Nov., control seeds showed pericarp splitting and germination, but only 5.6% of the seeds germinated (Figs. II-4C and 4D). In particular, seeds treated with ABA showed increased pericarp splitting compared to the control. GA_3 + Flu and GA_{4+7} + Flu stimulated pericarp splitting and germination, in particular more than 70% of seeds germinated in GA_{4+7} + Flu.

On 4 Jan., 58.9% of the control seeds germinated in 8 weeks (Fig. II-5A). However, solutions including GA_3, or GA_{4+7} stimulated germination, giving more than 80% germination. ABA delayed germination and Flu promoted germination. In addition, ABA + Flu was more effective on germination than the control. Pac delayed germination, but 36.3% of the seeds eventually germinated within 8 weeks. GA_{4+7} was more effective than GA_3 in promoting germination.

On 29 Jan., PGRs treatments showed similar pattern on germination compared to the seeds on 4 Jan. (Fig. II-5B).

On 3 Mar. (just before germination in nature), more than 80% of the control seeds germinated within 2 weeks (Fig. II-5C). ABA and Pac delayed germination,

but the seeds were recovered from the inhibitory effect.

Changes in Endogenous Phytohormones (ABA and GAs) in Buried Seeds

Endogenous ABA, GA_3, and GA_4 were detected in seed of *A. buergeriana* and *A. amurensis*. Seasonal changes were shown in Fig. II-6 and 7.

In *A. buergeriana*, the ABA content was high in freshly-harvested seeds (63.6 $ng \cdot g^{-1}$), but decreased after 6 days of burial (40.5 $ng \cdot g^{-1}$, Fig. II-6). At this time, embryo growth had occurred in nature (Fig. II-1). ABA content was low on 13 August (12 days after burial). At this time, 56.7% of the buried seeds had germinated in nature (Fig. II-1).

GA_4 content was about 47-fold higher than that of GA_3, indicating that total GA content presumably depended on GA_4 content (Fig. II-6). GA_4 content in freshly-harvested seeds was 4.3 $ng \cdot g^{-1}$ and then increased to 41.7 $ng \cdot g^{-1}$ at 12 days after burial. Therefore, the ratio of GA/ABA was elevated from seeds harvest to germination stage.

In *A. amurensis*, the ABA content was high in freshly-harvested seeds (80.7 $ng \cdot g^{-1}$) (Fig. II-7). However, the ABA content decreased drastically to non-detectable level on 28 Aug. 2012 and remained non-detectable on 26 Sep. and 31 Oct. 2012. During this period embryo growth grew rapidly (Fig. II-1).

The ABA content increased again to 4.1 $ng \cdot g^{-1}$ on 31 Dec. during which time germination did not occur despite the fully grown embryos (Fig. II-7). The ABA content decreased again to 0.6 $ng \cdot g^{-1}$ on 29 Jan.

GA_3 content ranged from non-detectable level to 0.72 $ng \cdot g^{-1}$ and GA_4 content ranged from 57.0 to 260.1 $ng \cdot g^{-1}$ (Fig. II-7). Therefore, total GA content was

presumably determined by GA_4 content.

GA_4 content in freshly-harvested seeds was 66.4 $ng \cdot g^{-1}$ and then decreased slightly to 57.0 $ng \cdot g^{-1}$ on 28 Aug. (Fig. II-7). However, the GA_4 content increased from 145.4 to 260.1 $ng \cdot g^{-1}$ from 26 Sep. to 31 Oct. 2012 during which time embryo grew rapidly in the seeds (Figs. II-1 and 7). And then the GA_4 content decreased again to 73.0 $ng \cdot g^{-1}$ on 31 Dec. 2012 and it remained thereafter at the similar levels until 29 Jan. 2013 during which time germination did not occur despite the fully grown embryos in nature (Figs. II-1 and 7). A higher GA_4 content (240.6 $ng \cdot g^{-1}$) was found on 28 Feb. (Fig. II-7). At this time the seeds started to germinate in nature (Fig. II-1). Therefore, embryo growth and germination pattern in seeds of *A. amurensis* was similar to that of the seasonal changes in total GA content and GA/ABA ratio.

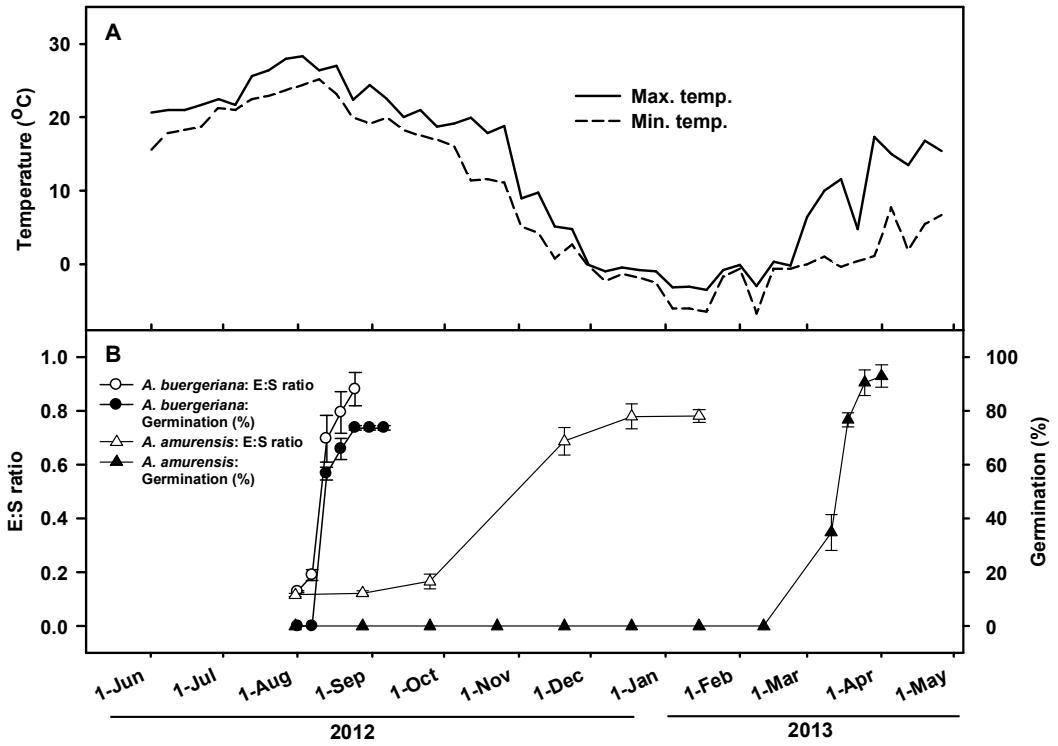

Fig. II-1. Mean daily maximum and minimum soil temperatures and embryo growth and germination of *Aquilegia buergeriana* and *Adonis amurensis* seeds buried at a depth of 3 cm in 2012. The E:S ratio is the ratio of embryo length to seed length. The soil temperature was measured at a depth of 3 cm. Vertical bars represent SE.

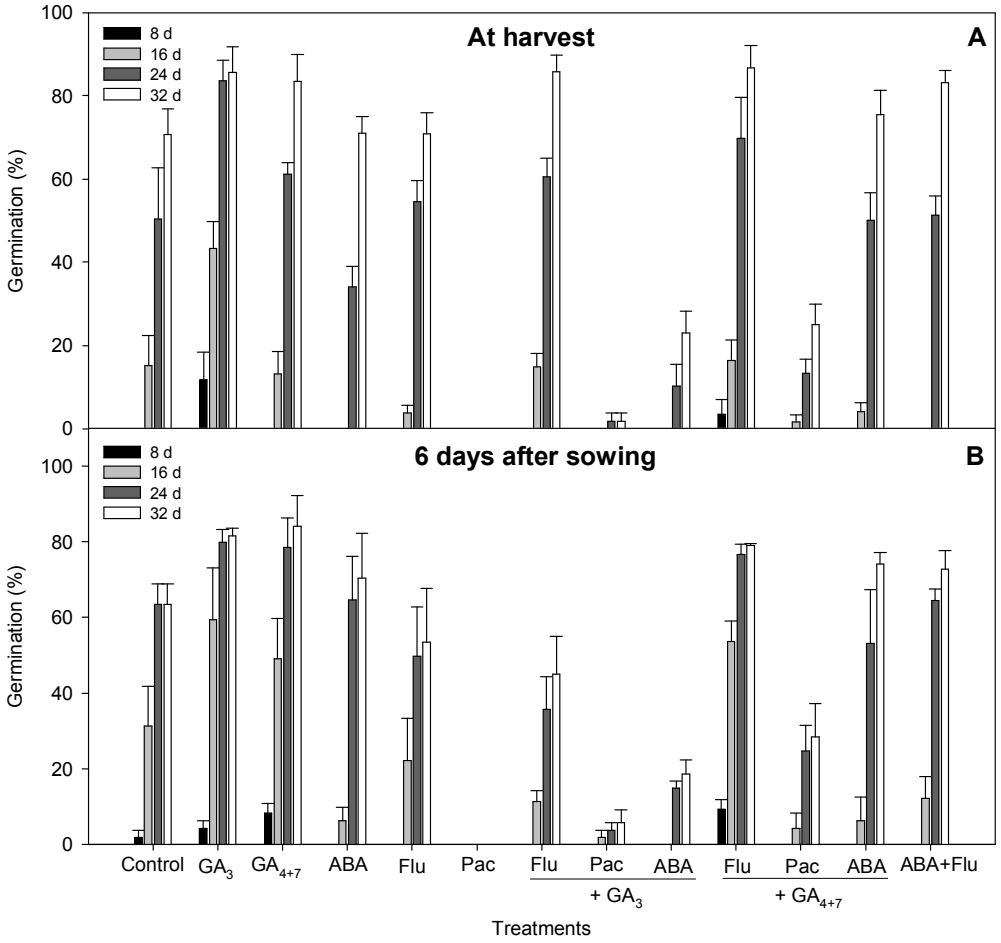

Fig II-2. Percent germination of *Aquilegia buergeriana* seeds as affected by combinations of plant growth regulators (PGRs). Seeds were collected on 18 Jun. 2012, after which the seeds were buried in an experimental garden on 1 Aug. 2012. PRGs were treated at harvest (A) and also treated with seeds that were exhumed at 6 days after sowing (on 7 Aug. 2012) (B). The seeds were soaked in a solution of each combination for 24 h and germinated under 25/15°C and 12h photoperiod conditions for 32 days.

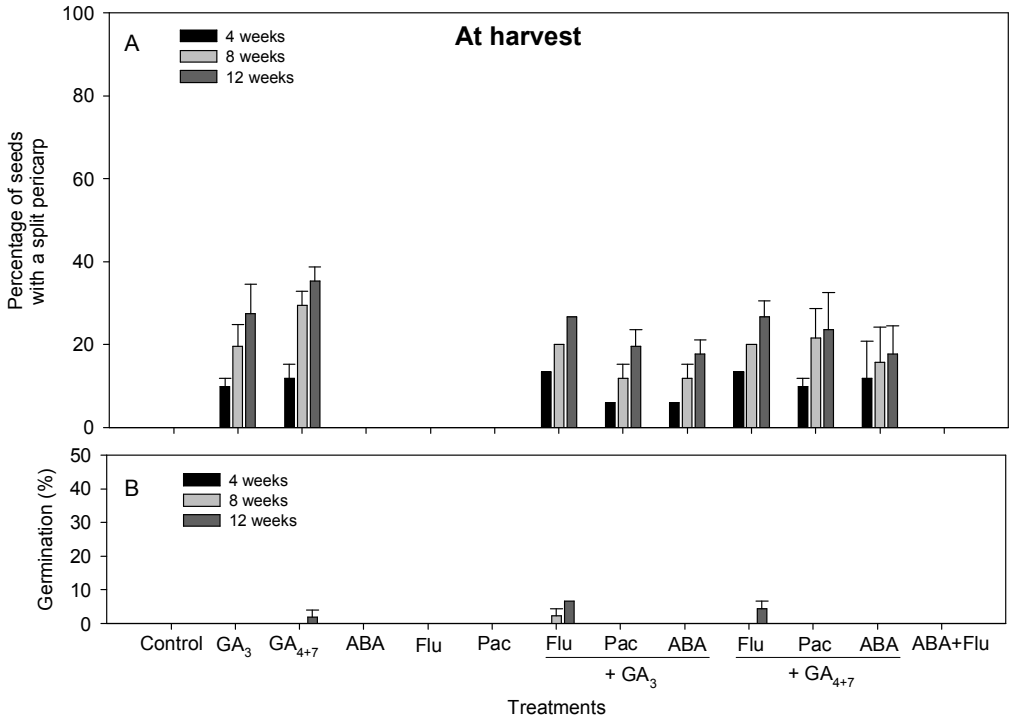

Fig II-3. Pericarp dehiscence (A) and germination (B) of *Adonis amurensis* seeds as affected by combinations of plant growth regulators (PGRs). Seeds were collected on 6~16 May 2012, and then treated with the PGRs. Seeds were soaked in a solution of each combination for 48 h and were germinated under 15/6°C and 12h photoperiod conditions for 16 weeks.

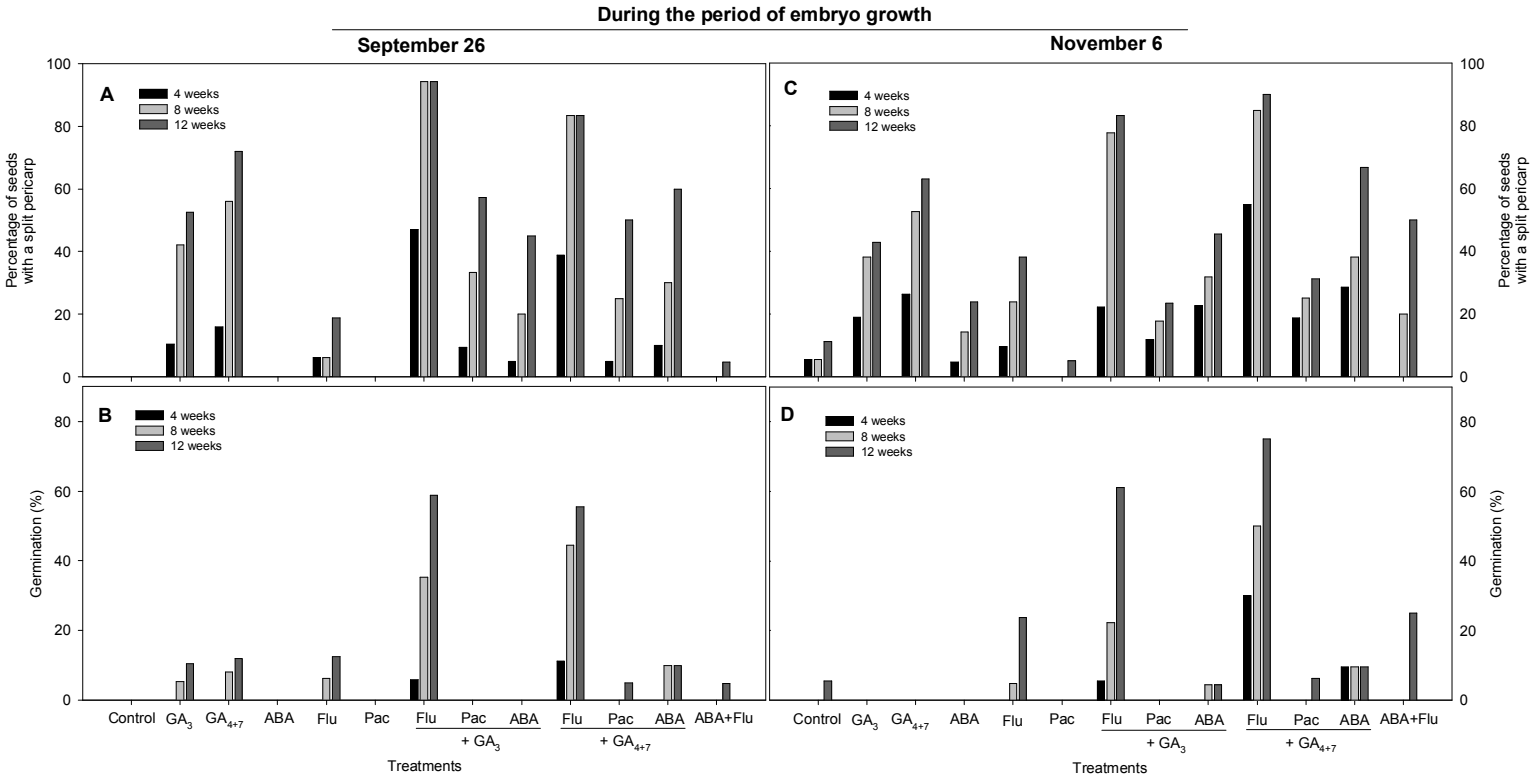
During the period of embryo growth
September 26
November 6
A
B
C
D
4 weeks
8 weeks
12 weeks
Percentage of seeds with a split pericarp
Germination (%)
Control
GA3
GA4+7
ABA
Flu
Pac
Flu + GA3
Pac + GA3
ABA + GA3
Flu + GA4+7
Pac + GA4+7
ABA + GA4+7
ABA+Flu
Treatments

Fig II-4. Pericarp dehiscence (A and C) and germination (B and D) of *Adonis amurensis* seeds as affected by combinations of plant growth regulators (PGRs). Seeds were collected on 6~16 May 2012 and buried in an experimental garden on 15 Jul. 2012, and were exhumed on 26 Sep. (A and B) and on 6 Nov. (C and D) in 2012, respectively, and then treated with the PGRs. Seeds were soaked in a solution of each combination for 48 h and were germinated under 15/6°C and 12h photoperiod conditions for 16 weeks.

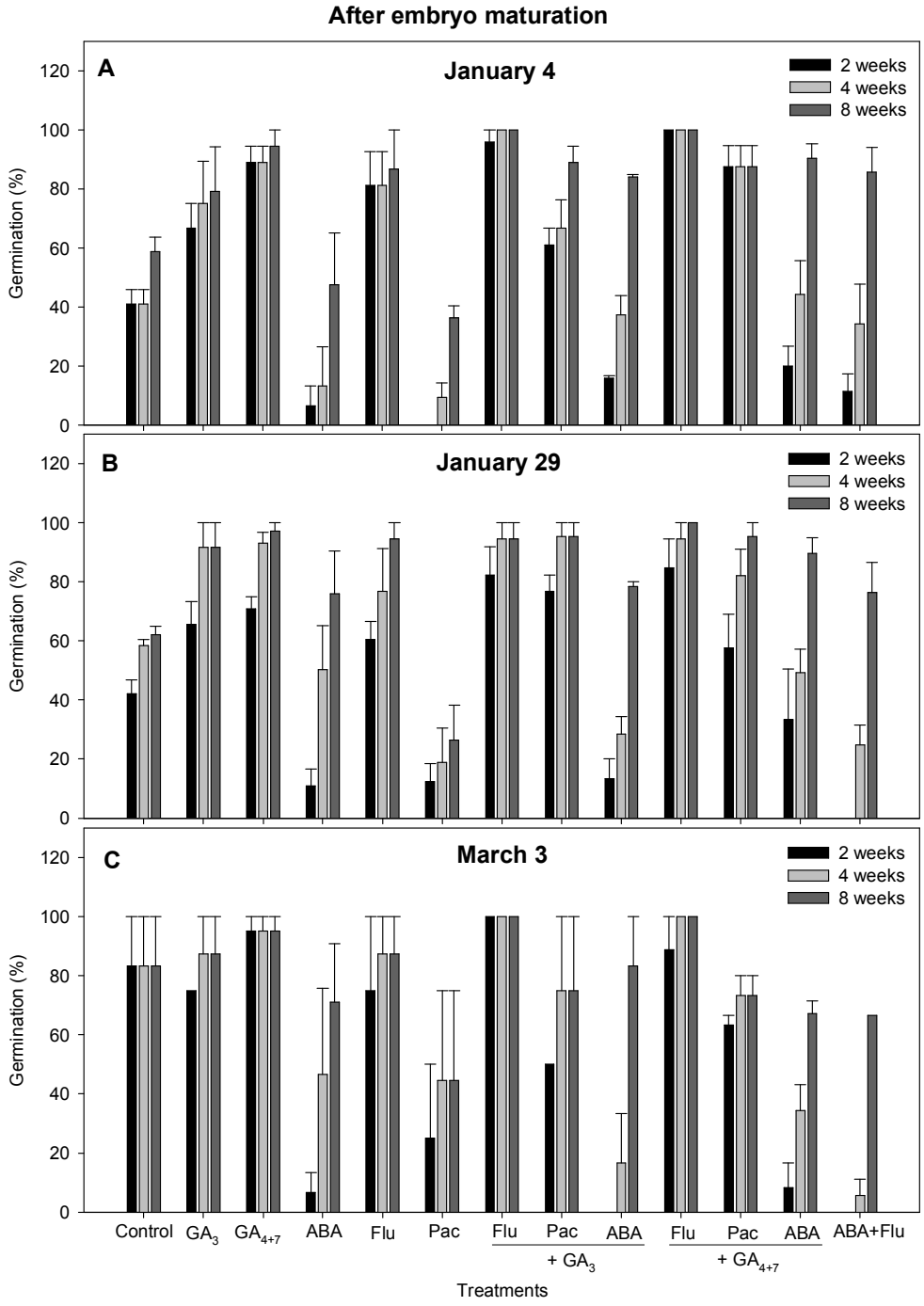
After embryo maturation
A
January 4
B
January 29
C
March 3
2 weeks
4 weeks
8 weeks
Germination (%)
0
20
40
60
80
100
120
Control
GA3
GA4+7
ABA
Flu
Pac
Flu
Pac
ABA
+ GA3
Flu
Pac
ABA
+ GA4+7
ABA+Flu
Treatments

Fig II-5. Germination of *Adonis amurensis* seeds as affected by combinations of plant growth regulators (PGRs). Seeds were collected on 6~16 May 2012 and buried in an experimental garden on 15 Jul. 2012, and then were exhumed on 4 Jan. (A), 29 Jan. (B) and on 3 Mar. (C) in 2013, and treated with the PGRs. Seeds were soaked in a solution of each combination for 48 h and were germinated under 15/6°C and 12h photoperiod conditions for 12 weeks.

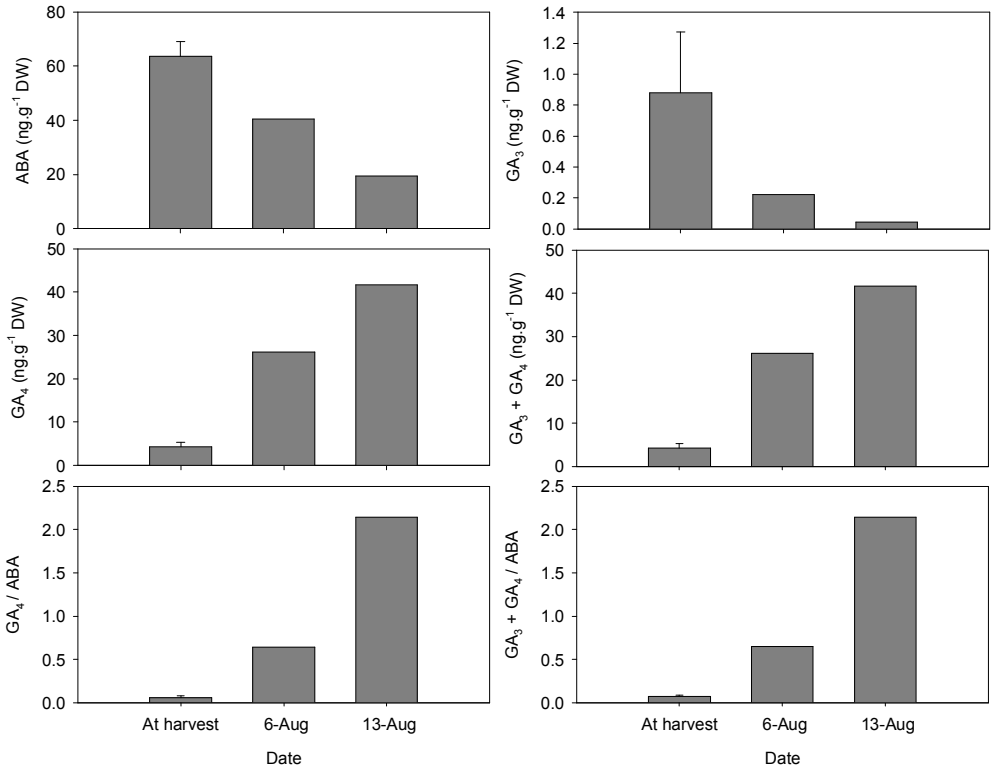

Fig II-6. Changes in the concentrations of endogenous phytohormones (ABA and GAs) in seeds of *Aquilegia buergeriana*. Seeds were collected on 18 Jun. 2012 and stored dry at 5°C. The seeds were buried in an experimental garden on 1 Aug. 2012, and then were exhumed on 6 Aug. (5 days after sowing) and 13 Aug. 2012 (12 days after sowing).

