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Nondeep simple morphophysiological dormancy in seeds of *Ilex maximowicziana* from northern (subtropical) and southern (tropical) Taiwan

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Abstract In this study, we show that seeds of *Ilex maximowicziana* collected from northern and southern Taiwan differ in germination responses to temperature. Seeds produced by plants growing in the tropical environment of southern Taiwan were more responsive (in a positive way) to higher incubation temperatures than those produced by plants growing in the subtropical environment of northern Taiwan. On the other hand, seeds produced in northern Taiwan were more responsive (in a positive way) to low incubation temperatures and to cold stratification than those from southern Taiwan. Germination percentages and rates of seeds from northern Taiwan were higher at 20/10°C than at 30/20°C, reaching a plateau of >80% germination after 12 weeks incubation, whereas germination of seeds from southern Taiwan reached >80% at 30/20 and 25°C but not at 20/10°C. Gibberellic acid (GA₃) increased germination rate but not germination percentage of seeds from both southern and northern Taiwan. Freshly matured seeds of *I. maximowicziana* have rudimentary embryos. During dormancy break, embryo length increased 11.5- and 8.0-fold in northern and southern seeds, respectively, before radicle emergence. Thus, seeds of *Ilex maximowicziana* have nondeep simple morphophysiological dormancy. This is the first detailed study of the germination requirements of a subtropical/tropical species of the large cosmopolitan genus *Ilex*.

Keywords Cold stratification · Embryo growth · *Ilex maximowicziana* · Nondeep morphophysiological seed dormancy · Rudimentary embryo

Introduction

There are many examples of species that exhibit variation in dormancy and germination characteristics when seeds are collected from sites that differ in elevation, longitude, latitude, and other attributes (see Table 8.2 in Baskin and Baskin 1998). Seeds of the same species collected in different locations may vary in germination percentages (e.g., Meyer et al. 1997), germination speed (Qaderi and Cavers 2000), sensitivity to moisture stress (Lindauer and Quinn 1972), temperature (Meyer et al. 1997), and light/dark (Andersson and Milberg 1998) requirements for germination, amount of cold stratification required to break dormancy (Meyer and Kitchen 1994), and rates of after ripening in dry storage (Hacker and Ratcliff 1989). In most species, the cause of variation in dormancy and germination characteristics is not known; however, it is well recognized that they can be influenced by genetics, maternal environment, or genetics × maternal environment (Roach and Wulff 1987; Baskin and Baskin 1998; Meyer and Pendleton 2000).

Many of the species in which site variation in dormancy and germination have been investigated have either physiological (physiological inhibiting mechanism in embryo) or physical (water-impermeable seed or fruit coat) dormancy. In fact, of 230 species listed as showing site variation in dormancy/germination (unpublished updated version of Table 10.2 in Baskin and Baskin 1998), only ten species belong to families known to have underdeveloped embryos (i.e., the embryo must grow inside the seed prior to radicle emergence). Thus, only a small number of the studies on site variation in dormancy/germination have been done using species whose seeds

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have morphological (embryo must grow) or morphophysiological (underdeveloped embryo also has a physiological inhibiting mechanism) dormancy. Consequently, our knowledge of site variation in dormancy/germination of species with underdeveloped embryos is limited.

Ilex is a genus in the Aquifoliaceae known to have seeds with small, globular to oval-oblong rudimentary (underdeveloped but differentiated) embryos that have to grow inside the seed before the radicle can emerge (Martin 1946; Hu 1975; Baskin and Baskin 2004). If embryo growth is the only prerequisite for radicle emergence, i.e., germination time ≤ 30 days, seeds have only morphological dormancy, whereas if intact seeds require >30 days to germinate or have a specific dormancy-breaking pretreatment, e.g., warm, cold, or warm-plus-cold stratification, seeds have morphophysiological dormancy (Baskin and Baskin 2004).

Cold stratification (moist chilling) and exogenous application of gibberellins can overcome physiological dormancy and promote germination of seeds of angiosperms and gymnosperms (Bewley and Black 1994; Baskin and Baskin 1998). Both cold stratification and the gibberellins such as GA₁, GA₃, GA₄, and GA₇, which are biologically active compounds, can shorten the time from sowing to germination of dormant seeds and thus the time required for seedling production. Cold stratification that simulates natural over-wintering of dispersed seeds in temperate climates is a practical and effective pre-treatment to overcome dormancy and promote spring germination and seedling establishment. It not only increases seed germination percentages and rates but also the temperature range over which seeds can germinate (Baskin and Baskin 1998).