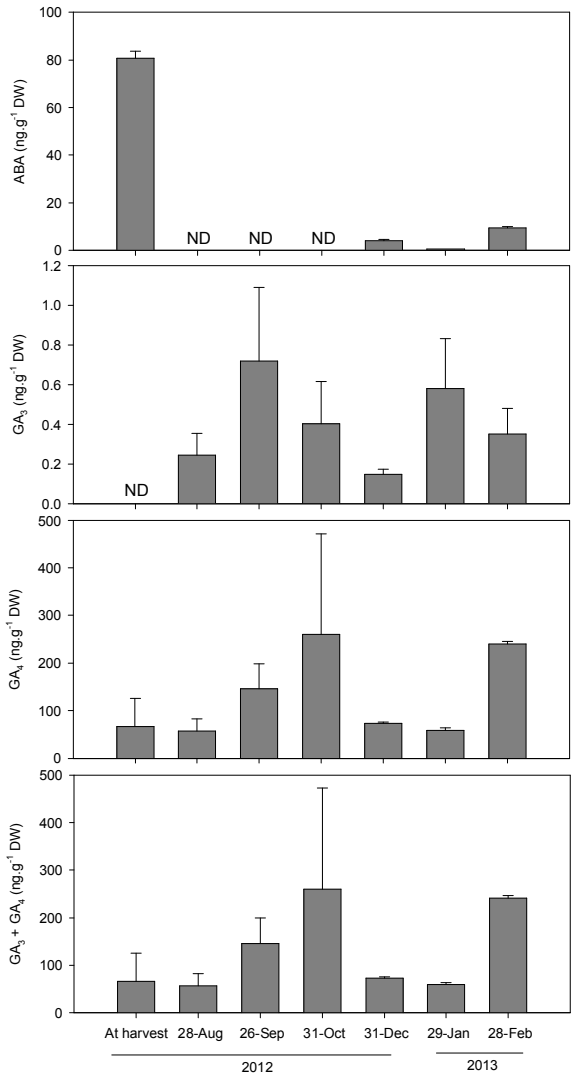

Fig II-7. Changes in the concentrations of endogenous phytohormones (ABA and GAs) in seeds of *Adonis amurensis*. Seeds were collected on 6~16 May 2012 and stored dry at 5°C. The seeds were buried in an experimental garden on 15 Jul. 2012, and then were exhumed on 28 Aug., 26 Sep., 31 Oct., and 31 Dec. in 2012 and 26 Jan. and 28 Feb. 2013.

DISCUSSION

Seeds of *A. buergeriana* and *A. amurensis* showed different pattern of embryo growth and germination in the natural environment. Embryo growth and germination of *A. buergeriana* seeds were completed within about 30 days. On the other hand, the embryo growth and germination of *A. emurensis* seeds required more than 8 months in nature in which embryo growth and germination occurred in autumn and spring next year, respectively.

This dormancy habit was regulated by endogenous phytohormones (ABA and GAs). Our results clearly show a relationship between the state of dormancy in seeds of the two species and hormones contents as well as their sensitivity.

In seeds of A. *buergeriana*, the ABA content was high at harvest. But it decreased rapidly after burial. At the same time, GA_4 content and GA/ABA ratio increased and then germination occurred during this time. Fluridone, an inhibitor in the carotenoid biosynthesis pathway (Bartels and Watson, 1978; Quatrano et al., 1997), did not affect germination rate and ABA had a similar effect on germination compared to the control. Likewise, paclobutrazol, which is an inhibitor of gibberellin biosynthesis at the oxidation of *ent*-kaurene to *ent*-karenoic acid step (Grossmann, 1990), strongly inhibited *A. buergeriana* seed germination. But this inhibition was slightly overcome by a treatment with GA_3 or GA_4 in the seeds. This implies that inhibition of ABA biosynthesis by fluridone was not the major cause of the stimulatory effect, but the GA biosynthesis and content had an important role in promoting embryo growth and germination. However, these hormones acted at the same time on embryo growth

and germination process, indicating that ABA and GA balance model was accepted in the seed with MD (Cadman et al., 2006; Finch-Savage and Leubner-Metzger, 2006).

In seeds of *A. amurensis*, embryo growth and germination process had similar pattern with the changes in ABA and GA contents. When embryo growth occurred in autumn in nature, ABA content drastically decreased into non-detectable levels and GA_4 gradually increased at this time. After embryos were fully grown, ABA content increased again in late December and GA_4 content decreased at the same time. During this time, seeds did not germinate immediately, although the seeds had fully grown embryos in nature. GA_4 increased markedly in late February during which time seeds started to germinate. Thus, both ABA and GA had antagonistic changes in seeds with MPD. Therefore, increased ABA and decreased GA content showed the reason why there was a time lag between embryo elongation and germination.

During embryo elongation in nature, when seeds were treated with ABA, there was no inhibitory effect on pericarp splitting, indicating low sensitivity to ABA (Figs. II-4C and 4D). Paclobutrazol application prevented pericarp splitting and germination, but this inhibition could be overcome slightly by a brief treatment with GA_4, indicating higher sensitivity to GA (Figs. II-4C and 4D).

In seeds of *A. amurensis*, although the inhibiting effect of ABA on freshly-harvested seeds was apparent, release of seed dormancy was not completed after the reduction in endogenous ABA content. As shown in Fig. II-7, ABA content was non-detectable in seeds in autumn but they still remained ungerminated, although embryos in the seeds were fully grown in this period. This agrees with

the view that a reduction in ABA content is not sufficient to break seed dormancy in some dormant seeds (Walton, 1980). It has been reported that GA might play a key role in germination of seeds with MPD. Chien et al. (1998) reported that ABA content drastically decreased after warm stratification but still ungerminated. After warm stratification, GA_4 or GA_{4+7} treatments promoted germination, indicating that although ABA content was low, GA content had to be increased at the same time. There was a fluctuation in the contents of ABA and GA. These changes in the content of ABA and GA probably resulted from balance changes between catabolism and biosynthesis (Corbineau et al., 2002)

In conclusion, *A. buergeriana* seeds had MD, and embryo growth and germination were completed within 30 days when temperature was high. On the other hand, *A. amurensis* seeds had MPD, and experienced high in summer followed by cold temperatures in winter to break seed dormancy in the natural environment. In *A. buergeriana* seeds, ABA content and sensitivity decreased rapidly, and GA content and sensitivity increased rapidly after burial. On the other hand, in *A. amurensis* seeds, ABA content decreased drastically after burial but GA content did not increase before the seeds experienced medium temperature in autumn. When underdeveloped embryos grew rapidly, ABA was non-detectable and GA content increased. But, the seeds remained ungerminated during cold season in winter. When the seeds started to germinate after cold period in winter, GA content increased rapidly with increasing soil temperatures outdoor. GA_4 played a key role in stimulating embryo growth and germination in the seeds with MD and MPD. The changes of GA/ABA ratio were similar to the changes of embryo growth and germination in these two basal angiosperms.

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PART II. Requirements for Dormancy Break and the Types of Morphophysiological Dormancy (MPD) in Seeds of Ranunculaceae Species Native to the Korean Peninsula

CHAPTER III. Embryo Morphology and Seed Dormancy in 14 Herbaceous Perennial Species Native to the Korean Peninsula

CHAPTER IV. Dormancy Types and Germination in Seeds of Five Genera of Ranunculaceae Native to the Korean Peninsula in Relation to Temperature and Gibberellic Acid

CHAPTER III

Embryo Morphology and Seed Dormancy in 14 Herbaceous Perennial Species Native to the Korean Peninsula

ABSTRACT

Many species in Berberidaceae, Ranunculaceae, Melanthiaceae and Liliaceae have underdeveloped embryos at seed dispersal from mother plants. The seeds with underdeveloped embryos at maturity have morphological (MD) or morphophysiological dormancy (MPD). This study was conducted to find basic information for the research of morphological dormancy of seeds in 14 herbaceous species of four plant families (Berberidaceae, Ranunculaceae, Melanthiceae, and Liliaceae) native to the Korean peninsula. Seeds of two Berberidaceae species (*Leontice microrhyncha* and *Jeffersonia dubia*), eight Ranunculaceae species (*Adonis amurensis*, *Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*), three Melanthiaceae species (*Heloniopsis koreana*, *H. tubiflora*, and *Trillium tschonoskii*), and one Liliaceae species (*Erythronium japonicum*) were collected, and embryo morphology and seed germination were investigated in the controlled laboratory conditions. All seeds of 14 species had underdeveloped embryos which occupied about 7-20% of the full seed length at maturity. The seeds of *L. microrhyncha*, *J. dubia*, *A. amurensis*, *A. buergeriana*, *R. crucilobus*, *R. franchetii*, *E. japonicum*, *T.*

tschonoskii, *H. koreana*, and *H. tubiflora* had rudimentary embryos at maturity. On the other hand, the seeds of *P. tonkangensis*, *T. rochenbrunianum*, *T. uchiyamai*, and *T. coreanum* had intermediate type between rudimentary and linear embryo. After 30 days, the seeds of *A. buergeriana*, *P. tonkangensis*, *H. koreana*, *H. tubiflora*, *T. rochenbrunianum*, and *T. uchiyamai* germinated to 92%, 84%, 22%, 40%, 12%, and 3%, respectively. On the other hand, no seeds of the other eight species germinated within 4 weeks. Seeds which did not germinate within 30 days are said to have MD. Thus, most of the seeds of *A. buergeriana* and *P. tonkangensis* have MD, whereas the seeds of *L. microrhyncha*, *J. dubia*, *A. amurensis*, *R. crucilobus*, *R. franchetii*, *E. japonicum*, *T. tschonoskii*, *T. coreanum* have MPD. On the other hand, the seeds of *H. koreana*, *H. tubiflora*, *T. rochenbrunianum*, and *T. uchiyamai* have about 78%, 60%, 87%, and 96% MPD. There was a different level of dormancy (MD and MPD) within the same seed population examined, indicating different adaptation strategies of wild plants in the natural environment.

Keywords: morphological dormancy, morphophysiological dormancy, native plants, temperate herbs, underdeveloped embryos

INTRODUCTION

Seed dormancy is considered to be one of the characteristics of seeds that determine the timing of germination and seedling establishment (Vandelook and Van Assche, 2008). Dormancy also plays a critical role in the plant life cycle because seeds represent the crucial link for species to persist across different locations and over time (Harper, 1977). A seed that does not germinate within 30 days under favorable physical conditions (temperature, light, etc.) is considered to be dormant (Baskin and Baskin, 1998). Dormancy-breaking and germination requirements are often species-specific and related to differences in the species' habitat preferences (Vandelook et al., 2008). In many plant species from temperate climates, seeds are dormant at the time of dispersal from the mother plant, and specific temperature requirements must be reached before they overcome dormancy (Baskin and Baskin, 1998).

An exquisite and experimentally useful classification system for seed dormancy has recently been proposed by Baskin and Baskin (2004) (Finch-Savage and Leubner-Metzger, 2006). According to Baskin and Baskin (2004), seed dormancy can be categorized into five classes [physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational dormancy (PY + PD)]. In temperate regions, many perennial herbs disperse seeds with underdeveloped embryos that must elongate to a critical length prior to radicle emergence (Baskin and Baskin, 1998). Seeds with an underdeveloped embryo have either MD or MPD (Baskin and Baskin, 1998, 2004). Seeds have MD if embryo growth and germination of seeds are

completed within 30 days under suitable incubation conditions without any dormancy-breaking pretreatment. However, if the seeds require more than 30 days and a dormancy-breaking treatment, such as warm and/or cold stratification, to germinate, they are classified as MPD (Baskin and Baskin, 1998; Nikolaeva, 1977). Nine levels of MPD have been proposed according to the requirements for breaking seed dormancy, the temperature requirements for embryo elongation and response to gibberellic acid (GA_3) (Baskin and Baskin, 1998, 2004; Baskin et al. 2008).

In the plant families of Berberidaceae, Ranunculaceae, Melanthiaceae, and Liliaceae, many seeds at the time of dispersal have nderdeveloped embryos and thus MD and/or MPD. According to Baskin and Baskin (1989), *Jeffersonia diphylla* (Berberidaceae) seeds have small, underdeveloped embryos and MPD. It has been reported that the seeds of *Helleborus niger* (Niimi et al., 2006), *Aconitum lycoctonum* L. (Vandelook et al., 2009), and *Thalictrum mirabile* (Walck et al., 1999) in Ranunculaceae had small embryos and MPD at dispersal. Embryos in seeds of Melanthiaceae species have been observed to be small in relation to the size of the endosperm (Baskin et al., 2001; Copete et al., 2011; Kondo et al., 2011). In *Trillium camschatcense*, seeds had underdeveloped embryos at dispersal and it took more than 1 year before radicle emergence occurred in the field conditions, and were therefore classified as having MPD (Kondo et al., 2011). MPD has also been identified in seeds of *Erythronium grandiflorum* (Baskin and Baskin, 1995) and *Erythronium japonicum* (Kondo et al., 2002) in Liliaceae.

Over the past several decades, extensive seed dormancy studies have been

conducted in herbaceous perennial species native to the Korean Peninsula. In particular, physiological seed dormancy breaking by cold stratification, GA treatment, after-ripening, and scarification has been reported in many species (Kang et al., 2010; Kim et al., 2010; La and Jeong, 2008; Yeam et al., 1985). However, more complex seed dormancy like MPD has not been reported in the seeds of the species native to Korean peninsula.

In this study, for seed dormancy experiment we selected 14 native perennial species of four plant families, Berberidaceae, Ranunculaceae, Melanthiaceae, and Liliaceae. To the best of our knowledge, no detailed studies have been conducted to determine whether seeds of the four plant families native to Korea have underdeveloped embryos, and if so, what kind of dormancy they have. Specifically, we investigated germination rate at 25/15℃, water absorption by intact seeds, and embryo morphology and size.

MATERIALS AND METHODS

Study Species

We selected 14 native plant species of four plant families, Berberidaceae, Ranunculaceae, Melanthiaceae, and Liliaceae for this study (Fig. III-1). In the Berberidaceae, seeds of *Leontice micorhyncha* and *Jeffersonia dubia* were used. *L. micorhyncha* and *J. dubia* are rare, and endangered species in Korea (Korea National Arboretum, 2008).

In Ranunculaceae, seeds of *Adonis amurensis*, *Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum* were used. *P. tonkangensis*, *R. crucilobus*, *T. rochenbrunianum*, and *T. uchiyamai* are endemic species, and *T. coreanum* is a rare, and endangered species in Korea (Korea National Arboretum, 2008).

In the Melanthiaceae, seeds of *Trillium tschonoskii*, *Heloniopsis koreana*, and *H. tubiflora* were used. *T. tschonoskii* is a native species in Ulleungdo Island and also an endangered species (Korea National Arboretum, 2008). Both *H. koreana* and *H. tubiflora* have recently been described as endemic species to Korea from information on morphological traits and molecular phylogeny (Fuse et al., 2004). *Erythronium japonicum* seeds were used for the family Liliaceae.

Seed Collection and General Procedures

In Berberidaceae, mature fruits from *L. micorhyncha* and *J. dubia* were collected on 1 May 2012 and 27 May 2009 from plants growing in the eco-

garden within the Hantaek Botanical Garden (37°09′N, 127°40′E), Yongin, Korea and within the Mulhyanggi Arboretum (37°17′N, 127°06′E), Osan, Korea, respectively.

In Ranunculaceae, mature fruits or seeds from *A. amurensis*, *A. buergeriana*, *R. crucilobus*, *T. rochenbrunianum*, *T. uchiyama*, and *T. coreanum* were collected on 22 May, 18 Jun., and 1 Jun. in 2011 and 20 Sep., 30 Sep., and 19 Sep. in 2012 from plants growing in the Hantaek Botanical Garden, respectively. *P. tonkangensis* seeds were collected on 7-18 May from plants growing within the glass house in agricultural technology center in Yeongwol-Gun, Korea (37°18′N, 128°46′E). *R. franchetii* seeds were collected on 19 May 2013 from plants growing in a natural population (37°69′N, 128°76′E) in Daegwallyeong, Gangwon Province, Korea.

In Melanthiaceae and Liliaceae, mature fruits from *T. tschonoskii*, *H. koreana*, and *E. japonicum* were collected on 1 Jun., 20 May, and 23 May in 2012 from plants growing in the eco-garden within the Hantaek Botanical Garden, respectively, and those of *H. tubiflora* were collected on 18 Jun. from plants growing in Hyangjeokbong (35°51′N, 127°44′E), Mt Deogyu, Muju, Korea.

Fruits or seeds were allowed to dry under laboratory conditions (20-25°C, 8-11 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light) for 1-2 weeks, then packed in sealed plastic bags and stored dry at 4°C until the beginning of the experiments. Three replicates of 20 or 30 seeds were used for imbibition test and germination test, respectively. The seeds were placed on two sheets of filter paper (Whatman No. 2, GE Healthcare Co., Ltd., Buckinghamshire, UK) in 90 × 15 mm Petri dishes and moistened with distilled water. All dishes were wrapped with parafilm to restrict water loss

during incubation. A 12-h light/dark photoperiod was provided by a cool white fluorescent lamps that provided a photon flux density of approximately 8-11 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the incubators (DS-13MCLP, Dasol Scientific Co., Ltd., Hwaseong, Korea). Radicle emergence was monitored weekly to calculate percent germination. Seeds were considered 'germinated' when radicles emerged at least 1 mm.

Germination Test

Seeds were incubated at 25/15°C in a 12-h light/dark photoperiod. Radicle emergence was recorded weekly for 8 weeks and results were expressed as mean percent germination.

Water imbibition Test

This experiment was conducted to determine whether seeds coat blocks water uptake. If no increase in seed mass is observed, they have physical dormancy (PY). Therefore, rate of water uptake by intact seeds was monitored in the experimental species. Three replications of 20 seeds each of the species were placed on two sheets of filter paper (Whatman No. 2, GE Healthcare Co., Ltd., Buckinghamshire, UK) in 90 × 15 mm Petri dishes, moistened with distilled water, and kept in the laboratory at room temperature (20-25°C). Initial (t_0) seed weight was determined for air-dry seeds and water-imbibed seed mass was measured after 3, 6, 9, 12, 24, 48, and 72 h incubation. Water uptake percentage was calculated as fresh mass increase by the seeds based on the formula:

$\%W_s = [(W_h - W_i)/W_i] \times 100$,

where W_s = increase in seed mass, W_h = mass of seeds after a given interval of imbibition, and W_i = initial seedd mass.

Morphological Observation of Embryos

During incubation, freshly imbibed seeds were cut into thin sections using a razor blade, and the length of seeds and embryos was measured under a dissecting microscope fitted with an ocular micrometer (KSZ-1B, Samwon Scientific Co., Ltd., Seoul, Korea). The ratio of embryo length to seed length (E:S ratio) was calculated to correct a positive correlation between seed length and embryo length (Vandelook et al., 2007). After measuring the length of seeds and embryos using the dissecting microscope, the sections of the seeds were viewed at 60 to 120× magnification and photographed with a Miview USB digital microscope (MV 1302U, CosView Technologies Co., Ltd., Shenzhen, China).

RESULTS

Germination

Berberidaceae

Fresh seeds of *L. microrhyncha* and *J. dubia* incubated at 25/15°C under 12-h alternating light conditions did not germinate for 8 weeks (Fig III-2).

Ranunculaceae

Fresh seeds of *A. buergariana*, *P. tonkangensis*, *T. rochenbrunianum*, and *T. uchiyamai* incubated at 25/15°C under 12-h alternating light conditions germinated to 92%, 84%, 12%, and 3%, respectively, in 4 weeks (Fig III-2). However, no seed germination was observed in *A. amurensis*, *R. crucilobus*, *R. franchetii*, and *T. coreanum* within 4 weeks. Extending the incubation time to 8 weeks increased seed germination to 86% in *P. tonkangensis*, 45% in *T. rochenbrunianum*, 22% in *T. uchiyamai* , and 3% in *T. coreanum* whereas no further increase in germination was observed in *A. amurensis*, *R. crucilobus*, *R. franchetii*, and *T. coreanum*.

Melanthiaceae

Fresh seeds of *H. koreana* and *H. tubiflora* incubated at 25/15°C under 12-h alternating light conditions germinated to 23% and 40%, respectively, in 4 weeks (Fig III-2). No seed germination was observed in *T. tschonoskii*. Extending the incubation time to 8 weeks increased seed germination to 49% in *H. koreana* and 61% in *H. tubiflora*, respectively, whereas no further increase in germination percentage was observed in *T. tschonoskii*.

Liliaceae

No germination was observed in seeds of *E. japonicum* for 8 weeks (Fig III-2).

Water Imbibition

Berberidaceae

After 24 h of imbibition, fresh mass in seed of *L. microrhyncha* increased by 124% (Fig III-3). The mass of seeds in *J. dubia* increased during incubation, indicating the uptake of water (data not shown).

Ranunculaceae

After 24 h of imbibition, fresh seeds of seven species in Ranunculaceae had imbibed water more than 30% (Fig III-3). Among the species, fresh mass in seeds of *T. rochenbrunianum* and *T. uchiyamai* increased more than 150% of their initial mass after 24 h of imbibition. The mass of seeds increased during incubation in *R. franchetii*, indicating the uptake of water (data not shown).

Melanthiaceae

After 24 h of imbibition, fresh mass in seed of *T. tschonoskii* increased by 40% (Fig III-3). The mass of seeds increased during incubation in two *Heloniopsis* species, indicating the uptake of water. Water moved from the outside of the seed coats to the inside of the endosperm through caudal appendages in both species (data not shown).

Liliaceae

After 9 h and 24 h of imbibition, fresh mass in seed of *E. japonicum* increased by 32% and 68%, respectively (Fig III-3). Further increase in the seed mass was observed from 24 h to 96 h.

Morphology of Embryo and Seed

Berberidaceae

Freshly matured seeds in two species in Berberidaceae had small embryos in length compared to the seed length (Fig III-4). The mean length of the embryos was 0.23 mm and 0.38 mm in seeds of *L. microrhyncha* and *J. dubia*, respectively (Table III-1). Thus, at seed harvest the ratio of the embryo to true seed length (E:S ratio) was 0.07 and 0.09 in *L. microrhyncha* and *J. dubia*, respectively.

Ranunculaceae

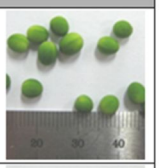
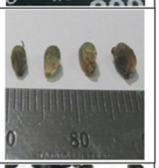
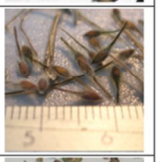
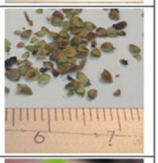
All seeds of eight species in Ranunculaceae had small embryos in length compared to the seed length (Fig III-4). The mean length of the embryos was between 0.20 mm and 0.52 mm (Table III-1). E:S ratio was 0.08 - 0.21. Thus, all seeds of the eight species had small embryos which occupied about 8-21% of the full seed length at maturity.

Melanthiaceae

The mean length of the embryos was 0.23 mm, 0.12 mm, and 0.16 mm in seeds of *T. tschonoskii*, *H. koreana*, and *H. tubuflora*, respectively (Table III-1). E:S ratio was 0.08, 0.09, and 0.11 in seeds of *T. tschonoskii*, *H. koreana*, and *H. tubuflora*, respectively.

Liliaceae

E. japonicum seeds had small embryos, and the mean length of the embryos was 0.43 mm (Fig III-4 and Table III-1). E:S ratio was 0.09. Thus, the embryos occupied 9% of the full seed length at maturity.

Scientific name (Korean name)	Family	Flowers	Fruits	Seed morphology	
Leontice microrhyncha S.Moore (한계령풀)	Berberidaceae		Berries		
Jeffersonia dubia (Maxim.) Benth. & Hook.f. ex Baker & S.Moore (깽깽이풀)			Capsules		
Adonis amurensis Regel & Radde (복수초)	Ranunculaceae		Achenes		
Aquilegia buergeriana var. *oxysepala* (Trautv. & Meyer) Kitam. (매발톱꽃)			Follicles		
Pulsatilla tongkangensis Y.N.Lee & T.C.Lee (동강할미꽃)			Achenes		
Ranunculus crucilobus H.Lev. (바위미나리아재비)			Achenes		
Ranunculus franchetii H.Boissieu (왜미나리아재비)			Achenes		

Continued.

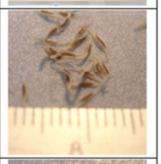
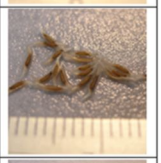
Scientific name (Korean name)	Family	Flowers	Fruits	Seed morphology	
Thalictrum rochebrunianum var. *grandisepalum* (H.Lev.) Nakai (금꿩의다리)	Ranunculaceae		Achenes		
Thalictrum uchiyamai Nakai (자주꿩의다리)			Achenes		
Thalictrum coreanum H.Lev. (연잎꿩의다리)			Achenes		
Trillium tschonoskii Maxim. (큰연영초)	Melanthiaceae		Capsules		
Heloniopsis koreana Fuse & N.S.Lee & M.N.Tamura (처녀치마)			Capsules		
Heloniopsis tubiflora Fuse & N.S.Lee & M.N.Tamura (숙은처녀치마)			Capsules		
Erythronium japonicum (Balrer) Decne. (얼레지)	Liliaceae		Capsules		

Fig. III-1. Morphology of flowers and seeds in 14 species native to the Korean Peninsula. All genera except three *Thalictrum* species (*T. rochenbrunianum*, *T uchiyamai*, and *T. coreanum*) are spring flowering perennials.

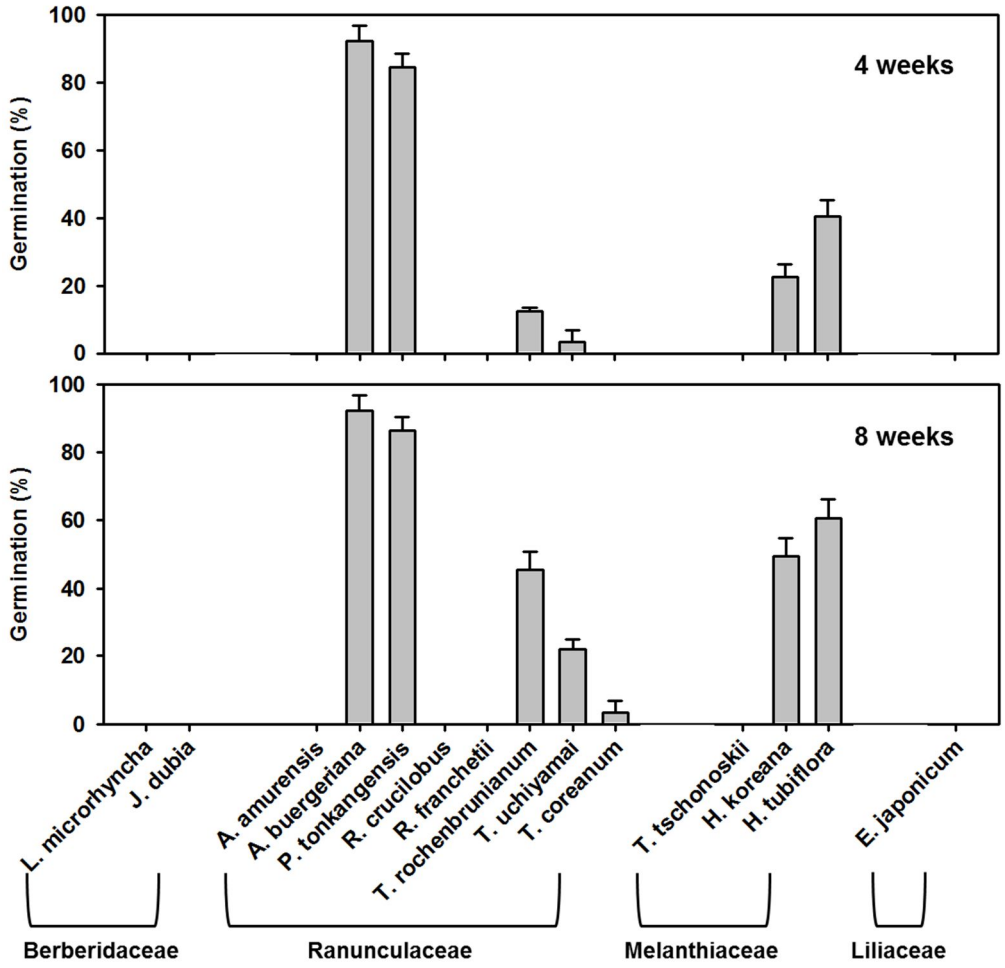


Fig. III-2. Percent germination in seeds of 14 species native to the Korean Peninsula. The seeds were incubated under 25/15℃ and 12h photoperiod conditions for 4 and 8 weeks.

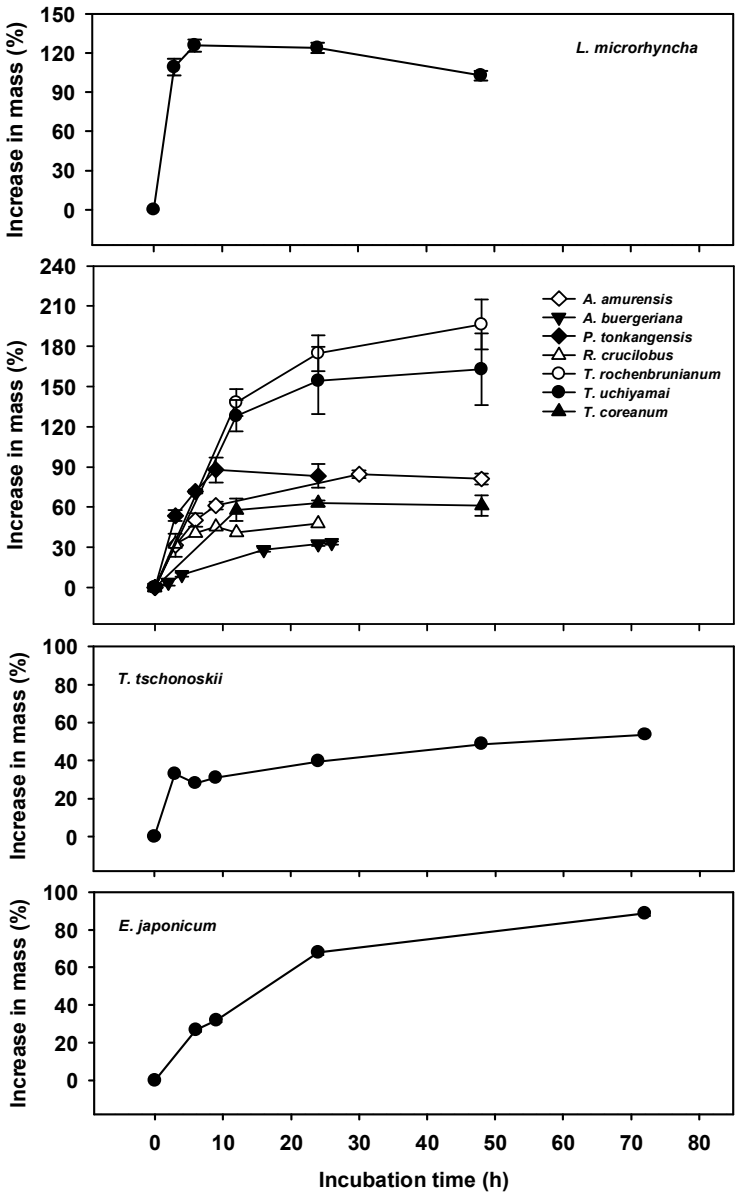
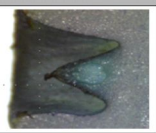
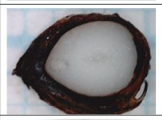
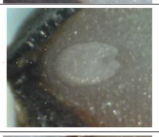
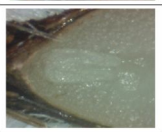
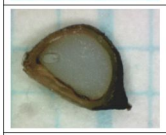
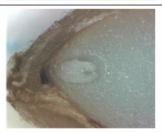
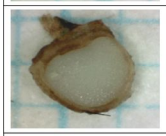
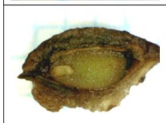
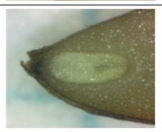
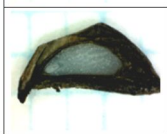
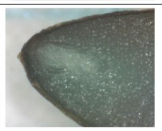


Fig. III-3. Water uptake of intact seeds of 10 species as represented by increase in mass. Seeds were incubated at room temperature (22-25°C) on filter paper moistened with distilled water for 72h. Vertical bars represent SE.

Scientific name (Korean name)	Family	Seed morphology		Embryo types
		Longitudinal sections	Embryos	
Leontice microrhyncha S.Moore (한계령풀)	Berberidaceae			Rudimentary
Jeffersonia dubia (Maxim.) Benth. & Hook.f. ex Baker & S.Moore (깽깽이풀)				Rudimentary
Adonis amurensis Regel & Radde (복수초)	Ranunculaceae			Rudimentary
Aquilegia buergeriana var. *oxysepala* (Trautv. & Meyer) Kitam. (매발톱꽃)				Rudimentary
Pulsatilla tongkangensis Y.N.Lee & T.C.Lee (동강할미꽃)				Between the rudimetary and linear types
Ranunculus cruciobus H.Lev. (바위미나리아재비)				Rudimentary
Ranunculus franchetii H.Boissieu (왜미나리아재비)				Rudimentary
Thalictrum rochebrunianum var. *grandisepalum* (H.Lev.) Nakai (금꿩의다리)				Between the rudimetary and linear types
Thalictrum uchiyamai Nakai (자주꿩의다리)				Between the rudimetary and linear types
Thalictrum coreanum H.Lev. (연잎꿩의다리)				Between the rudimetary and linear types

Continued.

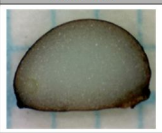
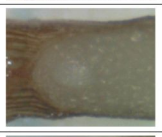
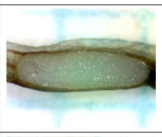
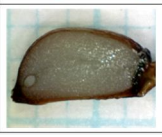
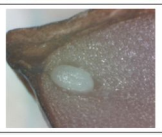
Scientific name (Korean name)	Family	Seed morphology		Embryo types
		Longitudinal sections	Embryos	
Trillium tschonoskii Maxim. (큰연영초)	Melanthiaceae			Rudimentary
Heloniopsis koreana Fuse & N.S.Lee & M.N.Tamura (처녀치마)				Rudimentary
Heloniopsis tubiflora Fuse & N.S.Lee & M.N.Tamura (숙은처녀치마)				Rudimentary
Erythronium japonicum (Balrer) Decne. (얼레지)	Liliaceae			Rudimentary

Fig. III-4. Longitudinal sections and underdeveloped embryos in seeds of 14 species native to the Korean Peninsula. Fresh seeds were cut into thin sections using a razor blade. The sections of the seeds were viewed at 60 to 120 × magnification and photographed with a Miview USB digital microscope.

Table III-1. Seed length, embryo length, and the ratio of embryo to seed length (E:S ratio) in seeds of 14 species native to the Korean Peninsula. Embryo and seed lengths were measured using a dissecting microscope fitted with an ocular micrometer.

Species	Mean initial value after seed collection		
	Seed length[z]	Embryo length	E:S ratio[y]
	(mm)		
Berberidaceae			
L. microrhyncha	3.20 ± 0.10[x]	0.23 ± 0.01	0.07 ± 0.00
J. dubia	4.30 ± 0.15	0.38 ± 0.01	0.09 ± 0.00
Ranunculaceae			
A. amurensis	2.55 ± 0.06	0.20 ± 0.01	0.08 ±0.01
A. buergeriana	2.17 ± 0.04	0.23 ± 0.01	0.11 ± 0.01
P. tonkangensis	2.60 ± 0.05	0.52 ± 0.03	0.20 ± 0.01
R. crucilobus	1.79 ± 0.03	0.29 ± 0.02	0.16 ± 0.01
R. franchetii	1.78 ± 0.03	0.29 ± 0.01	0.16 ± 0.01
T. rochenbrunianum	5.42 ± 0.19	0.50 ± 0.02	0.21 ± 0.01
T. uchiyamai	3.49 ± 0.15	0.34 ± 0.02	0.14 ± 0.01
T. coreanum	3.51 ± 0.07	0.48 ± 0.03	0.19 ± 0.01
Melanthiaceae			
T. tschonoskii	2.81 ± 0.06	0.23 ± 0.01	0.08 ± 0.00
H. koreana	5.51 ± 0.15	0.12 ± 0.01	0.09 ± 0.01
H. tubiflora	4.90 ± 0.16	0.16 ± 0.00	0.11 ± 0.01
Liliaceae			
E. japonicum	4.59 ± 0.10	0.43 ± 0.01	0.09 ± 0.00

[z]Seed length was measured with caudal appendage in two *Heloniopsis* species.

[y]Pericarp in seeds of berries and achenes, and caudal appendage in seeds of two *Heloniopsis* species were not included for calculating E:S ratio.

[x]Mean ± standard error (n=10).

DISCUSSION

Seeds of all species examined here imbibed water readily, increasing in fresh mass by more than 30% after 24 h of incubation (Fig. III-3). According to Baskin and Baskin (2003), if seed mass (fresh weight) does not increase, then the seed or fruit (for example, true seed + pericarp (achene) in *Thalictrum* species) coat is impermeable to water. On the other hand, if seed mass increases ≥ 20% based on air-dry seed or fruit mass, one can be assumed that the seed (or fruit) coat is permeable to water. Thus, all seeds in this study have no PY.

In temperate regions, many herbaceous plant species have small, underdeveloped embryos in seeds at the time of dispersal (Baskin and Baskin, 1988). Mature seeds from all species in this study had small embryos. According to Baskin and Baskin (1998), rudimentary or small, linear embryos must elongate before germination occurs; thus, the seeds generally are referred to as having underdeveloped embryos. Previous studies showed that seeds of Berberidaceae, Ranunculaceae, Melanthiaceae, and Liliaceae had small, underdeveloped embryos (Baskin and Baskin, 1988; Baskin et al., 2001; Kondo et al., 2011; Martin, 1946). It is suggested that all seeds of 14 species have underdeveloped embryos at maturity from available information on germination and characteristics of seeds in those families.

Underdeveloped embryos in seeds with MD are not physiologically dormant, and the seeds will typically germinate within 30 days. However, the embryos with MPD are dormant at the time of dispersal and need more than 30 days for germination (Baskin and Baskin, 1998, 2004; Nikolaeva, 1977).

The fact that embryos in freshly mature seeds of 14 species are small and underdeveloped implies that the seeds are morphologically or morphophysiologically dormant at dispersal. In this study, seeds of *A. buergeriana* and *P. tonkangensis* germinated more than 84% in 4 weeks, indicating that they have MD. On the other hand, no germination was observed in seeds of *L. microrhyncha* and *J. dubia* in Berberidaceae, *A. amurensis*, two *Ranunculus* species, and *T. coreanum* in Ranunculaceae, and *T. tschonoskii* in Melanthiaceae, and *E. japonicum* in Liliaceae in 4 weeks (Fig. III-2). This indicates that the seeds have MPD.

At 4 weeks after sowing, the seeds of *H. koreana* and *H. tubiflora* germinated to 22.7% and 40.7%, respectively. On the other hand, the seeds of *T. rochenbrunianum*, *T. uchiyamai*, and *T. coreanum* germinated to 12.5%, 3.3%, and 0%, respectively. Thus, the seeds of *H. koreana* and *H. tubiflora* had about 70% and 60% MPD. On the other hand, the seeds of *T. rochenbrunianum*, *T. uchiyamai*, amd *T. coreanum* had about 80%, 90%, and 100% MPD. This means that there was a different level of dormancy (MD and MPD) within the same seed population examined. Thus, some seeds had a large variation in time to germination. Seeds in the population that continued to germinate after 30 days were probably in various states of MPD, and thus needed different lengths of time at favorable temperatures and light to germinate (Adams et al., 2005; Baskin and Baskin, 2004). It is suggested that such a delay mechanism within the seed population can be an ecologically advantageous strategy for unpredictable environmental conditions (Alves-Da-Silva et al., 2011; Doussi and Thanos, 2002).

Nine levels of MPD have been proposed according to the requirements for breaking seed dormancy, the temperature requirements for embryo elongation and response to gibberellic acid (GA_3) (Baskin and Baskin, 1998, 2004; Baskin et al. 2008). Therefore, more detailed study to determine which types of MPD they have should be conducted.

Martin (1946) classified seeds into three divisions (basal, peripheral, and axile) and into 12 categories based on embryo morphology and size, relative amount of endosperm, and position of the embryo compared to the endosperm. Among the classification units, small, rudimentary embryos in basal division and small, linear embryos in axile division are considered as major types.

Seeds of two species in this study (*L. microrhyncha* and *J. dubia*) contained rudimentary, underdeveloped embryos, in agreement with what Martin (1946) reported and illustrated for Berberidaceae. In Ranunculaceae, seeds of four species (*A. amurensis*, *A. buergeriana*, and two *Ranunculus* species) had rudimentary embryos. On the other hand, seeds of the other four species (*P. tonkangensis* and three *Thalictrum* species) had intermediate type between rudimentary and linear embryo. Martin (1946) reported that this family is on the borderline between the rudimentary and linear types. All seeds of four species in Melanthiaceae and Liliaceae contained small, rudimentary embryos in this study. Martin's report (1946) corresponds with our findings that all studied species in the two families had seeds with small, rudimentary embryos at seed maturity.

In conclusion, 14 herbaceous perennial species studied had small, underdeveloped embryos at dispersal. There were differences in dormancy depth among the species. Two species (*A. buergeriana* and *P. tonkangensis*) had MD.

Five species (three *Thalictrum* species and two *Heloniopsis* species) showed a different level of dormancy (MD and MPD) within the same seed population examined. In the other seven species (*L. microrhyncha*, *J. dubia*, *A. amurensis*, two *Ranunculus* species, *T. tschonoskii*, and *E. japonicum*), all seeds had MPD. Therefore, germination of the seeds was delayed due to the underdeveloped embryos and/or physiological factors. These results could contribute to determine the kind of morphological seed dormancy and germination mechanisms in seeds of the Korean Peninsula.

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CHAPTER IV

Dormancy Types and Germination in Seeds of Five Genera of Ranunculaceae Native to the Korean Peninsula in Relation to Temperature and Gibberellic Acid

ABSTRACT

Few researches have been conducted on ecophysiological classification of seed dormancy in Korean native plants. Many species in Ranunculaceae (basal angiosperm family) have been known to have underdeveloped embryos at seed dispersal and morphological (MD) or morphophysiological dormancy (MPD). This study was conducted to classify the types of seed dormancy in eight Ranunculaceae species (*Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Adonis amurensis*, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*). Most seeds of *A. buergeriana* and *P. tonkangensis* germinated within 30 days and thus had MD. In *A. amurensis*, embryos grew at relatively warm temperatures in autumn with split pericarp, and seeds germinated after warm followed by cold temperature sequences, and GA did not overcome the dormancy. Thus, the seeds showed a special type of deep simple epicotyl MPD. In *R. crucilobus*, embryos growth occurred at cold temperature, and seed dormancy was broken by cold stratification, and GA did not overcome the dormancy. Thus, the seeds have deep complex MPD. On the other hand, in seeds of *R. franchetii*, embryo growth and germination occurred in

early and late autumn, respectively, after warm period in summer. However, germinated seeds in the field in autumn did not show seedling emergence at room temperatures when moved to laboratory conditions, indicating that the seeds had deep simple epicotyl MPD. In three *Thalictrum* species, embryo growth occurred at low temperatures, but it was more enhanced when seeds were moved from low temperatures to high temperatures. The seeds required only a cold stratification to break dormancy, and GA substituted for cold stratification. Therefore, it can be concluded that the seeds had non-deep simple MPD. We collected data of seed dormancy types in Ranunculaceae from available information and compared with congeners in different countries or continents. From the data, we found that there was a wide variety of dormancy types in five genera in Ranunculaceae. There were same types of MPD in eastern Asian - North American congeners (non-deep simple MPD) in the genus *Thalictrum* with an Arcto-Tertiary distribution pattern. This indicates that the same types of seed dormancy are at least as old as the Tertiary.

Keywords: Arcto-Tertiary, congeners, disjunct taxa, dormancy type, temperate herbs, underdeveloped embryos

INTRODUCTION

Many plant species, including members of Ranunculaceae, have underdeveloped embryos at maturity (Baskin and Baskin, 2014; Martin, 1946), and many studies on seeds germination ecophysiology of these species have shown that embryo growth to critical length is a prerequisite for germination (Baskin and Baskin, 1989; Baskin and Baskin, 2014; Vandelook and van Assche, 2009; Walck et al., 1999). Therefore, underdeveloped embryos are one of the reasons of seed dormancy that prevents germination in the natural environment.

Five kinds of dormancy are currently recognized in seeds [physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational dormancy (PY + PD)]. Among the dormancy types, seeds with embryos that are undifferentiated or underdeveloped at dispersal and require time for further development before germination are considered morphologically (MD) or morphophysiologically (MPD) dormant (Baskin and Baskin, 1998).

Dormancy is common in seeds of native wildflowers and may last anywhere from several weeks to more than one year (Heather et al., 2010). Among plant genera of Ranunculaceae, it has been reported that seeds of *Thalictrum mirabile*, *Isopyrum biternatum*, *Cimicifuga racemosa*, *Hepatica acutiloba*, *Delphinium tricorne* (native to USA), *Hepatica nobilis* (native to Japan), *Aquilegia barbaricina* and *A. nugorensis* (native to Italy), *Anemone nemorosa* (native to UK), *Aconitum lycoctonum* (native to Belgium), and *Aconitum napellus* (native to Spain) have underdeveloped embryos at maturity (Ali et al., 2007; Baskin and Baskin, 1985; Baskin and Baskin, 1986; Baskin and Baskin, 1994; Herranz et al.,

2010; Mattana et al., 2012; Nomizu et al., 2004; Vandelook et al., 2009; Walck et al., 1999). Thus, they have MD and/or MPD.