In this study, we ask if seeds of *Ilex maximowicziana* from different ecosystems differ in their dormancy breaking and germination requirements; seed germination of this species previously has not been studied. Thus, we (1) compared embryo growth and seed germination at various temperatures of seeds collected in northern and southern Taiwan, (2) tested the effects of cold stratification and gibberellic acid (GA₃) pretreatments on dormancy-break and germination, and (3) tested the effect of a sequence of temperatures that could occur in the natural habitat temperatures on timing of germination of seeds from Sutu. Our study is a contribution to the seed germination ecology of a large (ca. 400 species) cosmopolitan genus (Mabberley 1997). Little is known about the requirements for seed dormancy break and germination of *Ilex* species, especially those in tropical and subtropical regions (Baskin and Baskin 1998).

Materials and methods

Study species, fruit collecting, and seed handling

Ilex maximowicziana Loes. (Aquifoliaceae) is a small evergreen, dioecious tree distributed from the Ryukyu (Okinawa) islands to Taiwan. In Taiwan, this species

occurs in northern and southern regions at altitudes below 1,000 m (Lu 1993). In the northern region, *I. maximowicziana* mainly is distributed in northeastern Taipei and Ilan counties, where it is an understory tree in the *I. maximowicziana*–*Machilus thunbergii* (Lauraceae) vegetation type of the subtropical evergreen broad-leaved forest (Yu 2004). In the southern part of the Hengchun Peninsula (southern tip of Taiwan), the species occurs in the Hengchun and Manchou townships, Pingtung county, which has a tropical monsoon evergreen broad-leaved forest (project report from Kenting National Park).

Fruits of *I. maximowicziana* were collected from Sutu (24°52'N, 121°46'E, altitude 500 m), Taipei county, northern Taiwan, on 24 November 2008 and on 28 October 2009 (for GA₃ pretreatment only), and from near Hengchun (22°01'N, 120°46'E, altitude 200 m), Pingtung county, southern tip of Taiwan, on 19 January 2009. Mature black fruits (drupes) were collected from three to four trees at each site in natural forests. Each fruit contained one to four seeds (pyrenes), average 2.7 ± 0.94 ($n = 30$). Fruits were placed on a mesh (2.0 mm²) stainless-steel pan and rubbed on the steel top by hand. The exocarp, mesocarp, and the seed + endocarp (hereafter seeds) that floated on water were discarded, and only seeds that sank were used in the germination studies. The seeds were dried in the laboratory for 2 days and then stored temporarily at 5°C for 7 days before studies were initiated. There were about 18,200 and 16,350 seeds per liter from Sutu and Hengchun, respectively; moisture content of fresh seeds from Sutu and Hengchun was 17.3 and 10.2%, respectively.

Effect of temperature on embryo growth and seed germination

Fresh seeds of *I. maximowicziana* from Sutu and Hengchun were mixed with moist sphagnum moss, placed in sealable polyethylene bags (water content of the sphagnum moss inside the bags was about 400% of dry mass), and incubated at 12 h/12 h alternating temperatures regimes of 15/6, 20/10, 25/15, and 30/20°C and at a constant temperature of 25°C. The alternating temperature regimes approximate those that could occur during the year at Sutu and Hengchun (Central Weather Bureau of Taiwan). The daily photoperiod was 12 h in the incubators (80–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 400–700 nm, cool white fluorescent light) at the high temperature. The sphagnum moss used for seed germination was cut into small pieces. Each treatment consisted of three replications of 50 seeds from Sutu and of 25 seeds from Hengchun. Germination, i.e., radicle at least 2 mm long, was monitored weekly for 32 weeks. Results are expressed as germination percentage and as mean germination time (MGT) in days after seeds were placed at the five incubation temperatures. $\text{MGT} = (\sum n_i t_i)/N$, where n_i is the number of seeds germinated in t_i days from the beginning of the test, and n is the total number of germinated seeds at the end of the test (Naylor 1981). MGT

is a measure of the rate of germination and of the sharpness of the germination peak.

Growth of embryos was measured at weekly or bi-weekly intervals until they were fully grown. To monitor embryo growth, seeds were incubated at the above temperatures in moist sphagnum moss. The seeds were dissected using a razor blade, and ten embryos from each of the two collection sites and incubation time and temperature were measured under a dissecting microscope equipped with a calibrated micrometer. Due to a low number of seeds from Sutu, embryo growth was measured only at 30/20, 25/15, and 20/10°C.

To simulate seed germination in the *I. maximowicziana* habitat in northern Taiwan after seed dispersal in winter and spring, three replications of 50 seeds each from Sutu were mixed with moist sphagnum moss and incubated at a 12-h photoperiod in the following temperature sequence: (1) 5/5°C for 4 weeks → 15/6°C for 4 weeks → 20/10°C for 8 weeks → 25/15°C for 12 weeks. These temperatures approximate those that could occur at Sutu in winter, spring, and summer (data from Central Weather Bureau of Taiwan). All seeds were monitored for radicle emergence weekly for 28 weeks. Embryo growth was measured at weekly or bi-weekly intervals until they were fully grown.