Prior to this study, relatively few data had been collected on the dormancy breaking, germination requirements, and ecophysiology of seed dormancy types in species of Ranunculaceae native to the Korean peninsula. Lee et al. (2003) reported that seeds of *Megaleranthis saniculifolia* have underdeveloped embryos and warm (15-30^{o}C) for at least 2 weeks followed by cold (4^{o}C) stratification for 16 weeks was required for breaking seed dormancy. Kim et al. (1998) reported that no seeds of *Adonis amurensis* germinated in light or darkness at 15, 20, 25, and 30^{o}C and a cold stratification for 10, 20, or 30 days at 4^{o}C did not overcome seed dormancy, and GA treatment with 100, 200, 1000 mg·L^{-1} had no effect for breaking the dormancy. Cold stratification for more than 4 weeks at 4^{o}C in *Thalictrum rochenbrunianum* and 400 mg·L^{-1} GA_3 soaking for 24 h in *T. coreanum* promoted seed germination to 26.7% and 36%, respectively (Lee et al., 2007). However, whether or not embryos were underdeveloped and needed to grow inside the seed before they germinate was not specifically determined. Further, they did not identify the kind of seed dormancy in those species.

From the results of dormancy study in seeds of Berberidaceae, Ranunculaceae, Melanthiaceae, and Liliaceae (in Chapter III), we found that seeds in these plant families have MD and/or MPD. Nine types of MPD (Baskin and Baskin, 2014) have been described based on 1) temperatures required to break MD and PD, 2) temperatures for embryo growth, and 3) whether gibberellic acid overcomes the dormancy.

Therefore, we conducted this study to determine 1) whether seeds have MPD

and which one of the nine types they have and 2) how timing of germination is controlled by various temperature sequences. Specifically, we investigated 1) the effect of warm, cold, or warm followed by cold temperature sequences for dormancy break and embryo growth, 2) effect of GA_3 on dormancy break and germination in the seeds by field and controlled laboratory experiments and finally 3) compared the types of seed dormancy with disjunct species of genera on different countries or continents.

MATERIALS AND METHODS

Experiment 1: Embryo Growth and Germination According to Various Temperature regimes

Study Species and Seed Collection

We selected eight native plant species of Ranunculaceae including *Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Adonis amurensis*, *Ranunculus crucilobus*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*. Among them, *P. tonkangensis*, *R. crucilobus*, *T. rochenbrunianum*, and *T. uchiyamai* are endemic species, and *T. coreanum* is a rare and endangered species in Korea (Korea National Arboretum, 2008).

Mature fruits were collected on 12 May and 24 May in 2010 and on 22 May 2011 and 6-16 May 2012 and 12 May 2013 from *A. amurensis* and on 18 Jun. in 2011 from *A. buergeriana* from plants growing in the Hantaek Botanical Garden (37°09′N, 127°40′E), Yongin-si, Korea. *P. tonkangensis* seeds were collected on 7-18 May 2012 from plants growing within the glass house in the Yeongwol Agricultural Technology Center, Korea (37°18′N, 128°46′E).

Mature fruits were collected on 12-24 Sep. 2011 from *T. rochenbrunianum* and on 24-30 Sep. 2011 and 20-30 Sep. 2012 from *T. uchiyama*, and on 30 Aug. 2012 from *T. coreanum*, respectively, from plants growing in the Hantaek Botanical Garden.

Mature fruits of *R. crucilobus* were collected on 30 May and 1 Jun. 2012 and 6 Jun. 2013 from plants growing in the Hantaek Botanical Garden and those of *R.*

franchetii were collected on 29 May 2013 from plants growing in a natural population (37°69′N, 128°76′E) in Daegwallyeong, Gangwon Province, Korea.

Fruits or seeds were allowed to dry in laboratory conditions (20-25°C, 8-11 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light) for 1-2 weeks, then packed in sealed plastic bags and stored dry at 5°C until the beginning of the experiment. Preliminary experiments indicated that dry storage at 5°C for about several months (more than 2 months) did not affect seed dormancy status.

Laboratory Experiments

For embryo growth and germination study, seeds were treated with 500 $mg \cdot L^{-1}$ benomyl for more than 3 h for fungal control before being used in laboratory experiments. Unless otherwise stated, each of the three replicates of 30 seeds was used. The seeds were placed on two sheets of filter paper (Whatman No. 2, GE Healthcare Co., Ltd., Buckinghamshire, UK) in 90 × 15 mm Petri dishes and moistened with distilled water. All dishes were wrapped with parafilm (Pechiney Plastic Packaging, Menasha, WI, USA) to restrict water loss during incubation. At all temperature regimes, a 12-h light/dark photoperiod was provided by a cool white fluorescent lamps that provided a photon flux density of approximately 30-40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the incubators (DS-13MCLP, Dasol Scientific Co., Ltd., Hwaseong, Korea). Radicle emergence was monitored to calculate percent germination. Seeds were considered “germinated” when radicles emerged at least 1 mm.

Effect of Temperature Regimes on Embryo Growth

In *A. buergeriana*, four Petri dishes with 20 seeds were placed into 5, 20, 25, 15/6, 20/10, or 25/15°C. At weekly intervals for 4 weeks, 20 seeds were removed at random from each of the four dishes at each temperature and the length of each embryo was measured. The embryo length of each seed was measured using a dissecting microscope equipped with a micrometer. In *P. tonkangensis*, seeds were incubated at 5, 15/6, 20/10, 25/15, or 30/20°C. At 4 days intervals for 12 days, the embryo length was measured.

In *A. amurensis*, seeds collected in 2010 and 2011 were incubated at a constant 5°C and at alternating 25/15°C for 34 weeks in 2010 and 28 weeks in 2011. And another seeds were subjected to the following temperature sequences in 2010: (i) beginning with warm temperature 25/15°C for 16 weeks, and then kept under cold temperature conditions (5°C) for 12 weeks; and (ii) 25/15°C for 28 weeks, and then kept at 5°C for 6 weeks; and (iii) 25/15°C for 28 weeks, and then kept at 15/6°C for 6 weeks. In 2011 seeds, two temperature sequences were used: (i) beginning with warm temperature 25/15°C for 16 weeks, and then kept under cold temperature conditions (5°C) for 12 weeks; and (ii) 25/15°C for 16 weeks, and then kept under medium temperature conditions (15/6°C) for 12 weeks. Embryo length was measured as previously described.

In *R. crucilobus*, seeds collected in 2013 were used. To determine if warm, cold, or warm plus cold stratification treatments were required for embryo growth, seeds were incubated at a constant 5°C and at alternating 25/15°C for 16 weeks. And another seeds were subjected to the following three temperature sequences: (i) beginning with warm temperature 25/15°C for 8 weeks, and then kept at 5°C for 8 weeks; and (ii) 25/15°C for 8 weeks, and then kept at 15/6°C

for 8 weeks; and (iii) beginning with cold temperature 5°C for 8 weeks, and then kept at 25/15°C for 8 weeks. Embryo length was measured as previously described.

In *T. rochenbrunianum* and *T. uchiyamai*, seeds collected in 2011 were used. Seeds were incubated at a constant 5°C and at alternating 25/15°C for 17 weeks. And another seeds were subjected to the following two temperature sequences: (i) beginning with warm temperature 25/15°C for 9 weeks, and then kept at 5°C for 11 weeks; and (ii) beginning with cold temperature 5°C for 9 weeks, and then kept at 25/15°C for 5 weeks. In *T. coreanum*, seeds were incubated at 5 or 25/15°C for 21 weeks and another seeds were subjected to the following two temperature sequences: (i) beginning with warm temperature 25/15°C for 8 weeks, and then kept at 5°C for 13 weeks; and (ii) beginning with cold temperature 5°C for 8 weeks, and then kept at 25/15°C for 4 weeks. Embryo length was measured as previously described.

Effect of Temperature Regimes on Germination: move-along experiment

A move-along experiment (Baskin and Baskin, 2003) adapted to seasonal temperatures in Korea was used to determine whether seeds required warm, cold, or warm followed by cold stratification; seeds of *A. buergeriana* and *P. tonkangensis* were excluded because the seeds required only warm temperatures for germination (see Chapter III).

In *A. buergeriana*, seeds were incubated at 5, 20, 25, 15/6, 20/10, or 25/15°C. Seeds in another three Petri dishes were wrapped with two layers of aluminum foil and incubated at those temperatures to create a dark treatment. In *P.*

tonkangensis, seeds were incubated at 5, 15, 20, 25, 15/6, 20/10, 25/15, or 30/20°C. Radicle emergence was monitored weekly for 4 weeks, after which time the percentage of seeds with an emerged radicle was calculated. Seeds in another three Petri dishes were wrapped with two layers of aluminum foil and incubated at only 25/15°C to create a dark treatment.

In *A. amurensis*, seeds collected in 2011 were used. The treatments consisted of four control chambers set at 5 (for winter), 15/6 (for early spring and late autumn), 20/10 (for late spring and early autumn), or 25/15°C (for winter) and two move-along treatments that began with the summer temperature (25/15°C) or the winter temperature (5°C). All seeds were incubated for 52 weeks. For the move- along test, seeds were subjected to the following two temperature sequences: (i) beginning with warm temperature 25/15°C for 12 weeks, slightly cooler temperatures (20/10°C) for 4 weeks, further reduced temperature conditions (15/6°C) for 4 weeks and cold conditions (5°C) for 12 weeks, and then moving the seeds back to 15/6, 20/10, and 25/15°C; and (ii) beginning with cold temperature 5°C for 12 weeks, and slowly warming the temperature to 15/6°C for 4 weeks, then 20/10°C for 4 weeks, 25/15°C for 12 weeks, and finally moving the seeds back to 20/10, 15/6, and 5°C. Germination was monitored at 2 weeks intervals.

In *R. crucilobus*, for the move-along experiment, seeds collected in 2013 were used. Seeds were incubated at the four control temperatures for 28 weeks. Seeds for the move-along were incubated at two temperature sequences: (i) beginning with warm temperature 25/15°C for 10 weeks, and slightly cooler temperatures (20/10°C) for 4 weeks, further reduced temperature conditions (15/6°C) for 4

weeks, and and cold conditions (5°C) for 8 weeks, and then moving the seeds back to 15/6°C; and (ii) beginning with cold temperature 5°C for 10 weeks, slowly warming the temperature to 15/6°C for 4 weeks, then 20/10°C for 4 weeks, 25/15°C for 8 weeks, and finally moving the seeds back to 20/10°C.

Seeds of *T. rochenbrunianum* in 2011, *T. uchiyamai* in 2011 and 2012, and *T. coreanum* in 2012 were used for the move-along experiment. For the experiment in 2011, seeds were incubated at the four control temperatures for 24 weeks. Another seeds for the move-along were incubated at two temperature sequences: (i) beginning with warm temperature 25/15°C for 10 weeks, and slightly cooler temperatures (20/10°C) for 4 weeks, further reduced temperature conditions (15/6°C) for 4 weeks , and cold conditions (5°C) for 8 weeks, and then moving the seeds back to 15/6 and 20/10°C; and (ii) beginning with cold temperature 5°C for 10 weeks, and slowly warming the temperature to 15/6°C for 4 weeks, then 20/10°C for 4 weeks, 25/15°C for 10 weeks, and finally moving the seeds back to 20/10°C and 15/6°C. For the experiment in 2012, seeds were incubated at the four control temperatures for 32 weeks. In the move-along sequences in 2012, seeds were subjected at 5°C for 10 weeks for winter simulation temperature and the other temperatures were the same as 2011.

Experiment 2: Dormancy Breaking and Germination as Affected by Temperature and Gibberellic Acid

Study Species and Seed Collection

All seeds in experiment 2 were the same as the seeds in the experiment 1 (see

study species and seed collection in experiment 1).

High Temperature Requirements for Embryo Growth and Germination

In this study, seeds of *A. buergeriana*, *P. tonkangensis*, and *A. amurensis* were used because they required warm temperatures for embryo growth and germination.

In *A. buergeriana* (seeds in 2011) and *P. tonkangensis* (seeds in 2012), effect of short period warm stratification on germination was determined. Seeds of *A. buergeriana* and *P. tonkangensis* were warm stratified at 25/15^{o}C for 7 and 6 days, respectively, and then they were moved to 15/6, 20/10, or 25/15^{o}C. After the warm stratification, radicle emergence was monitored weekly for 3 weeks in *A. buergeriana* and at 4 day intervals for 24 days in *P. tonkangensis*.

In *A. amurensis*, this study determined the optimum duration for warm stratification before being moved to cold temperatures. Seeds collected in 2012 were first placed at 25/15^{o}C for 0, 3, 6, 9, or 15 weeks. After each duration conditions, all seeds were moved to 15/6^{o}C for 8 weeks for embryo growth. This sequence of temperatures simulated natural conditions, where high summer temperatures are followed by autumn temperatures. Embryo growth occurred in autumn in natural environments (see Chapter I; phenology of embryo growth). Seeds were buried on 23 Jun., 23 Jul., 23 Aug., 28 Sep., and 31 Oct. in 2013. Three replicates of 20 seeds each for each burial dates were used. After the burial, seeds were exhumed on 27 Dec. in 2013 and then percentage of seeds with a split pericarp was measured. We found that embryo growth was almost completed at the time of pericarp dehiscence in Chapter I.

Cold Temperature Requirements for Embryo Growth and Germination

In *A. amurensis*, to determine if cold stratification is required for radicle emergence in seeds with fully elongated embryos, and if so, how long of cold stratification is required, seeds were put in a fine-mesh polyester bag and placed in an experimental garden on 19 Jun. in 2011. The seeds were removed from the bag on 16 Nov. and 17 Dec. in 2011 and on 21 Jan. and 11 Feb. in 2012. Thereafter, seeds were incubated at 20/10^{o}C and checked for germination for 4 weeks.

In *R. crucilobus*, seed required cold stratification only for germination. Seeds collected in 2013 were cold stratified for 0, 4, 8, or 12 weeks at 5^{o}C and then the seeds were incubated at 25/15^{o}C. Germination percentage was calculated after 4 weeks of incubation.

In *R. franchetii*, we observed emerged radicles in autumn from seeds sown on 23 Jul. in 2013. To determine if germinated seeds have epicotyl dormancy, and if so, cold stratification is required for breaking epicotyl dormancy, the germinated seeds were moved from field to laboratory and then the seeds were incubated at room temperatures (20-25^{o}C). Cotyledon emergence was observed in the seeds for 14 weeks.

In *T. rochenbrunianum* and *T. uchiyamai*, seeds were stratified at 5 or 1^{o}C for 0, 4, 8, or 12 weeks and then incubated at 25/15^{o}C for 12 weeks. Since seeds of *Thalictrum* species are dispersed in late summer and early autumn, the seeds could experience several weeks of warm temperatures (≥15^{o}C) before receiving cold stratification in winter in nature. Thus, seeds were subjected at 25/15^{o}C (warm stratification) for 6 weeks and then cold stratified at 5 or 1^{o}C for another

12 weeks. After each of the cold stratification, seeds were incubated at 25/15°C for 12 weeks. In *T. coreanum*, seeds were cold stratified at 5°C for 0, 3, 6, 9, or 12 weeks and then incubated at 25/15°C for 6 weeks. In addition, seeds of *T. rchenbrunianum* and *T. uchiyamai* in 2011 and *T. coreanum* in 2012 were buried on 28 Sep. in an experimental garden. Seeds of *T. rchenbrunianum* and *T. uchiyamai* were exhumed on 31 Oct., 20 Dec. in 2011 and 21 Jan. in 2012 and seeds of *T. coreanum* were exhumed on 30 Oct. and 28 Dec. in 2012 and 30 Jan. in 2013. Exhumed seeds were incubated at 25/15°C for 2 weeks and then germination percentage was calculated.

Effect of GA Treatment on Embryo Growth and Germination

In *A. buergeriana* and *P. tonkangensis*, seeds were soaked in solutions at concentrations of 0 (distilled water, control), 10, 100, or 1000 $mg \cdot L^{-1}$ GA_3 for 24 h at room temperature, and then incubated at 5, 15/6, 20/10, and 25/15°C for 4 weeks.

In *A. amurensis*, seeds collected in 2011 were used for GA experiment. Seeds were soaked in solutions at concentrations of 0 (with distilled water, control), 10, 100, or 1000 $mg \cdot L^{-1}$ GA_3 for 48 h at room temperature, and then incubated at 5, 15/6, 20/10, and 25/15°C for 16 weeks, during which time germinated seeds were counted at 2 weeks intervals and re-sterilized with 500 $mg \cdot L^{-1}$ benomyl. And the number of seeds with a split pericarp but no radicle protrusion was determined. A split pericarp in *A. amurensis* is indicative of the embryo growth, although the length of embryos vary at the moment the pericarp splits (see Chapter I). After 14 weeks of incubation at 15/6°C, seeds treated with GA and

those soaked with distilled water (control) were cut in half and embryo morphology of them was observed because a split pericarp was shown in seeds treated with GA.

In *R. crucilobus*, seeds collected in 2012 were soaked in solutions at concentrations of 0 (with distilled water, control), 10, 100, or 1000 $mg \cdot L^{-1}$ GA_3 for 24 h at room temperature, and then incubated at 25/15°C for 12 weeks. Seeds collected in 2013 were soaked in solutions at concentrations of 0 (with distilled water, control), 10, 100, or 1000 $mg \cdot L^{-1}$ GA_3 and in solutions of 20 mgL^{-1} fluridone or 20 $mg \cdot L^{-1}$ fluridone plus 100 $mg \cdot L^{-1}$ GA_3 for 24 h at room temperature, and then incubated at 15/6 and 25/15°C for 12 weeks.

In three *Thalictrum* species, seeds were soaked in solutions at concentrations of 0 (with distilled water, control), 10, 100, or 1000 $mg \cdot L^{-1}$ GA_3 for 24 h at room temperature, and then incubated at 5, 15/6, 20/10, or 25/15°C for 16 weeks in which seeds of *T. coreanum* were incubated at 5, 15/6, or 25/15°C. Germination percentages at 4, 8, and 12 weeks of incubation were calculated.

Data Collection and Statistical Analysis

During incubation, seeds were cut into thin sections using a razor blade, and the length of seeds was measured under a dissecting microscope fitted with an ocular micrometer (KSZ-1B, Samwon Scientific Co., Ltd., Seoul, Korea). After measuring the length of seeds using the dissecting microscope, the sections of the seeds were viewed at 60 to 120× magnification and photographed with a Miview USB digital microscope (MV 1302U, CosView Technologies Co., Ltd., Shenzhen, China). Means and standard errors were calculated for germination

percentages and embryo length. The percent germination after treatment was analyzed using GLM followed by Tukey's honestly significant different test at $P < 0.05$ (SAS Institute, Cary, NC, USA) to compare treatment differences within the germination data.

RESULTS

Experiment 1: Embryo Growth and Germination According to Various Temperature regimes

Effect of Temperature Regimes on Embryo Growth

Aquilegia buergeriana

Embryos in fresh matured seeds of *A. buergeriana* were 0.24 ± 0.01 mm long. They grew faster at warm temperatures (25 and 25/15°C) than the other incubation temperatures (Fig. IV-1-1A). The embryo growth was completed within 3 weeks at warm temperatures.

Pulsatilla tonkangensis

Embryo growth pattern in seeds of *P. tonkangensis* was similar to those of *A. buergeriana* (Fig. IV-1-1B).

Adonis amurensis

In 2010, none of the seeds that were incubated at 25/15°C and 5°C had reached the critical embryo length after 28 weeks (Fig. IV-1-2A). A warm stratification pretreatment at 25/15°C was required for embryo growth. Embryo growth in seeds receiving 16 or 28 weeks at 25/15°C began immediately after seeds were transferred to 5 or 15/6°C. After 6 weeks at 5 or 15/6°C, embryos length in seeds that previously had been at 25/15°C for 16 or 28 weeks had increased more than 200%. However, standard errors of mean embryo length at 5°C were high than

those at 15/6°C. Embryo growth pattern in seeds of 2011 was similar to those in 2010 (Fig. IV-1-2B). However, embryo growth was faster at 15/6°C than at 5°C. Standard errors of mean embryo length were smaller at 15/6°C than at 5°C.

Ranunculus crucilobus

A warm stratification pretreatment was not required for embryo growth (Fig. IV-1-3). The 8 weeks of cold stratification at 5°C increased the rate of embryo growth at 25/15°C. Embryo growth in seeds receiving 8 weeks at 5°C began immediately after the seeds were transferred to 25/15°C. After 4 weeks at 25/15°C, embryo length in seeds that previously had been at 5°C for 8 weeks increased more than 200%. In addition, embryos at a constant 5°C increased to the critical length for germination after 14 weeks of incubation.

Thalictrum rochenbrunianum

In seeds of *T. rochenbrunianum,* cold stratification was required for embryo growth (Fig. IV-1-4A). After a cold stratification at 5°C for 9 weeks, embryos in the seeds grew rapidly when transferred to warm temperature at 25/15°C, and embryo length increased by more than 300%. At a constant 5°C, the embryos started to grow from 9-12 weeks of incubation. But, they did not reach to the critical length for germination during 17 weeks of incubation.

Thalictrum uchiyamai

Embryo growth pattern in seeds of *T. uchiyamai* was similar to those of *T. rochenbrunianum* (Fig. IV-1-4B).

Thalictrum coreanum

Embryo growth pattern in seeds of *T. coreanum* also was similar to those of *T. rochenbrunianum* and *T. uchiyamai* (Fig. IV-1-4C). After a cold stratification at 5°C for 8 weeks, embryos in the seeds grew rapidly when transferred to warm temperature at 25/15°C, and embryo length increased by more than 300%. In seeds of *T. coreanum*, embryos stared to grow from 6-8 weeks of incubation at 5°C, and the length of the embryos reached to the critical length for germination at 16 weeks of incubation.

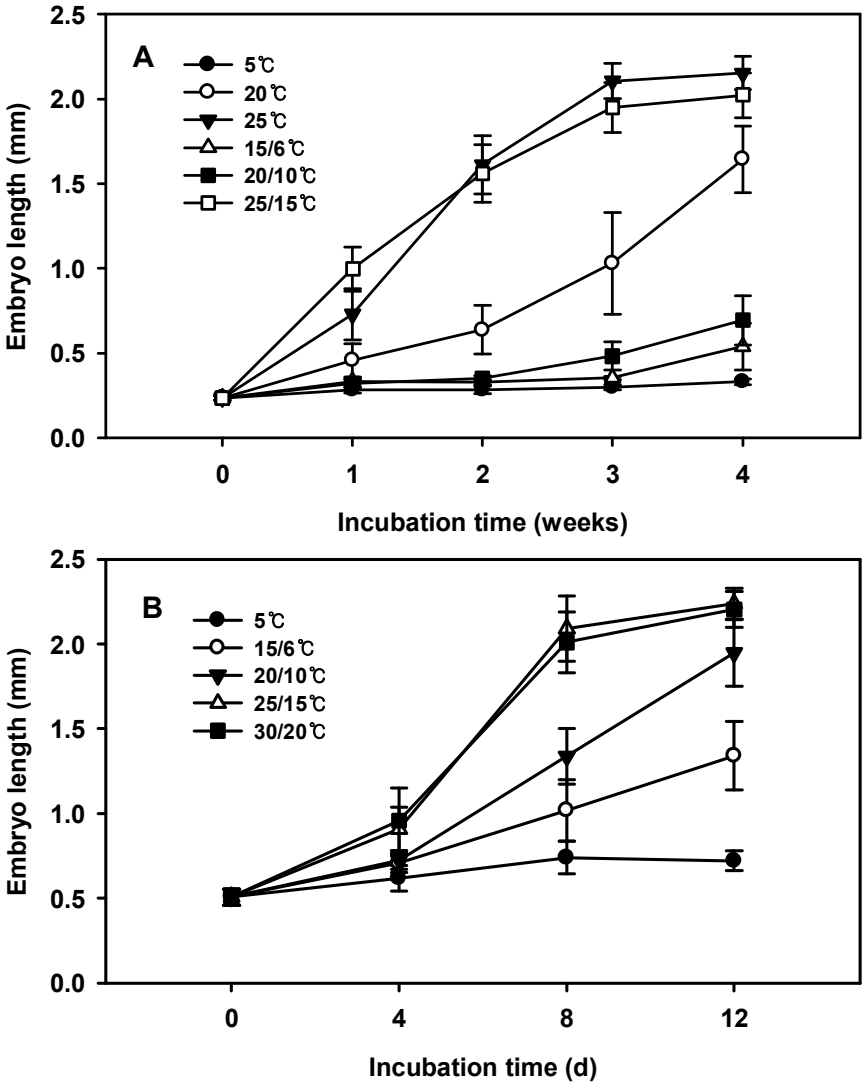

Fig. IV-1-1. Effect of constant and alternating temperature regimes on embryo growth in seeds of *Aquilegia buergeriana* (A) in 2011 and *Pulsatilla tonkangenesis* (B) in 2012. Vertical bars represent SE.

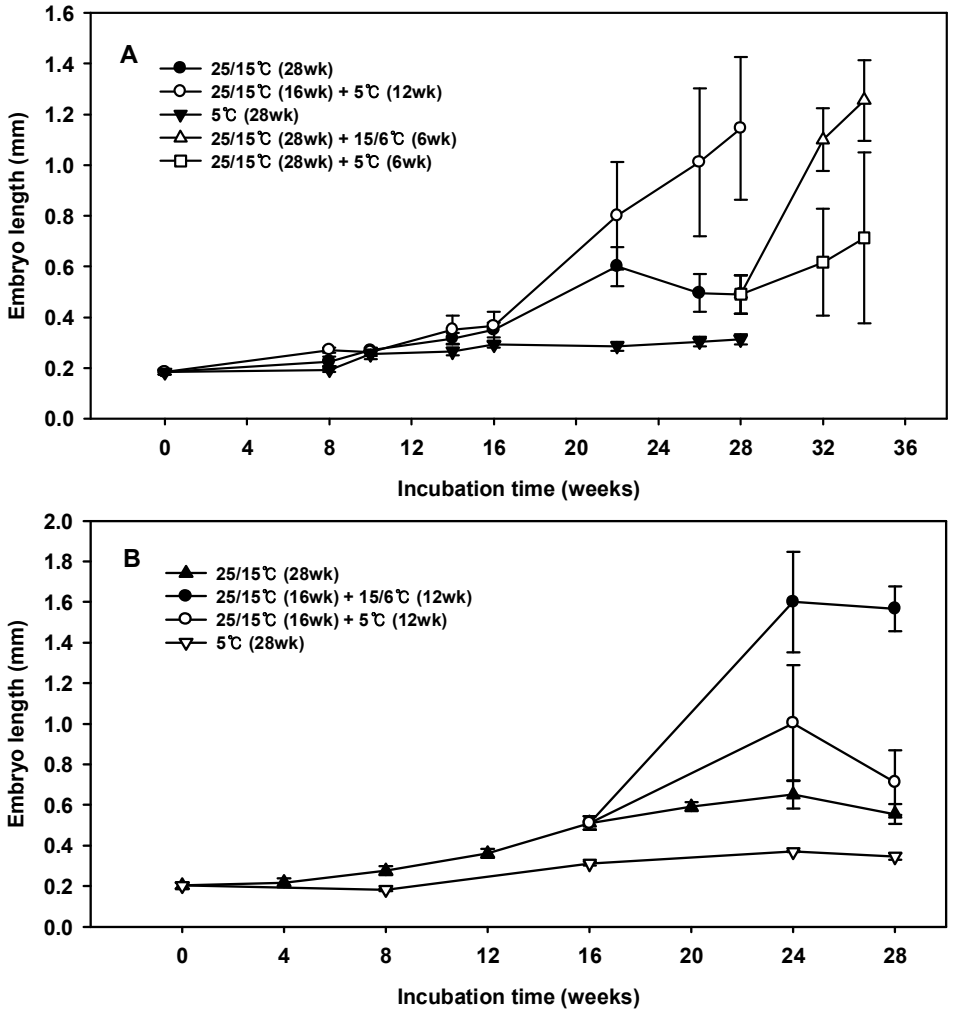

Fig. IV-1-2. Embryo length in seeds of *Adonis amurensis* as affected by temperature treatments. Seeds collected in 2010 (A) and 2011(B) were used for embryo growth study. Vertical bars represent SE.

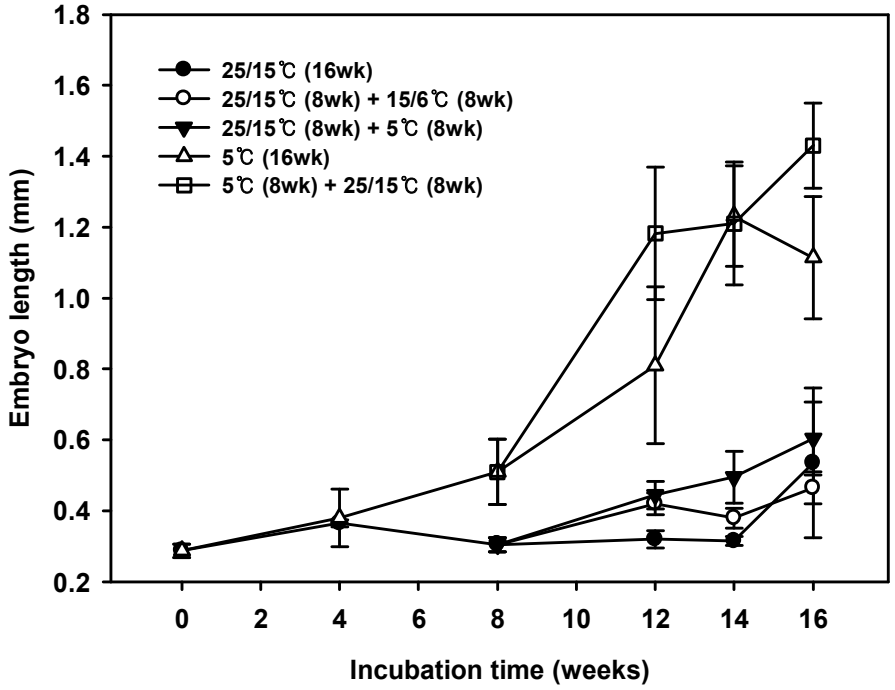

Fig. IV-1-3. Embryo length in seeds of *Ranunculus crucilobus* as affected by temperature treatments. Seeds collected in 2013 were used for this study. Vertical bars represent SE.

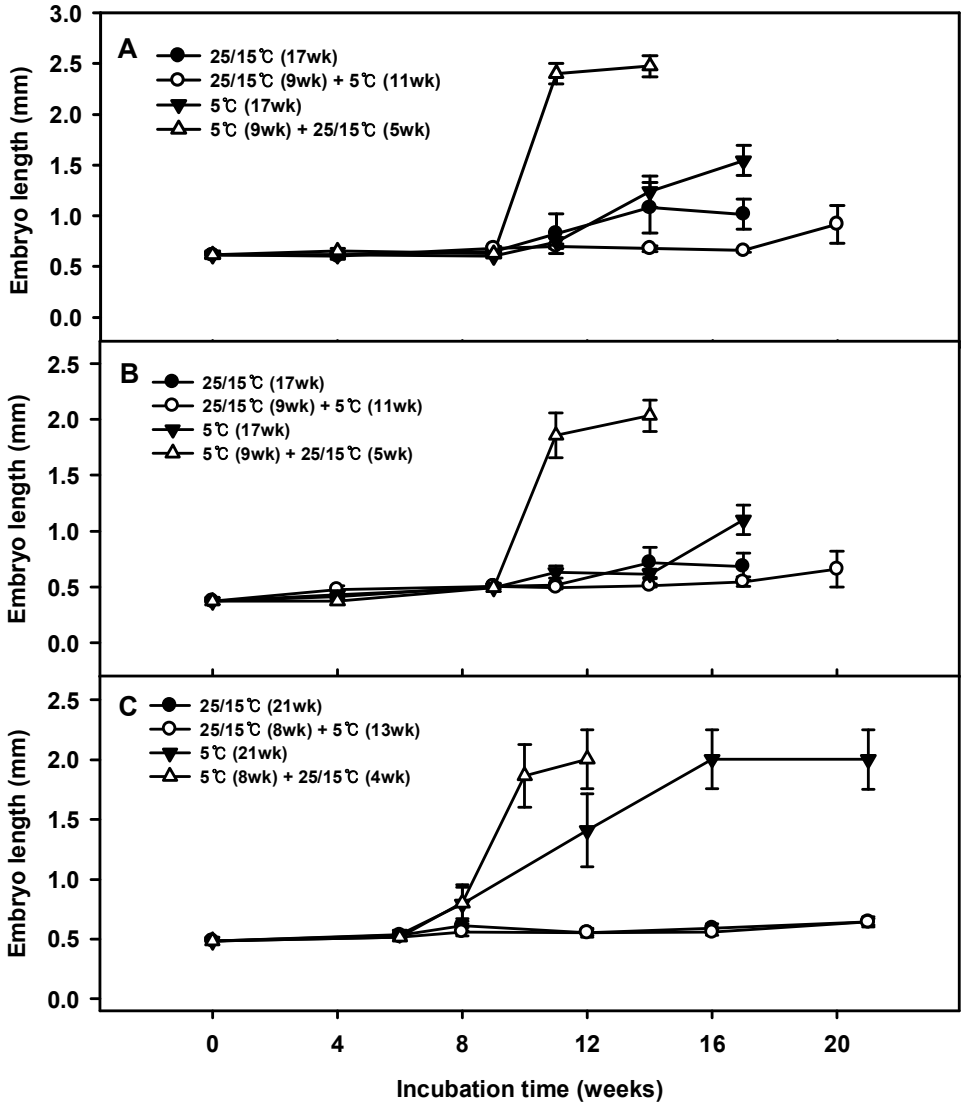

Fig. IV-1-4. Embryo length in seeds of *Thalictrum rochenbrunianum* (A), *T. uchiyamai* (B), and *T. coreanum* (C) as affected by temperature treatments. Seeds collected in 2011 for *T. rochenbrunianum* and *T. uchiyamai* and in 2012 for *T. coreanum* were used for this study. Vertical bars represent SE.

Effect of Temperature Regimes on Germination: move-along experiment

Aquilegia buergeriana

The percent germination of *A. buergeriana* seeds significantly increased at 25/15°C (light/dark) compared to other examined temperatures (Fig. IV-1-5A). At constant dark conditions, this alternating temperature showed greater percentage germination (Fig. IV-1-5B). No or few seeds germinated at 5 or 15/6°C, under both light and constant dark conditions. Percentage germination at constant 20 and 25°C under constant dark conditions was lower than that under alternating light conditions.

Pulsatilla tonkangensis

The highest germination was observed at 25/15°C under both light and dark conditions (Fig. IV-1-6). More than 70% of seeds incubated at constant 15, 20, and 25°C germinated under alternating light within 4 weeks. No seeds germinated at 5°C and germination remained below 30% at 15/6°C under alternating light conditions.

Adonis amurensis

No or few seeds germinated within 52 weeks at the constant temperature of 5°C, or at any of the three daily alternating temperature regimes of 15/6, 20/10, or 25/15°C (Fig. IV-1-7). For seeds incubated in the temperature sequence beginning at 25/15°C, germination began near the end of the 5°C treatment and increased rapidly to 43.3 ± 12.0% at 15/6°C. In the temperature sequence beginning at 5°C, no seeds germinated after 44 weeks of incubation. Seeds started to germinate

during the second exposure to 5°C. At the end of the second 5°C, seeds germinated to 6.7%.

Ranunculus crucilobus

For seeds incubated in the temperature sequence beginning at 5°C, germination began after 10 weeks of incubation at 5°C and increased rapidly to 21.9 ± 6.5% at 15/6°C, and then to 49.0 ± 6.7% at 20/10°C (Fig. IV-1-8). In the temperature sequence beginning at 25/15°C, no seeds germinated after 14 weeks of incubation. Seeds started to germinate during the incubation at 15/6°C. But, only 6.4 ± 2.2% of the seeds germinated after 28 weeks of incubation. No or few seeds germinated within 28 weeks at the two daily alternating temperature regimes of 20/10 or 25/15°C. On the other hand, 37.8 ± 7.4% and 12.5 ± 3.1% of the seeds germinated at the constant temperature of 5°C and daily alternating temperature regimes of 15/6°C, respectively within 28 weeks.

Thalictrum rochenbrunianum

In the three *Thalictrum* species, cold stratification for several weeks was required for dormancy break and germination (Figs. IV-1-9, 10, and 11). In *T. rochenbrunianum*, seeds incubated in the temperature sequence beginning with 5°C, germination began after 10 weeks of incubation at 5°C treatment and increased rapidly to 92.9 ± 1.9% at 15/6°C (Fig. IV-1-9). For seeds incubated in the temperature sequence beginning at 25/15°C, germination was 24.7 ± 10.4% after 26 weeks of incubation, and then increased rapidly to 91.6 ± 2.6% at the second 15/6°C. Although the percentage germination increased at the constant

temperature of 5°C, germination speed and final percentage germination were higher in temperature sequences including 5°C followed by 15/6°C than in the incubation at constant 5°C.

Thalictrum uchiyamai

In *T. uchiyamai*, seeds incubated in the temperature sequence beginning at 5°C germinated to 34.6 ± 3.3% at 15/6°C, and then increased to 43.2 ± 2.5% at 20/10°C (Fig. IV-1-10A). For seeds incubated in the temperature sequence beginning at 25/15°C, germination was 4.9 ± 3.3% after 26 weeks of incubation, and then increased rapidly to 41.0 ± 1.9% at the second 15/6°C and to 52.8 ± 6.3% at the second 20/10°C. Although the percentage germination increased at the constant temperature of 5°C, both germination speed and final percentage germination were higher in temperature sequences including 5°C followed by 15/6°C than in the constant 5°C. In seeds collected in 2012, percentage germination in seeds incubated at constant alternating temperature of 25/15°C was higher than that of 2011 (Fig. IV-1-10B). But, the germination pattern in the seeds incubated in the temperature sequence beginning at 5°C was similar to that of 2011.

Thalictrum coreanum

In *T. coreanum*, no seed germinated within 32 weeks at the three daily alternating temperature regimes of 15/6, 20/10, or 25/15°C (Fig. IV-1-11). For seeds incubated in the temperature sequence beginning at 5°C, germination began after 10 weeks of incubation, and increased rapidly to 80.9 ± 4.8% at

15/6°C, and then to 91.3 ± 1.8% at 20/10°C. In the temperature sequence beginning at 25/15, no seeds germinated within 28 weeks. The seeds germinated to 54.0 ± 4.8% after the seeds were transferred from 5°C to the second 15/6°C.

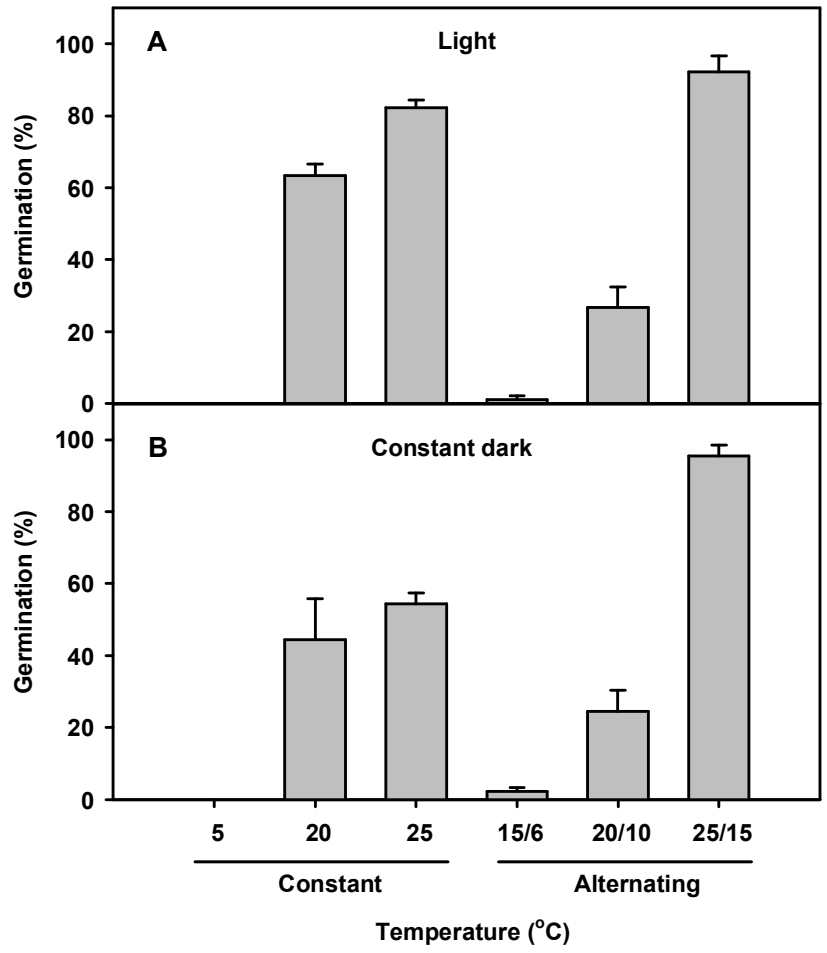

Fig. IV-1-5. Effect of different temperature regimes on seed germination after 4 weeks of incubation in *Aquilegia buergeriana* under light (12h photoperiod) (A) and constant dark conditions (B). Seeds collected in 2011 were used. Vertical bars represent SE.

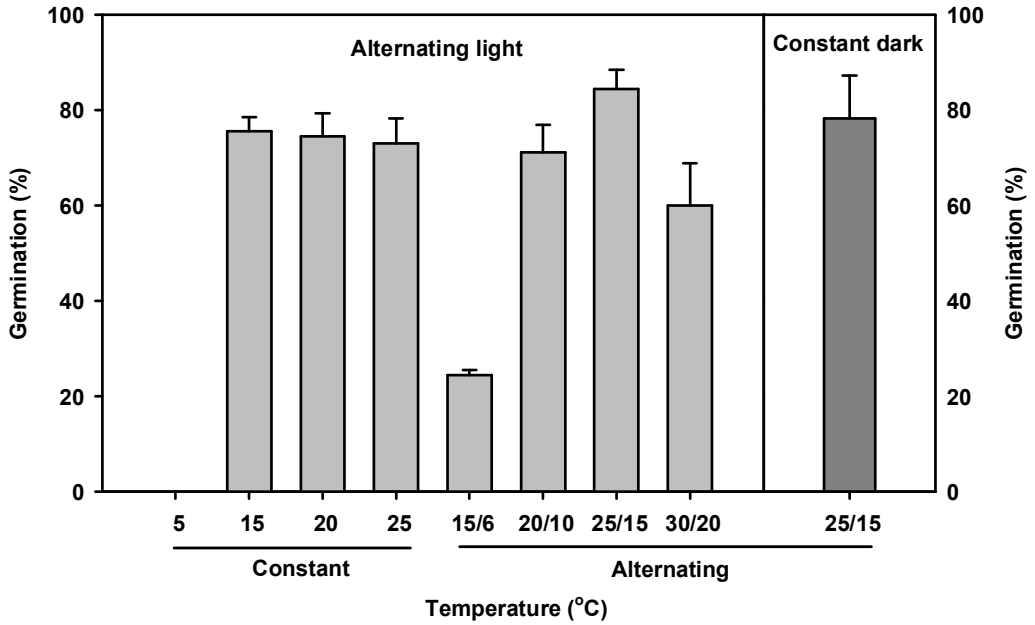

Fig. IV-1-6. Effect of different temperature regimes on seed germination after 4 weeks of incubation in *Pulsatilla tonkangensis* under alternating light (12h photoperiod) and constant dark conditions. Seeds collected in 2012 were used. Vertical bars represent SE

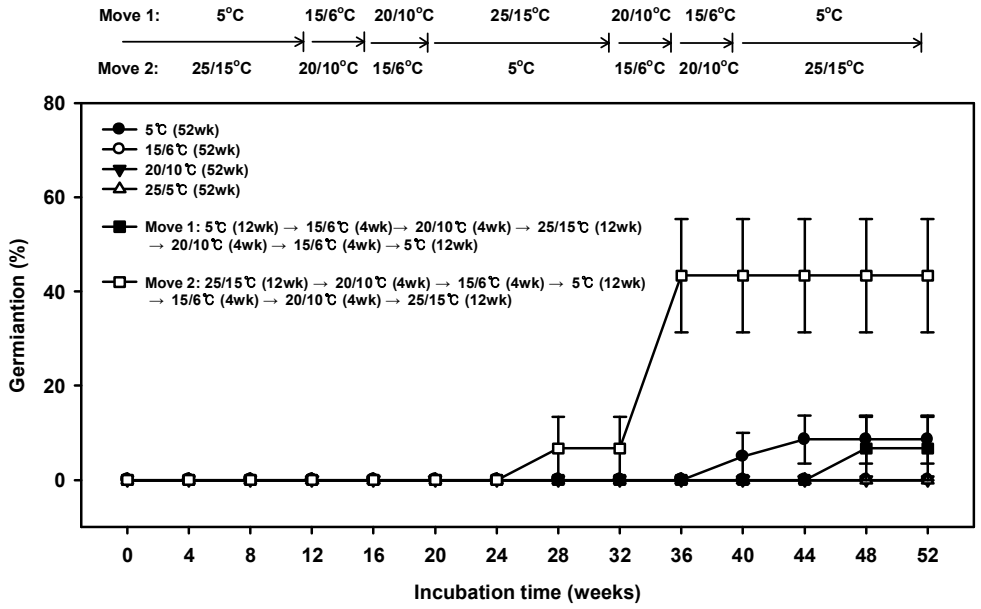

Fig. IV-1-7. Germination of *Adonis amurensis* seeds incubated under a constant temperature or a temperature sequence beginning at 25/15°C or at 5°C. Seeds collected in 2011 were used for this study. Vertical bars represent SE.

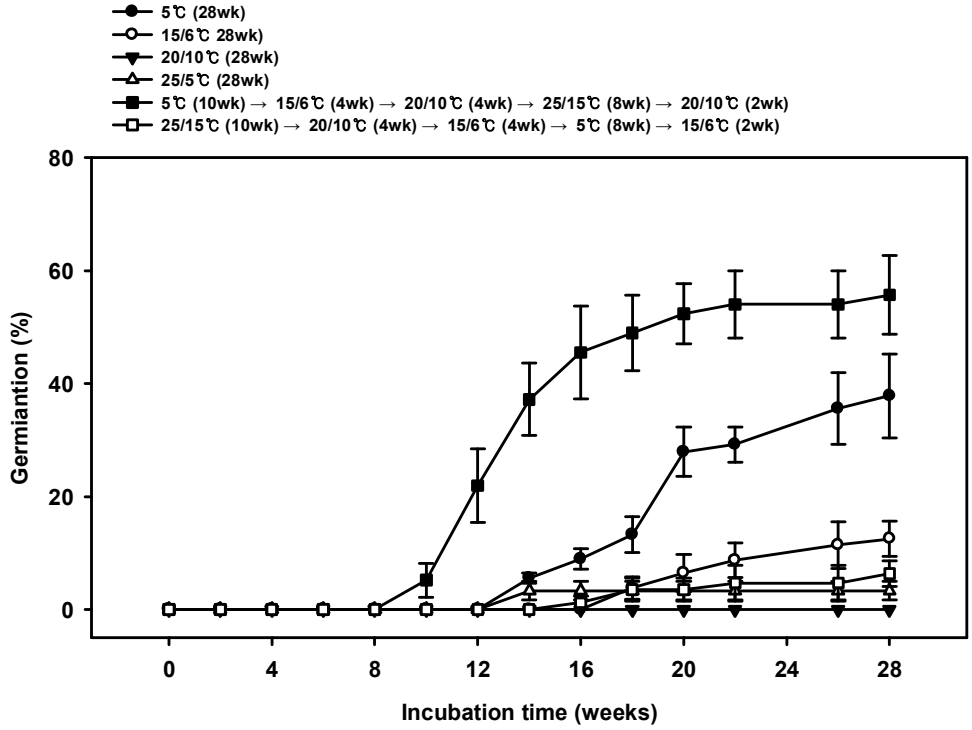

Fig. IV-1-8. Germination of *Ranunculus crucilobus* seeds incubated under a constant temperature or a temperature sequence beginning at 25/15^{o}C or at 5^{o}C. Seeds collected in 2013 were used for this study. Vertical bars represent SE.

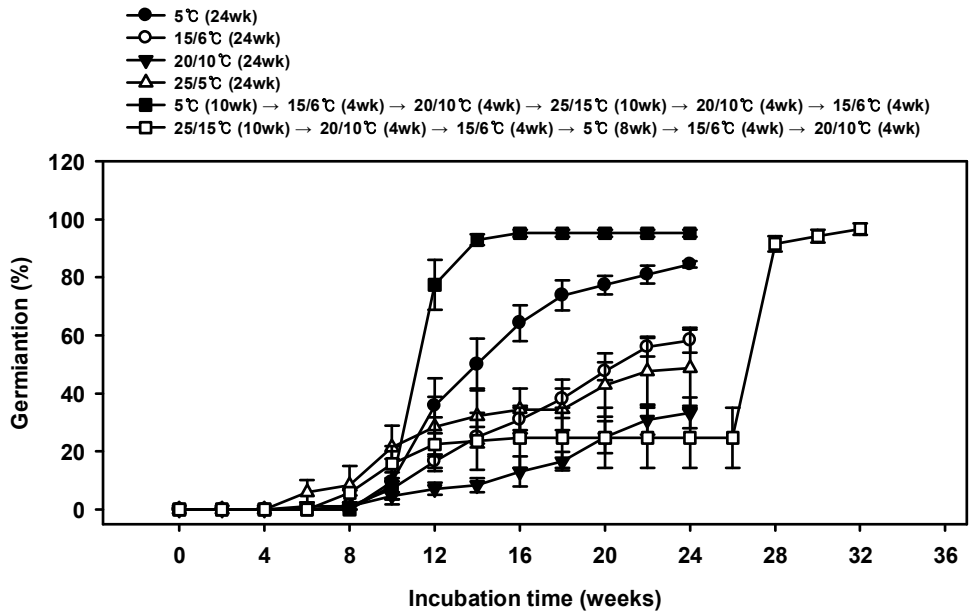

Fig. IV-1-9. Germination of *Thalictrum rochenbrunianum* seeds incubated under a constant temperature or a temperature sequence beginning at 25/15°C or at 5°C. Seeds collected in 2011 were used for this study. Vertical bars represent SE.

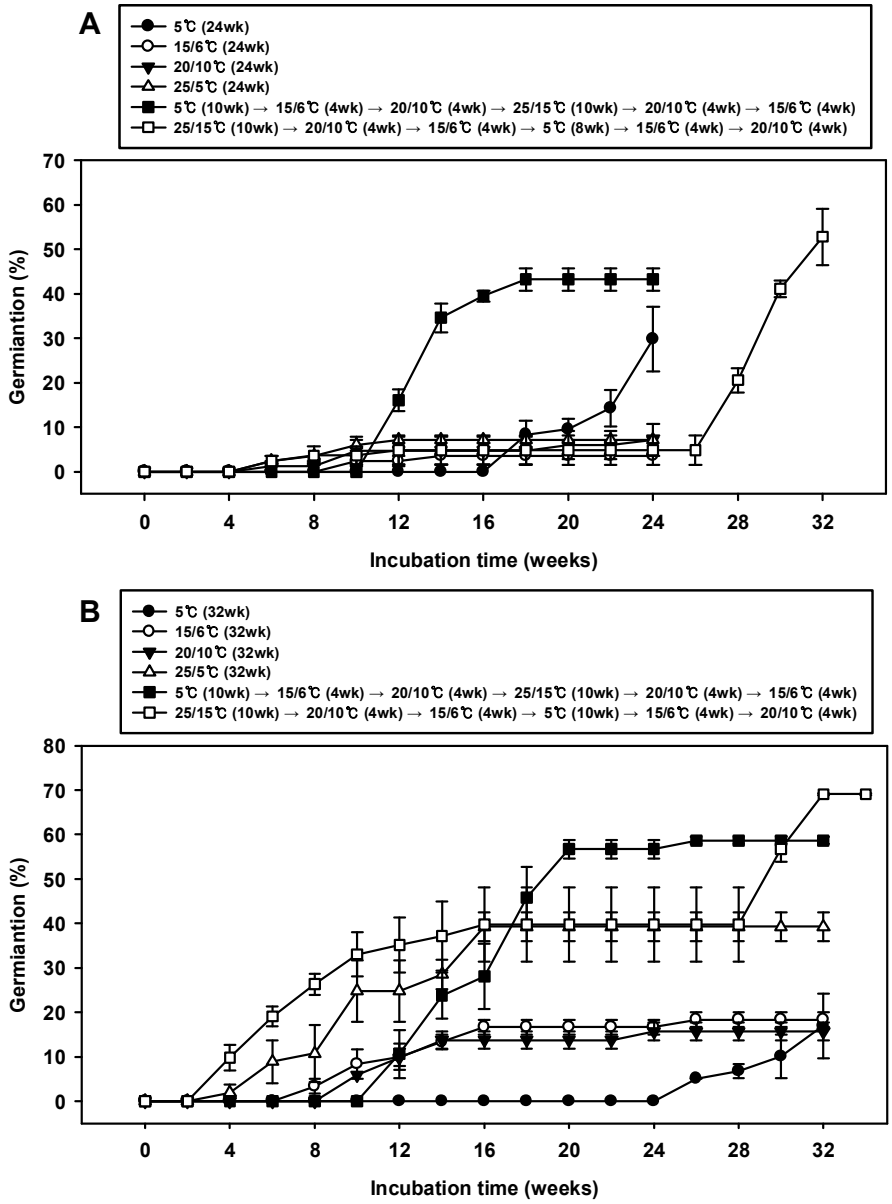

Fig. IV-1-10. Germination of *Thalictrum uchiyamai* seeds incubated under a constant temperature or a temperature sequence beginning at 25/15°C or at 5°C. Seeds collected in 2011 (A) and 2012 (B) were used for this study. Vertical bars represent SE.

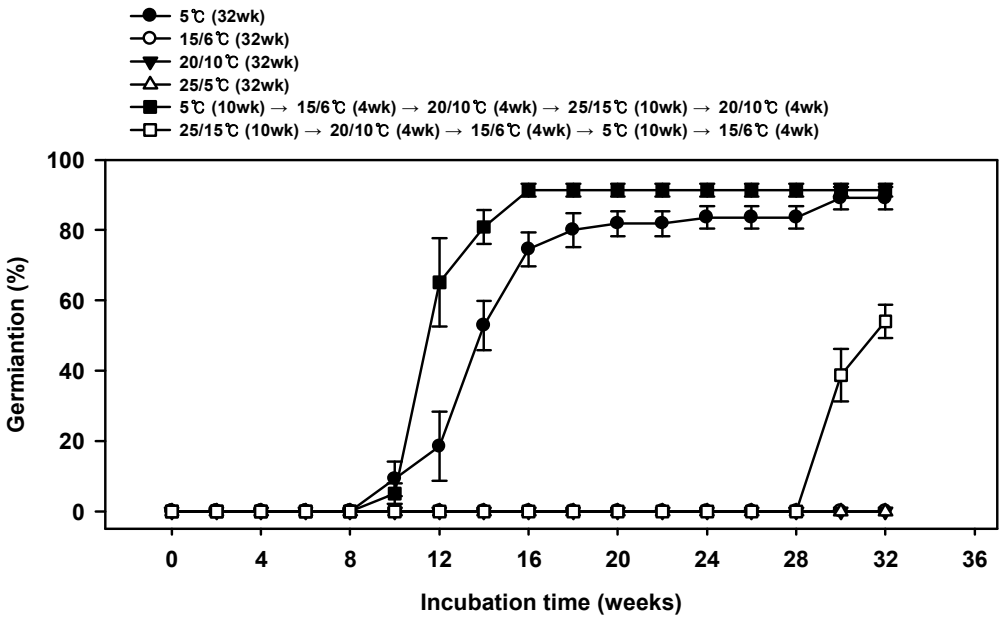

Fig. IV-1-11. Germination of *Thalictrum coreanum* seeds incubated under a constant temperature or a temperature sequence beginning at 25/15^{o}C or at 5^{o}C. Seeds collected in 2012 were used for this study. Vertical bars represent SE.

Experiment 2: Dormancy Breaking and Germination as Affected by Temperature and Gibberellic Acid

High Temperature Requirements for Embryo Growth and Germination

Aquilegia buergeriana

Following 7 days of warm stratification, seeds of *A. buergeriana* germinated to 35.6, 43.3, and 94.4% at 15/6, 20/10, and 25/15°C, respectively, after 3 weeks of incubation (Fig. IV-2-1A). Therefore, short period warm stratification promoted germination under relatively low temperatures (15/6 and 20/10°C) because without warm stratification few seeds of *A. buergeriana* germinated at 15/6 °C (Fig. IV-1-5).

Pulsatilla tonkangensis

Following 6 days of warm stratification, seeds *P. tonkangensis* germinated to 86.4, 86.8, and 87.9% at 15/6, 20/10, and 25/15°C, respectively, after 24 days of incubation (Fig. IV-2-1B). Therefore, short period warm stratification promoted germination under relatively low temperatures (15/6 and 20/10°C) because without warm stratification less than 30% of *P. tonkangensis* seeds germinated at 15/6°C (Fig. IV-1-6).

Adonis amurensis

Embryos grew at 15/6°C for 8 weeks after warm stratification at 25/15°C for 0, 3, 6, 9, or 16 weeks (Fig. IV-2-2A). When the seeds were warm stratified for more than 6 weeks, the embryo length was longer than that in 0 or 3 weeks of

warm stratification. Effect of burial date on pericarp dehiscence was determined. When seeds were buried from 23 Jun. to 23 Aug. in 2013, more than 70% of the seeds had a split pericarp (Fig. IV-2-2B). However, when the burial date was delayed to 23 Sep. through 31 Oct., the percentage of a split pericarp decreased, indicating that the seeds did not have enough warm stratification before being moved to medium to low temperatures.

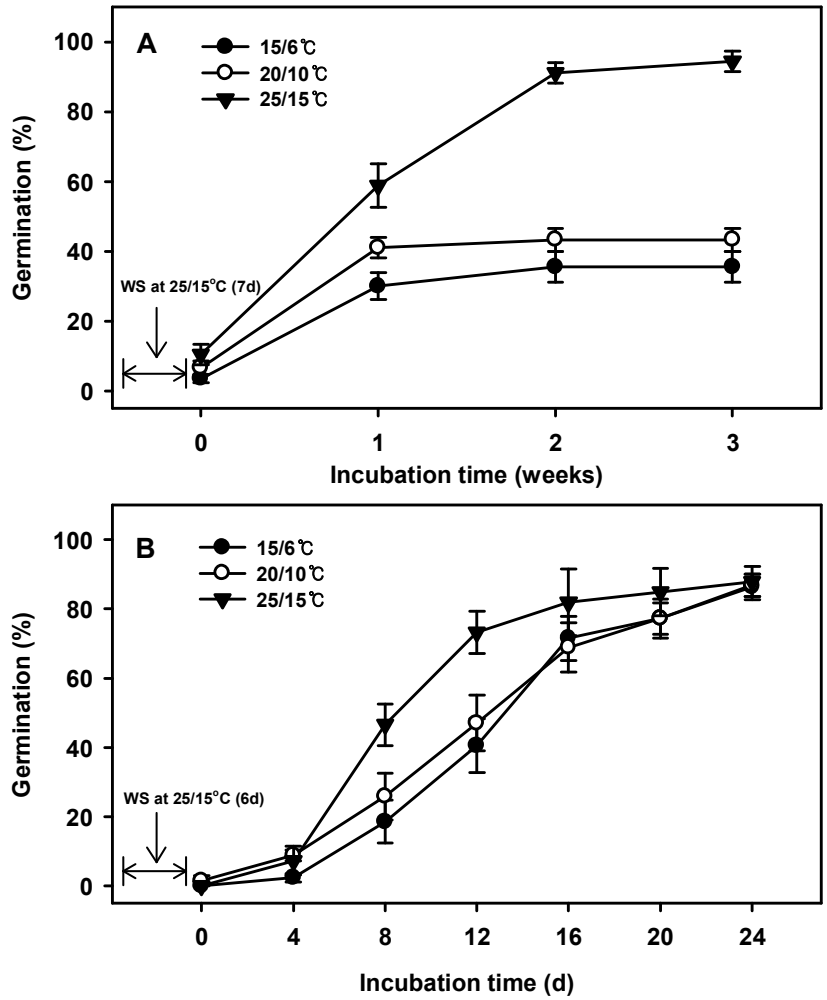

Fig. IV-2-1. Effect of warm stratification (WS) at 25/15°C on germination of *Aquilegia buergeriana* (A) and *Pulsatilla tonkangensis* (B) seeds. Seeds were warm stratified for 7 or 6 days, respectively, before being moved to 15/6, 20/10, or 25/15°C. Seeds collected in 2011 for *A. buergeriana* and 2012 for *P. tonkangensis* were used for this study. Vertical bars represent SE.

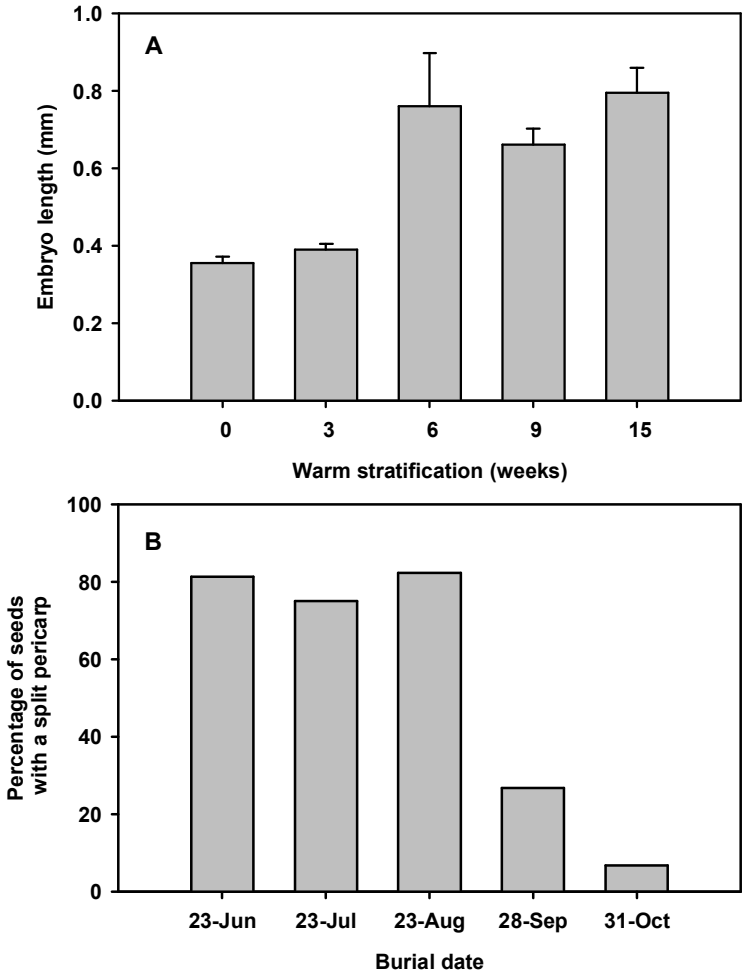

Fig. IV-2-2. Embryo length and mean percentage of seeds with a split pericarp in seeds of *Adonis amurensis* as affected by warm stratification period (A) and burial date (B), respectively, in a open field. Seeds were warm stratified at 25/15°C for 0, 3, 6, 9, or 15 weeks and then moved to 15/6°C. After 8 weeks of incubation at 15/6°C, embryo length was measured. For the effect of burial date on pericarp dehiscence, seeds were buried at five date each in 2013 and a split pericarp was observed on 27 Dec. 2013. Vertical bars represent SE.

Cold Temperature Requirements for Embryo Growth and Germination

Adonis amurensis

In the natural environment, embryo growth in seeds of *A. amurensis* was completed (see phenology in Chapter I) in autumn. After embryo maturation, the seeds experienced cold period in winter. When seeds were exhumed on 16 Nov. and 17 Dec. from a field, they germinated to 0 and 11.1%, respectively, after 4 weeks of incubation at 20/10°C (Table IV-2-1). However, when they were transferred from field to laboratory on 21 Jan. and 11 Feb., the seeds germinated to 46.6 and 82.2%, respectively, after 4 weeks of incubation at 20/10°C.

Ranunculus crucilobus

In *R. crucilobus*, cold stratification pretreatment was required for germination (Fig. IV-2-3). No seed germinated after 4 weeks at 25/15°C. However, seeds after cold stratification at 5 for 4, 8, or 12 weeks germinated to 41.7, 61.9, or 83.8%, respectively.

Ranunculus franchetii

In *R. franchetii*, seeds germinated in autumn after warm period in summer in an open field (Fig. IV-2-4). However, seedling emergence was not observed in the germinated seeds when the seeds were incubated at room temperature (20-25°C) for more than 100 days.

Thalictrum rochenbrunianum

In the three *Thalictrum* species, cold stratification pretreatment had

significant effect on germination (Table IV-2-2). In *T. rochenbrunianum*, seeds incubated at 25/15°C following 4-12 weeks of cold stratification germinated to 67-95% after 2 weeks of incubation whereas few seeds germinated without cold stratification pretreatment. Seeds were exhumed from a field and then incubated at 25/15°C (Table IV-2-3). When the seeds were exhumed on October, December, and January, they germinated to 16.1, 97.8, and 91.7%, respectively, after 2 weeks of incubation.

Thalictrum uchiyamai

In *T. uchiyamai*, seeds incubated at 25/15°C following 8-12 weeks of cold stratification at 5°C germinated to 56-66% after 2 weeks of incubation whereas cold stratified seeds for 0 and 4 weeks germinated to 0 and 8.4%, respectively, after 2 weeks of incubation (Table IV-2-2). Cold stratification at 5°C was more effective than at 1°C. When seeds were warm stratified for 6 weeks before they were transferred to 5 or 1°C for cold stratification, final germination percentages at 25/15°C after 12 weeks of incubation were higher than those without warm stratification pretreatment. When the seeds were exhumed on October, December, and January, they germinated to 0, 64.4, and 85.5%, respectively, after 2 weeks of incubation (Table IV-2-3).

Thalictrum coreanum

In *T. coreanum*, seeds were cold stratified at 5°C only. The seeds germinated to 69-72% after 2 weeks of incubation at 25/15°C following 6-12 weeks of cold stratification at 5°C. However, cold stratified seeds for 0 and 3 weeks germinated

to 0 and 15.0%, respectively after 2 weeks of incubation at 25/15^{o}C. When the seeds were exhumed on October, December, and January, they germinated to 0, 77.7, and 84.7%, respectively, after 2 weeks of incubation (Table IV-2-3).

Table IV-2-1. Effect of exhumed dates on germination of *Adonis amurensis* seeds. Seeds were collected on 22 May in 2011 and buried in a field soil on 19 Jun. 2011. Exhumed seeds were incubated at 20/10°C and then germination percentage at 2 and 4 weeks of incubation was calculated.

Exhumed dates	Incubation time (weeks)	
	2	4
2011		
November 16	0.00 b[z]	0.00 c
December 17	0.00 b	11.11 c
2012		
January 21	26.67 b	46.67 b
February 11	76.67 a	82.22 a

[z]Germination percentages followed by different letters in a column indicate significant differences at $P < 0.05$ (Tukey's honestly significant difference test).

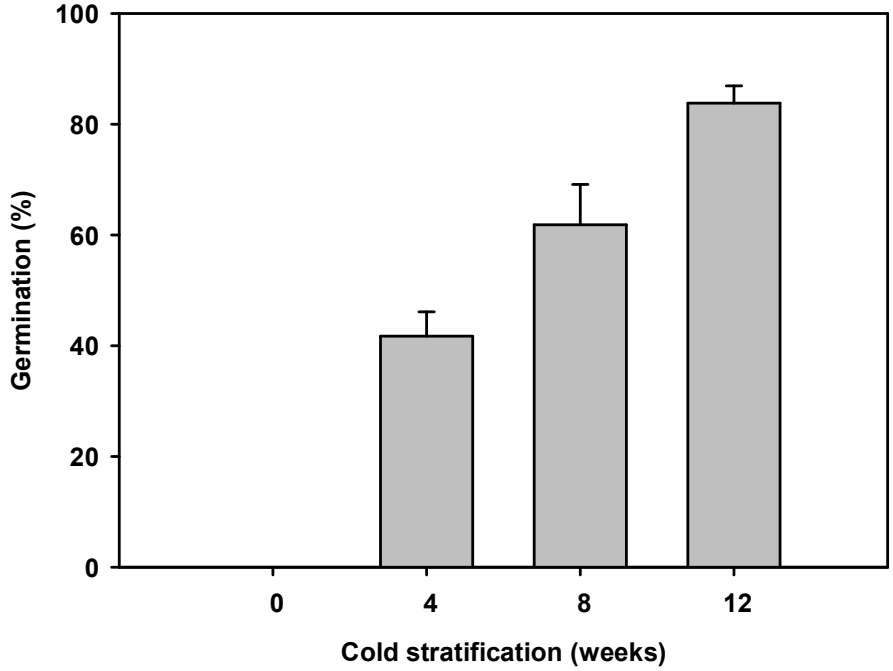

Fig. IV-2-3. Percent germination of *Ranunculus crucilobus* seeds after 4 weeks of incubation at 25/15°C as affected by 0, 4, 8, or 12 weeks of cold stratification at 5°C. Seeds collected in 2013 were used. Vertical bars represent SE.

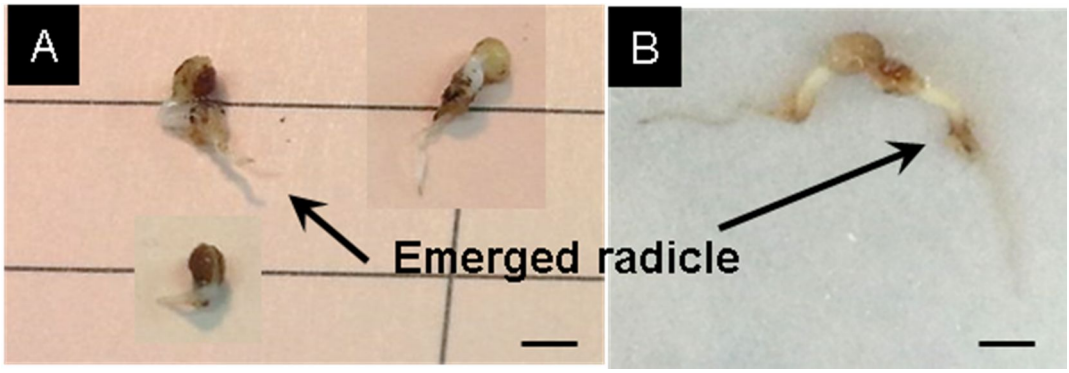

Fig. IV-2-4. Germinated seeds (A) on 14 Nov. 2013 in a field and the seeds (B) incubated for 80 days at room temperatures (20-25^{o}C) after moving them to laboratory in *Ranunculus franchetii*. Although germinated seeds in the field in autumn were moved to warm temperature conditions in laboratory, no seedlings emerged after more than 100 days. Seeds collected in 2013 were used. Vertical bars = 2mm.

Table IV-2-2. Effect of a cold (1 or 5°C) stratification period on germination percentages in seeds of three *Thalictrum* species. Stratified seeds were incubated at 25/15°C.

Stratification weeks at			Incubation time after warm and/or cold stratification (weeks)			
25/15°C	5°C	1°C	2	4	6	12
				T. rochenbrunianum[z]		
0	0	0	0.0 e[y]	1.1 c	12.2 b	42.2 b
0	4	0	89.8 abc	90.9 ab	93.2 a	96.6 a
0	8	0	79.2 cd	94.7 ab	94.7 a	96.0 a
0	12	0	95.4 ab	95.4 ab	95.4 a	96.6 a
0	0	4	79.8 bcd	84.6 b	88.1 a	90.5 a
0	0	8	67.7 d	94.2 ab	94.2 a	94.2 a
0	0	12	94.2 abc	96.5 ab	97.7 a	97.7 a
6	12	0	96.5 a	98.8 a	98.8 a	98.8 a
6	0	12	94.3 abc	98.9 a	98.9 a	98.9 a
				T. uchiyamai		
0	0	0	0.0 c	0.0 e	4.4 d	11.1 e
0	4	0	8.4 c	27.0 d	35.5 c	36.7 de
0	8	0	66.4 a	74.4 a	77.1 ab	77.1 abc
0	12	0	56.3 a	70.7 ab	72.0 ab	73.1 abc
0	0	4	7.4 c	50.0 bc	56.7 bc	61.1 bcd
0	0	8	23.2 b	36.8 cd	40.5 c	41.6 d
0	0	12	12.1 bc	39.8 cd	51.9 bc	51.9 cd
6	12	0	55.5 a	76.0 a	77.7 ab	84.0 ab
6	0	12	68.4 a	87.5 a	88.6 a	88.6 a
				T. coreana		
	0		0.0 c	0.0 c	0.0 c	-
	3		15.0 b	18.3 b	18.3 b	-
	6		69.0 a	77.8 a	77.8 a	-
	9		70.5 a	81.6 a	81.6 a	-
	12		72.1 a	90.8 a	90.8 a	-

[z]Seeds collected in 2011 for *T. rochenbrunianum* and *T. uchiyamai* and in 2012 for *T. coreanum* were used for the stratification experiment.

[y]Mean separation within columns by Tukey's honestly significant difference test at $P < 0.05$.

Table IV-2-3. Effect of exhumed dates on germination in seeds of three *Thalictrum* species. Buried seeds were exhumed at each date and then incubated at 25/15°C. Germination percentage at 2 weeks of incubation was calculated.

Exhumed dates	Germination (%) after 2 weeks of incubation at 25/15°C		
	T. rochenbrunianum[z]	*T. uchiyamai*	*T. coreanum*
September	0.0 c[y]	0.0 c	0.0 b
October	16.1 b	0.0 c	0.0 b
December	97.8 a	64.4 b	77.7 a
January	91.7 a	85.5 a	84.7 a

[z]Seeds collected in 2011 for *T. rochenbrunianum* and *T. uchiyamai* and in 2012 for *T. coreanum* were used.

[y]Germination percentages among the exhumed dates followed by different letters indicate significant differences at $P < 0.05$ (Tukey's honestly significant difference test).

Effect of GA Treatment on Embryo Growth and Germination

Aquilegia buergeriana

In *A. buergeriana* seeds, for all temperature conditions, the final germination percentage increased with increasing concentration of GA_3 (Fig. IV-2-5). In particular, the effect of GA_3 on germination promotion was high at 15/6 and 20/10^{o}C.

Pulsatilla tonkangensis

In *P. tonkangensis* seeds, germination percentage decreased with increasing concentration of GA_3 at 25/15^{o}C. However, the final germination percentage increased with increasing concentration of GA_3 at 15/6^{o}C. In the two species, seeds without GA_3 treatment germinated up to 75-80% during 4 weeks of incubation at 25/15^{o}C.

Adonis amurensis

None of the seeds showed a split pericarp and germination without GA_3 treatment at any of the incubation temperatures for 16 weeks (Figs. IV-2-6 and 7). However, percentage of seeds with a split pericarp increased with increasing concentration of GA_3 from 0 to 100mg·L^{-1} (Figs. IV-2-6A and 7). Among the incubation temperatures, the percentage of seeds with a split pericarp was the highest at 15/5^{o}C. Although the pericarp dehiscence was observed in seeds treated with GA_3, no or few seeds germinated in all incubation temperatures for 16 weeks (Fig. IV-2-7). In addition, fluridone treatment was not effective on germination (data not shown).

Ranunculus crucilobus

In seeds collected in 2012, GA_3 treatment increased percentage germination at 25/15°C (Fig. IV-2-8A). The final germination proportions recorded at 0, 10, 100, and 1000 $mg \cdot L^{-1}$ GA_3 were 1.7, 4.2, 3.3, and 25.1%, respectively. In seeds collected in 2013, seeds treated with 1000 $mg \cdot L^{-1}$ GA_3 germinated to 19.7% after 12 weeks of incubation. Therefore, effect of GA_3 on germination was similar to 2012 and 2013. However, when the seeds were soaked in solutions of 20 $mg \cdot L^{-1}$ fluridone or fluridone + 100 $mg \cdot L^{-1}$ GA_3, germination percentages were 60.4 and 84.7% at 25/15°C and 55.4 and 89.5% at 15/6°C, respectively (Fig. IV-2-8B).

Thalictrum rochenbrunianum

In *T. rochenbrunianum*, for all temperature regimes, the final germination percentage increased with increasing concentration of GA_3 (Figs. IV-2-9A, 9B, and 9C). At 4 weeks after incubation, seeds treated with 1000 $mg \cdot L^{-1}$ GA_3 germinated to more than 80% at 15/5, 20/10 and 25/15°C. However, no seeds germinated at 5°C (Fig. IV-2-9A). After 12 weeks of incubation, seeds treated with 1000 $mg \cdot L^{-1}$ GA_3 germinated to more than 80% at 5°C (Fig. IV-2-9C).

Thalictrum uchiyamai

In *T. uchiyamai*, for all temperature regimes, the final germination percentage increased with increasing concentration of GA_3 from 0 to 100 $mg \cdot L^{-1}$ (Figs. IV-2-9D, 9E, and 9F). After 12 weeks of incubation, seeds treated with 100 $mg \cdot L^{-1}$ GA_3 germinated to more than 70% at 15/6, 20/10 and 25/15°C (Fig. IV-2-9F).

However, a solution of 1000 $mg \cdot L^{-1}$ GA_3 was not effective on germination.

Thalictrum coreanum

In *T. coreanum*, the final germination percentage increased with increasing concentration of GA_3 from 0 to 1000 $mg \cdot L^{-1}$ at 25/15°C after 4 weeks of incubation (Fig. IV-2-9G). But, no or few seeds germinated at 5 and 15/6°C. After 12 weeks of incubation, seeds treated with 1000 $mg \cdot L^{-1}$ GA_3 germinated to more than 70% at 25/15°C (Fig. IV-2-9I). In addition, seeds incubated at 5°C germinated to 33.3% without GA_3 treatment.

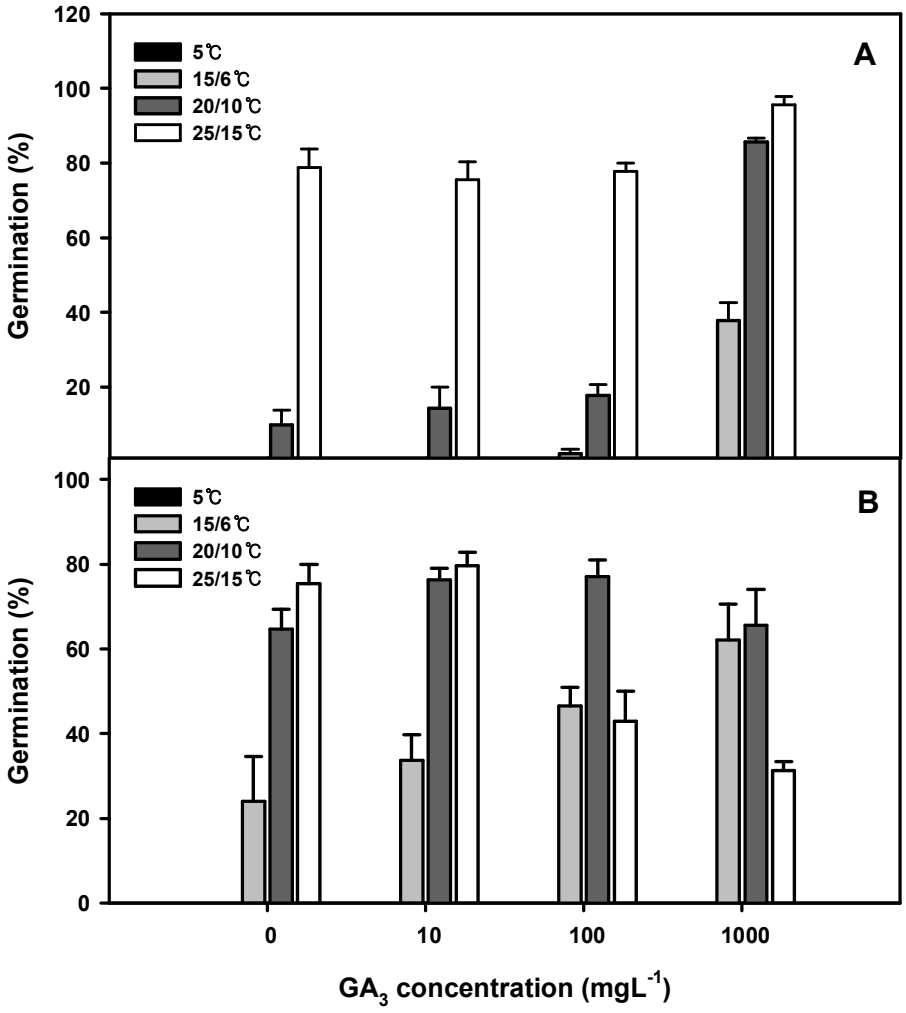

Fig. IV-2-5. Effect of GA_3 and temperature regimes on germination after 4 weeks of incubation in seeds of *Aquilegia buergeriana* (A) and *Pulsatilla tonkangensis*. Seeds collected in 2011 for *A. buergeriana* and in 2012 for *P. tonkangensis* were used. Vertical bars represent SE.

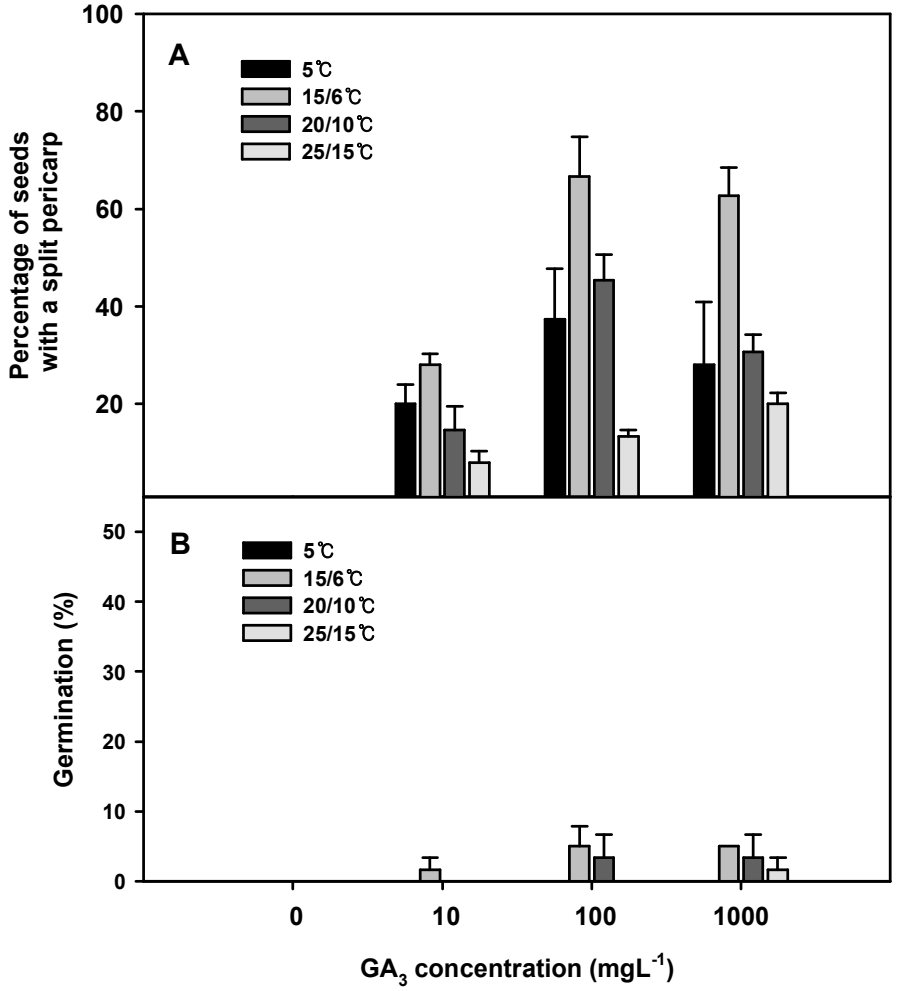

Fig. IV-2-6. Effect of a 0, 10, 100, or 1000 mg·L^{-1} GA_3 treatment on mean percentage of seeds with a split pericarp (A) and mean final percentage germination (B) in seeds of *Adonis amurensis* after 16 weeks of incubation at four different temperature regimes. Seeds collected in 2011 were used. Vertical bars represent SE.

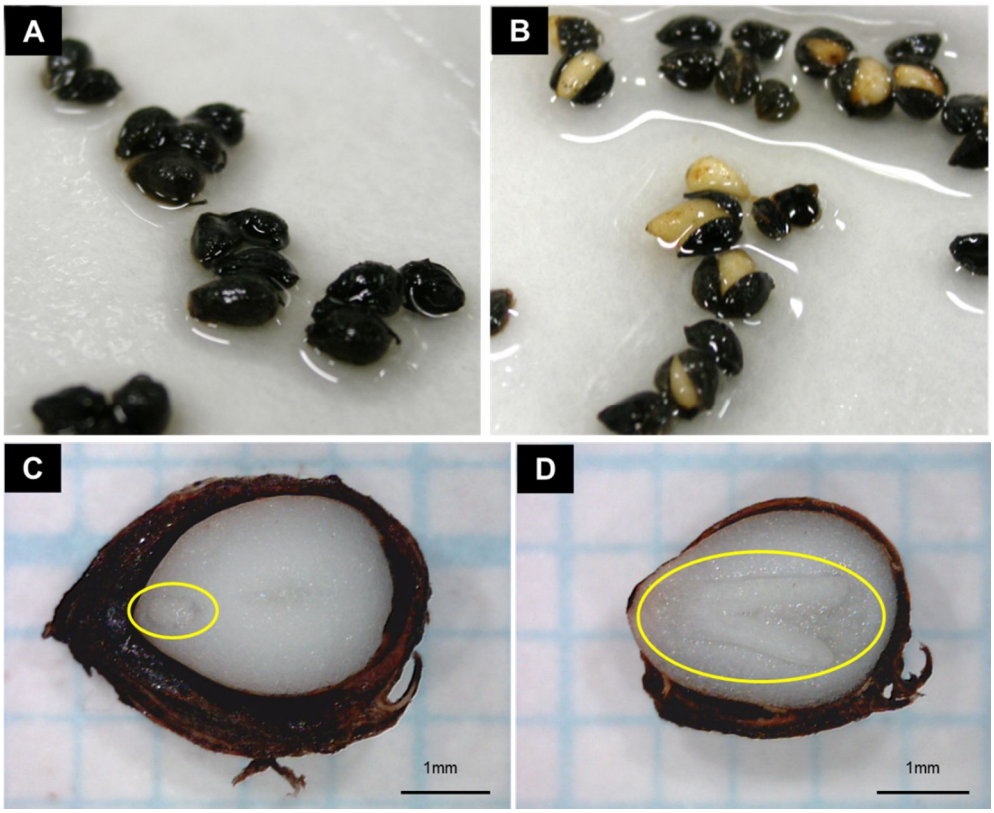

Fig. IV-2-7. Seed morphology without (A and C) or with GA treatment (B and D) in *Adonis amurensis*. Outside appearance (A and B) and longitudinal section (C and D) of the seeds treated 0 or 100 $mg \cdot L^{-1}$ GA_3 were observed at 14 weeks of incubation at 15/6°C. Seeds collected in 2011 were used. GA increased the rate of embryo growth, but it did not substitute for cold stratification. Thus, no or few seeds germinated at spring temperatures (15-20°C).

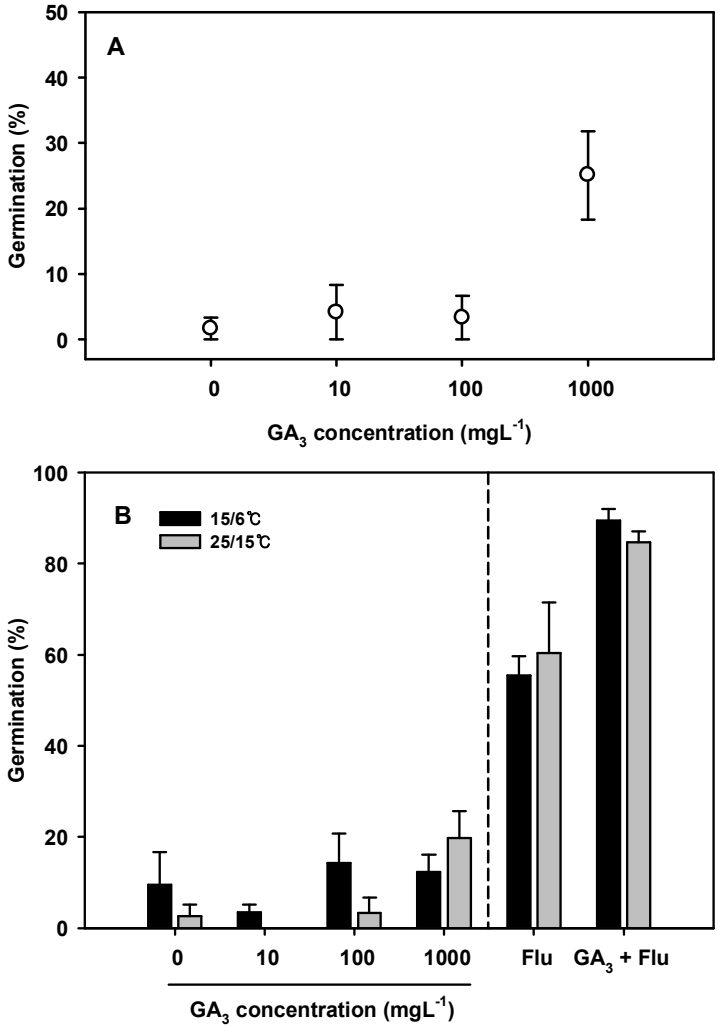

Fig. IV-2-8. Effect of GA_3 and Fluridone (Flu) treatment on mean percentage germination in seeds of *Ranunculus crucilobus* after 12 weeks of incubation. In GA_3 + Flu, 100 mg·L^{-1} of GA_3 and 20 mg·L^{-1} of fluridone were applied. Seeds collected in 2012 (A) and 2013 (B) were used. Seeds in 2012 (A) were incubated at only 25/15^{o}C after the treatments. Vertical bars represent SE.

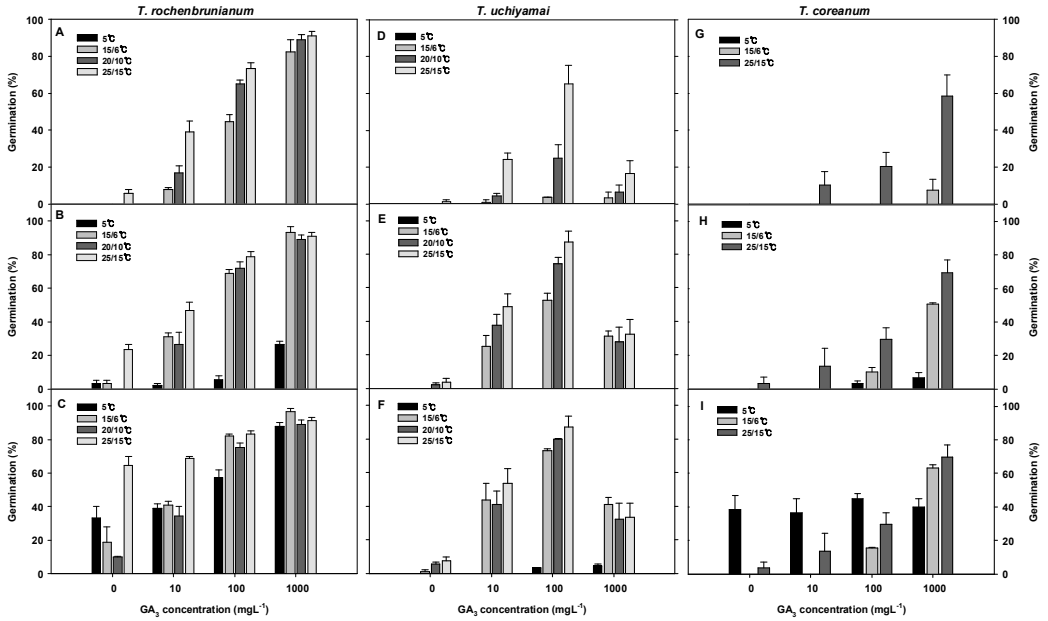

Fig. IV-2-9. Effect of a 0, 10, 100, or 1000 mg·L^{-1} GA_3 treatment on mean percentage germination in seeds of *Thalictrum rochenbrunianum* (A, B, and C), *T. uchiyamai* (D, E, and F) and *T. coreanum* (G, H, and I) after 4 (A, D, and G), 8 (B, E, and H), and 12 (C, F, and I) weeks of incubation at different temperature regimes. Seeds collected in 2011 for *T. rochenbrunianum* and *T. uchiyamai* and in 2012 for *T. coreanum* were used. Vertical bars represent SE.

DISCUSSION

Eight species in Ranunculaceae showed different dormancy breaking requirements in controlled laboratory conditions. We found that the seeds of *A. buergeriana* and *P. tonkangensis* showed morphological dormancy (MD), whereas the seeds of the other study species (*A. amurensis*, *R. crucilobus*, *R. franchetii*, *T. rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*) showed morphophysiological dormancy (MPD).

Nine types of MPD have been distinguished based on i) temperatures required for dormancy break, ii) temperatures at the time of embryo growth and iii) whether GA overcomes dormancy (Baskin and Baskin, 1998, 2004, 2014). These are non-deep simple, intermediate simple, deep simple, non-deep simple epicotyl, deep simple epicotyl, deep simple double, non-deep complex, intermediate complex, and deep complex MPD.

Based on temperatures at the time of embryo elongation, seeds with MPD have been divided into two categories: simple and complex (Baskin and Baskin, 1998, 2004). Embryo growth occurs at relatively warm temperatures (≥ 15°C) in simple MPD; whereas, in complex MPD, embryo growth occurs at low temperatures (0-10°C) (Baskin and Baskin, 1998, 2004). Each type of MPD (simple or complex) can be subdivided into non-deep, intermediate, and deep MPD depending on the physiological states of seeds (Baskin and Baskin, 1998, 2004; Nikolaeva 1977). The dormancy of seeds with non-deep simple MPD can be broken by warm or cold stratification, and GA can be used to overcome the dormancy (Baskin and Baskin, 1998, 2004). Seed dormancy in intermediate

simple MPD can be broken by warm plus cold stratification and by GA treatment. On the other hand, in seeds with deep simple MPD, warm plus cold stratification is essential and GA is ineffective for breaking the dormancy.

In seeds with deep simple epicotyl MPD, radicle emergence occurs before the seeds receive cold stratification in winter. After radicle emergence, epicotyl needs cold stratification for cotyledon emergence. In addition, seeds with deep simple double MPD need more than two years for dormancy break and germination (Baskin and Baskin, 1998; Kondo et al., 2002; Kondo et al., 2011).

In this study, underdeveloped embryos in seeds of *A. amurensis* grew at relatively high temperatures in autumn although further elongation of the embryos occurred during early winter in Korea, indicating that the seeds have a simple type MPD. Next, information on temperatures required for dormancy break and on whether GA_3 treatment overcomes dormancy is needed for additional classification of the type of MPD in *A. amurensis*. Seeds of *A. amurensis* required warm followed by cold temperature sequences to break dormancy. Although GA_3 treatment promoted embryo growth, it did not overcome dormancy. Thus, few seeds treated with GA_3 germinated during 16 weeks. Therefore, the seeds have deep simple MPD.

In addition to the nine types of MPD, Baskin and Baskin (1998) documented a special type of deep simple epicotyl MPD for seeds of *Hydrastis canadensis* that have some characteristics of both deep simple and deep simple epicotyl MPD. Seeds with this kind of dormancy are characterized by embryo growth and splitting of the pericarp in autumn and emergence of radicle and seedlings in spring next year. In seeds of *A. amurensis*, pericarp splitting and endosperm

exposure outside the seeds were observed from November (see Figs. I-1 and 2 in Chapter I). Furthermore, radicles grew inside the seeds at that time. But, emergence of radicle and cotyledon from the seeds occurred in spring next year (see Figs. I-1 and 2 in Chapter I). Thus, seeds of *A. amurensis* have a special type of deep simple epicotyl MPD. This kind of dormancy has also been reported in seeds of *Corydalis ambigua*, a native perennial species in Japan (Kondo et al., 2005). Baskin and Baskin (2014) documented that this kind of dormancy represents a transitional stage between deep simple and deep simple epicotyl.

Seeds of the two *Ranunculus* species showed different dormancy breaking requirements although they are in the same genus. In *R. crucilobus*, embryos grew consistently at cold temperature during winter in the natural environment (see Figs. I-5 and 6 in Chapter I). In the controlled laboratory experiment, the embryos grew at 5^{o}C although the rate of embryo growth increased at warm temperature (25/15^{o}C) following cold stratification at 5^{o}C. Thus, the seeds have complex type MPD. Seeds of *R. crucilobus* required only a cold stratification for dormancy breaking. GA_3 treatment did not completely overcome the dormancy although the percentage germination increased with increasing the concentration of GA_3. Therefore, the seeds have deep complex MPD. In the Ranunculaceae, deep complex MPD have been reported in seeds of *Delphinium tricorne* (Baskin and Baskn, 1994).

We found that germination of *R. franchetii* had already ended in November after warm season in summer in the natural environment (see Figs. I-7 and 8 in Chapter I). But, we did not find cotyledons emergence after radicle emergence.

Germinated seeds in the field did not show seedlings emergence in laboratory conditions when moved to room temperatures (Fig. IV-2-4). Many plants whose cotyledons do not emerge immediately after radicle emergence are known. This phenomenon is referred to as 'epicotyl dormancy' (Baskin and Baskin, 1998). Similar results were found in *Erythronium japonicum* native in Japan (Kondo et al., 2002) and *Narcissus hispanicus* native in the Iberian Peninsula (Copete et al., 2011).

There are two types of epicotyl MPD; non-deep simple epicotyl and deep simple epicotyl (Baskin and Baskin, 2014; Copete et al., 2011; Dhyani et al., 2013; Kondo et al., 2002). In seeds with non-deep simple epicotyl MPD, cold stratification is not essential for dormancy break. However, in seeds with deep simple epicotyl MPD, the seeds require both warm and cold stratification for dormancy break (Baskin and Baskin, 2014). In *R. franchetii*, seeds with emerged radicles did not show shoot emergence before cold in winter, indicating the seeds need cold stratification for breaking epicotyl dormancy. Epicotyl is the embryonic shoot and leaves containing the growing point (apical meristem) and the first two leaves (Mullen et al., 2008). Mesocotyl are called the first internode, and hypocotyl is the stem tissue between epicotyl and the radicle (Mullen et al., 2008). In *R. franchetii* seeds, although emerged radicles grew continuously at room temperatures, they did not produce leaves and shoot. Thus, the seeds might have epicotyl dormancy. Therefore, the seeds of *R. franchetii* are considered to have deep simple epicotyl MPD.

In three *Thalictrum* species, embryo growth occurred at low temperatures. But, the rate of embryo growth was more enhanced at warm temperature

(25/15°C) following cold stratification. Thus, seeds of them have a simple type of MPD. Seeds of the three *Thalictrum* species required only a cold stratification to break dormancy, and GA_3 substituted for cold stratification. Therefore, it can be concluded that the MPD is non-deep simple.

T. mirabile is another North American species in the genus *Thalictrum* (Walck et al., 1999). Its seeds have non-deep simple MPD, requiring only cold stratification (1°C) for more than 8 weeks to break dormancy (Walck et al., 1999). Furthermore, embryos grew at warm temperature after cold stratification, and GA_3 substituted for cold stratification in seeds of this species. Thus, it appears that these two eastern North America-eastern Asia disjuncts of *Thalictrum* exhibit the same dormancy type.

At the genus level of seed dormancy, evolutionary trends have been viewed in many species which show eastern Asia-eastern North America disjunct distribution patterns. According to Baskin and Baskin (1995, 1998), the presence of the same type of MPD in disjunct species in the same genera (congeners) with an Arcto-Tertiary distribution pattern indicates that the type of MPD is at least as old as Tertiary in Cenozoic. On the other hand, if disjunct species have different types of MPD, the differences may have been present in the Tertiary or evolved since that time. Regardless of the time of origin, each type of MPD in a genus should be considered as the product of selective pressures in each habitat. Thus, if congeners have different types of MPD, one could be derived from the other, or both could be derived from ancestors with even another type of MPD. This concept has recently been reported in many plant species with Arcto-Tertiary distribution patterns such as *Sambucus* spp. (Hidayati et al., 2000) *Osmorhiza*

aristata (Walck et al., 2002), *Aristolochia* spp. (Adams et al., 2005), and *Chaerophyllum temulum* (Vandelook et al., 2007). Therefore, the same types of MPD in eastern Asian-North American disjuncts that are members of the genus *Thalictrum* with an Arcto-Tertiary distribution are evidence that the same types of dormancy are at least as old as the Tertiary.

Seed dormancy is also controlled by other plant hormones such as ethylene, brassinosteriods, auxins, jasmonic acid, and cytokinins (Blake et al., 2002; Kucera et al., 2005, Pence et al., 2006; Socolowski and Cicero, 2011). In *Xylopia aromatica*, Promalin (GA_{4+7} + BA) treatments at concentrations of 250 and 500 $mg \cdot L^{-1}$ broke seed dormancy effectively (Socolowski and Cicero, 2011). In *Rotippa sububellata* Rollins, BAP effectively broke seed dormancy (Pence et al., 2006). In this study, we treated *A. amurensis* seeds with Promalin at a concentration of 18, 180, or 1800 $mg \cdot L^{-1}$ (data not shown). However, the treatments did not affect seed dormancy in *A. amurensis*. Thus, the response of seed dormancy by PGRs may be different depending on plant species. More detailed study is needed to determine the effect of other PGRs and to find more practical methods for propagation in the study species.

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CONCLUSION

The main aim of this dissertation research was to explore the diversity of seed dormancy and seedling emergence strategies in Korean temperate forest herbs and to study the environmental factors regulating the dormancy. The requirements for dormancy break and germination of 14 species were studied. The testing procedure consisted of a combination of field observations with laboratory experiments in which stratification requirements for dormancy break were analyzed. The discussion of the results is mainly focused on comparing differences and similarities in dormancy syndromes from an ecophysiological perspective.

Embryo morphology and seed dormancy of two Berberidaceae species (*Leontice microrhyncha* and *Jeffersonia dubia*), eight Ranunculaceae species (*Adonis amurensis*, *Aquilegia buergeriana*, *Pulsatilla tonkangensis*, *Ranunculus crucilobus*, *R. franchetii*, *Thalictrum rochenbrunianum*, *T. uchiyamai*, and *T. coreanum*), three Melanthiaceae species (*Heloniopsis koreana*, *H. tubiflora*, and *Trillium tschonoskii*), and one Liliaceae species (*Erythronium japonicum*) were studied, and the types of seed dormancy were classified, and its regulation by phytohormones was discussed. Furthermore, we collected seed dormancy data of five genera of Ranunculaceae from available information, and the types of seed dormancy were compared with congeners in different countries or continents.

All seeds of 14 species we studied had underdeveloped embryos which occupied about 7-20% of the full seed length at maturity. Most seeds of *A.*

buergeriana and *P. tonkangensis* had morphological dormancy (MD), whereas the seeds of *L. microrhyncha*, *J. dubia*, *A. amurensis*, *R. crucilobus*, *R. franchetii*, *E. japonicum*, *T. tschonoskii*, *T. coreanum* had morphophysiological dormancy (MPD). On the other hand, the seeds of *H. koreana*, *H. tubiflora*, *T. rochenbrunianum*, and *T. uchiyamai* had both MD and MPD, indicating that there was a different level of dormancy (MD and MPD) within the same seed population examined.

Among the 14 species, the types of MPD were classified in seeds of Ranunculaceae. All six species, which had MPD, emerged seedlings mainly in spring after dormancy was broken. However, different dormancy mechanisms to attain this pattern of spring emergence were observed regardless of the time of seed dispersal. Seeds of *A. amurensis* had a special type of deep simple epicotyl MPD. Embryos grew at relatively warm temperatures in autumn with split pericarp, and seeds germinated after warm followed by cold temperature sequences. Although GA promoted embryo growth, it did not fully overcome the dormancy. Seeds of *R. crucilobus* had deep complex MPD. Embryo growth occurred at cold temperature (5^{o}C), and seed dormancy was broken by cold stratification. GA did not overcome the dormancy. On the other hand, seeds of *R. franchetii* had deep simple epicotyl MPD. Embryos in the seeds grew at relatively warm temperature in early autumn, and germination occurred in late autumn. Germinated seeds required cold stratification for seedling emergence. In three *Thalictrum* species, seeds had non-deep simple MPD. Embryo growth occurred at low temperatures, but it was more promoted when the seeds were transferred from

low to high temperatures. Seeds required only a cold stratification to break dormancy, and GA substituted for cold stratification.

MD and MPD were regulated by phytohormones (ABA and GAs). In *A. buergeriana* seeds (MD), ABA content and sensitivity decreased rapidly, and GA content and sensitivity increased rapidly after burial. On the other hand, in *A. amurensis* seeds (MPD), ABA content decreased drastically after burial, but GA content did not increase before the seeds experienced temperature changes from high temperatures in summer to medium temperatures in autumn in the natural environment. When underdeveloped embryos grew rapidly, ABA was non-detectable and GA content increased. But, the seeds remained ungerminated during cold season in winter. When the seeds started to germinate after cold period in winter, GA content increased rapidly. GA_4 played a key role in stimulating embryo growth and germination in both MD and MPD. The changes of GA/ABA ratio were similar to the changes of embryo growth and germination in the buried seeds. These results indicate that MD or MPD in the basal angiosperm taxa also could be controlled by hormone balance model.

The Ranunculaceae form a relatively large family, consisting of about 1700 species in about 60 genera distributed in most parts of the world. We collected data of seed dormancy types in Ranunculaceae from available information and compared with congeners in different countries or continents (Table 5). A literature review of 30 species in the genus *Aquilegia*, *Pulsatilla*, *Adonis*, *Ranunculus*, and *Thalictrum* in Ranunculaceae and their most probable dormancy type reveals that a wide variety of dormancy mechanisms occurs (Table 5). We

found that although epicotyl MPD has not been reported in the genus *Ranunculus* so far, *R. franchetii* showed epicotyl dormancy in this study. All species in the genus *Pulsatilla* had MD. On the other hand, all species in the genus *Thalictrum* expressed non-deep simple MPD. This indicates that there has been trait stasis (i.e. little or no change in morphology or ecology) in seed dormancy within the genera. There were the same types of MPD in eastern Asian - North American congeners in the genus *Thalictrum* with an Arcto-Tertiary distribution pattern. This implies that identical types of dormancy are evidence that this type of dormancy is at least as old as the Tertiary.

Table 5. List of seed dormancy type of the genus *Aquilegia*, *Pulsatilla*, *Adonis*, *Ranunculus*, and *Thalictrum* in Ranunculaceae. A literature review of 30 species in five genera of Ranunculaceae shows a wide variety of dormancy mechanisms (types).

Species	Dormancy type[z]	Native in	Reference[y]
Adonis amurensis	A specialized type of deep simple epicotyl MPD	East Asia	**Chapter I and IV**
Adonis ramosa	MPD	Asia	Kondo et al., 2006
Adonis vernalis	MD	Europe and Asia	Poluyanova and Lyubarskii, 2008
Aquilegia barbaricina	Intermediate or deep simple MPD	endemic to Italy	Mattana et al., 2012
Aquilegia buergeriana	MD	Northeastern Asia	**Chapter I and III**
Aquilegia nugorensis	Intermediate or deep simple MPD	endemic to Italy	Mattana et al., 2012
Aquilegia pubescens	MD	North America	Chabot and Billings, 1972
Aquilegia vulgaris	Non-deep simple MPD or MD	Europe	Grime et al., 1981
Pulsatilla slavica	MD	East Europe	Lhotska and Moravcova, 1989
Pulsatilla tongkangensis	MD	Endemic to Korea	**Chapter I and III**
Pulsatilla vulgaris	MD	Europe	Wells and Barling, 1971
Ranunculus acris	Non-deep simple MPD or MD	Europe and Asia	Roberts and Boddrell, 1985
Ranunculus auricomus	Non-deep complex MPD	Europe and Asia	Vandelook, 2009
Ranunculus bulbosus	Non-deep simple MPD or MD	Western Europe and North America	Roberts and Boddrell, 1985
Ranunculus crucilobus	Deep complex MPD	Endemic to Korea	**Chapter I and IV**
Ranunculus ficaria	Non-deep complex MPD	Europe and west Asia	Vandelook, 2009

Ranunculus flammula	Non-deep simple MPD or MD	North America and Eurasia	Roberts and Boddrell, 1985
Ranunculus franchetii	Deep simple epicotyl MPD	Asia	**Chapter I and IV**
Ranunculus glacialis	MPD	Europe	Schwienbacher et al., 2011
Ranunculus ollissiponensis	Non-deep simple MPD or MD	Europe	Gimenéz-Benavides, 2005
Ranunculus peltatus	MD	Europe, southwestern Asia and northern Africa	Carta et al., 2012
Ranunculus repens	Non-deep simple MPD or MD	Europe, Asia and northwestern Africa	Roberts and Boddrell, 1985
Ranunculus sabinei	MD	North America	Bell and Bliss, 1980
Ranunculus sceleratus	Non-deep simple MPD or MD	North America and Eurasia	Probert et al., 1987
Ranunculus testiculatus	Non-deep simple MPD or MD	Europe and Asia	Young et al., 1992
Thalictrum coreanum	Non-deep simple MPD	Endemic to Korea (?)	**Chapter I and IV**
Thalictrum minus	Non-deep simple MPD or MD	Europe, Northwest Africa, Yemen, Ethiopia, South Africa, Southwest Asia, and Siberia	Grime et al., 1981
Thalictrum mirabile	Non-deep simple MPD	North America	Walck et al., 1999
Thalictrum rochenbrunianum	Non-deep simple MPD	Endemic to Korea	**Chapter I and IV**
Thalictrum uchiyamai	Non-deep simple MPD	Endemic to Korea	**Chapter I and IV**

[z]MD, morphological dormancy; MPD, morphophysiological dormancy.

[y]Information was collected from Scopus, Web of Science, ScienceDirect, Wiley Online Library, Springer, JSTOR, Taylor & Francis Online, Google Scholar. Class of dormancy in some species is inferred from available information on germination and on characteristics of seeds. Therefore, this table could be only a small subset of the species in the five genera of Ranunculaceae.

ABSTRACT IN KOREAN

본 연구는 한반도 자생 매자나무과, 미나리아재비과, 멜란티움과 및 백합과에 속하는 야생 숙근초들의 종자휴면과 발아 및 유묘출현의 다양성과 그것을 조절하는 요인들을 생태생리적 관점에서 이해하는 것에 목적을 두었다. 따라서 본 연구에서는 매자나무과 2종(한계령풀과 깽깽이풀), 미나리아재비과 8종(매발톱꽃, 동강할미꽃, 복수초, 왜미나리아재비, 바위미나리아재비, 금꿩의다리, 자주꿩의다리 및 연잎꿩의다리), 멜란티움과 3종(처녀치마, 숙은처녀치마, 및 큰연영초), 그리고 백합과 1종(얼레지)을 대상으로 종자휴면을 분류하고, 식물호르몬에 의한 종자휴면의 조절 원리를 구명하였다. 한편, 다른 국가 또는 다른 대륙에 자생하는 동일 속 식물들의 종자휴면 유형과 비교하여 고찰하였다. 연구대상 14종 모두 종자가 탈리되는 시점에 배의 크기가 종자크기의 약 7~20% 정도 되는 미숙배를 가지고 있었다. 일반적으로 미숙배 종자의 발아와 유묘출현이 30일 정도 이내에 완료되는 경우를 형태적휴면(MD, morphological dormancy)이라 하고, 반면에 30일에서 수개월의 긴 시간을 필요로 할 경우, 이를 형태생리적휴면(MPD, morphophysiological dormancy)이라 한다. 미나리아재비과의 매발톱꽃과 동강할미꽃은 한달 이내에 거의 모든 종자가 발아하였고, 유묘가 곧바로 출현하였는데, 따라서 형태적휴면 종자임을 알 수 있었다. 그러나 매자나무과의 한계령풀, 깽깽이풀, 미나리아재비과의 복수초, 왜미나리아재비, 바위미나리아재비 및 연잎꿩의다리, 멜란티움과의 큰연영초, 그리고 백합과의 얼레지는 8주가 지나도 거의 발아하지 않았다. 따라서 형태생리적휴면 종자임을 알 수

있었다. 반면, 멜란티움과의 처녀치마와 숙은처녀치마, 미나리아재비과의 금꿩의다리와 자주꿩의다리 종자는 4주 후에 각각 22, 40, 12, 3%가 발아하였고, 배양 기간이 8주까지 길어지면서, 발아율이 점차 증가하는 경향이었다. 이는 같은 개체군 내에서도 종자 휴면의 깊이(형태적 또는 형태생리적 휴면)를 달리하는 야생식물의 특징이라 할 수 있다. 연구대상 14종 중, 미나리아재비과 8종을 대상으로 구체적인 형태생리적휴면 유형을 분류하였다. 미나리아재비과의 연구대상 종들은 대부분 봄에 유묘를 출현시키는 전략을 보였지만, 서로 다른 메커니즘의 종자휴면 유형을 가지고 있었다. 복수초의 경우, 여름철의 고온기를 지나고 가을철의 중온기가 되면서 배가 급속히 신장하였다. 배가 신장되면서 과피가 열개되었고, 그 상태를 유지하면서 겨울철의 저온기를 지나 휴면이 타파되었다. GA처리로는 휴면을 타파시키지 못했다. 따라서 복수초 종자는 휴면을 타파시키기 위해, 고온에서 저온으로 이어지는 온도의 순차적 변화가 필수적이으며, 이를 통하여 a special type of deep simple MPD로 분류하였다. 바위미나리아재비 종자는 저온에서 배가 신장하였고, 또한 저온처리를 통해 휴면이 타파되었다. 그러나 GA처리는 휴면을 타파시키지 못했다. 따라서 deep complex MPD로 분류하였다. 반면, 왜미나리아재비의 경우, 여름철의 고온에서 가을철의 중온으로 가면서 배가 신장하였고, 곧바로 발아하였다. 그러나 유묘출현이 지연되었고, 겨울철의 긴 저온기를 지나고 이듬해 봄에 유묘가 출현하였다. 가을철에 발아된 종자를 실험실 내로 가져와 배양하였을 때, 유근은 지속적으로 신장하였으나, 떡잎이 종자의 외부로 출현하지 않았다. 이는 발아된 이후에도 추가적인 저온을 필요로 하는 배축휴면의 일종으로 deep simple epicotyl

MPD로 분류할 수 있었다. 꿩의다리 3종의 경우, 미숙배가 저온에서 발달하였으나, 저온처리 이후에 고온으로 이동할 경우, 배발달이 촉진되어 휴면이 타파되었다. GA처리는 휴면을 효과적으로 타파시켰다. 따라서 non-deep simple MPD로 분류할 수 있었다. 휴면을 분류한 후, 다양한 문헌들을 통하여 다른 국가 또는 대륙에 자생하는 미나리아재비과의 동일속 식물들의 종자휴면 유형을 조사하였다. 조사 후에 연구 대상 종들의 종자휴면 유형과 비교하였다. 이를 통하여, 미나리아재비과 내에서 종에 따라서 다양한 휴면유형을 보이는 것을 확인할 수 있었다. 특히 미나리아재비속(*Ranunculus*) 내에서는 그간 배축휴면은 보고된 바가 없는 것을 확인할 수 있었다. 대상 식물들 중, 할미꽃속(*Pulsatilla*)과 꿩의다리속(*Thalictrum*) 종들은 모두 같은 종자휴면 유형을 보유하고 있었다. 이는 같은 종자휴면 유형이 동일속에 내에서 잘 보존되어 있음을 의미한다. 꿩의다리속(*Thalictrum*)의 경우, 북미지역에 자생하는 *Thalictrum mirabile*와 한반도에 자생하는 꿩의다리 3종이 생물지리적으로 대륙이 분리되는 시점인 Arcto-Tertiary distribution patterns (신생대 제 3기, 약 6500~200만년전)을 보이는 것으로 알려져 있는데, 자생지는 다르지만 같은 휴면유형을 보였다. 이는 동일한 휴면 유형이 적어도 Tertiary시점에 존재했음을 의미한다. 본 연구에서는 이러한 형태적 또는 형태생리적 휴면이 자연상태에서 어떻게 조절되는지 알아보고자, 종자의 내생호르몬을 분석하였다. 미숙배를 갖는 종자였지만 발아와 유묘출현이 30일 이내에 완료되었던 매발톱꽃의 경우, 종자가 자연상태에 파종된 이후, ABA함량과 민감도가 급속히 감소하였고, 반면에 GA함량은 급속히 증가하였다. 그러나 배달달과 발아가 자연상태에서 상당히 지연되었던 복수초의 경우

는, 종자가 파종된 후에 ABA함량은 곧바로 감소하였으나, GA함량이 증가되지 않았다. 그러나 여름철의 고온기를 지나고 가을철의 중온기로 접어들면서 GA함량이 증가되었다. 이 시점에 배신장은 촉진되었다. 배발달이 완성된 이후에 곧바로 발아하지 못하였는데, 이 시점에 다시 ABA함량은 증가하였고, 겨울철의 저온기를 지나면서 다시 감소하였다. 반면, GA함량은 배발달 이후에 다시 감소하였는데, 겨울철의 저온기를 지나면서 그 함량은 다시 증가하였다. GA/ABA 비율은 자연상태에서 배발달과 발아의 양상과 비슷한 패턴을 보여주었다. 이러한 결과들은 미숙배 종자를 갖는 basal angiosperms에서도 호르몬 밸런스 모델이 적용된다는 것을 의미한다.