Effect of cold stratification on embryo growth and seed germination

Fresh seeds from Sutu and Hengchun were mixed with moist sphagnum moss (cut into small pieces and water content about 400% of dry mass), sealed inside polyethylene bags, and stored at 5°C in darkness for 4, 8, and 12 weeks prior to testing them for germination. After treatment, seeds were incubated for 20 weeks at a (12/12 h) day/night temperature of 25/15°C with a 12-h photoperiod (ca. 80–100 $\mu\text{mol m}^{-2}\text{s}^{-1}$, 400–700 nm, cool white fluorescent light) at the higher temperature. Thus, total incubation time of seeds stratified (incubated at 5°C) for 0 (control), 4, 8, and 12 weeks was 20, 24, 28, and 32 weeks, respectively. Each treatment consisted of three replications of 50 seeds from Sutu and of 25 seeds from Hengchun. Germination, i.e., radicle at least 2 mm long, was recorded weekly for 20 weeks. Results are expressed as germination percentage (%) and MGT.

Embryo growth for ten seeds was measured immediately after cold stratification and then for ten seeds at bi-weekly intervals during incubation at 25/15°C.

Effect of GA₃ on seed germination

Fresh seeds harvested from Hengchun and Sutu were soaked in concentrations of 0 (water control), 26, 260, and 2,600- μM solutions of GA₃ (95% purity, Sigma, USA) for 24 h at room temperature and then mixed with moist sphagnum moss for incubation at 25/15°C in a 12-h daily photoperiod (ca. 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$,

400–700 nm, cool white fluorescent light). Results are expressed as germination percentage (%) and MGT.

Statistical analysis

To examine the effect of seed collection site, various incubation temperatures, and cold stratification at 5°C for 4, 8, and 12 weeks on germination percentages and mean germination time (germination rate) of *I. maximowicziana* seeds, a two-way analysis of variance (ANOVA) was conducted to test for significant interactions and strength of associations between factors using SAS. Germination percentages (mean \pm SE) in the experiment testing the effect of GA₃ on germination were compared by a one-way ANOVA and by least significant difference (LSD) test at the 5% level of significance using SAS and Microsoft Office Excel 2003. Germination percentage data were arcsine square-root transformed prior to analysis. A *t* test was used to compare embryo length in fresh seeds and in those in which the embryo had grown to its maximum length (seed coat had split) prior to radicle emergence for seeds from the two collection sites.

Results

Effect of temperature on embryo growth and seed germination

Embryos were small and heart-shaped, and they occupied a small cavity in the endosperm. Thus, most of the seed interior was filled with endosperm (Fig. 1a). Mean (\pm SE) lengths of fresh seeds and embryos ($n = 10$) were 4.89 ± 0.33 and 0.41 ± 0.04 mm, respectively, from Sutu and 5.74 ± 0.28 and 0.49 ± 0.09 mm, respectively, from Hengchun. Thus, the embryo length/seed length (E:S) ratios were 0.08 ± 0.01 (Sutu) and 0.09 ± 0.02 (Hengchun). Length of embryos in fresh seeds from Hengchun and Sutu differed significantly (*t* test, $p < 0.05$). The length of embryos with a split seed coat before radicle emergence was 4.70 ± 0.42 mm (Sutu) (Fig. 1d) and 3.94 ± 0.55 mm (Hengchun) (Fig. 1c). Thus, embryo length in seeds from Sutu and Hengchun increased about 11.5- and 8.0-fold, respectively. Length of embryos in seeds with a fully developed embryo from Hengchun and Sutu differed significantly (*t* test, $p < 0.05$).

After 32 weeks incubation, 96–100% of Sutu seeds had germinated at 15/6, 20/10, and 25/15°C, and 79–84% at 30/20 and 25°C (Fig. 2a). By week 12 of incubation, 90% of Sutu seeds at 20/10°C had germinated, whereas $< 60\%$ of those had done so at the other four temperatures (Fig. 2a). Mean (\pm SE) germination time (MGT) of Sutu seeds at 15/6, 20/10, 25/15, 30/20, and 25°C was 95.6 ± 1.2 , 72.9 ± 0.4 , 78.8 ± 1.7 , 103.9 ± 11.5 , and 94.8 ± 7.5 days, respectively. Thus, the optimum

Fig. 1 Embryo growth in seeds of *Ilex maximowicziana*. Longitudinal section of fresh seeds with a rudimentary embryo (**a, c**) and of seeds with a fully grown embryo at the time the seed coat splits (**b, d**) for seeds collected at Hengchun (**a, b**) and at Sutu (**c, d**). Embryo (*em*) in cavity (*cv*), endosperm (*es*), seed coat (*sc*), endocarp (*ec*). Scale bar 1 mm. Arrow in (**b**) points to the position of the split in the seed coat at the tip of the radicle

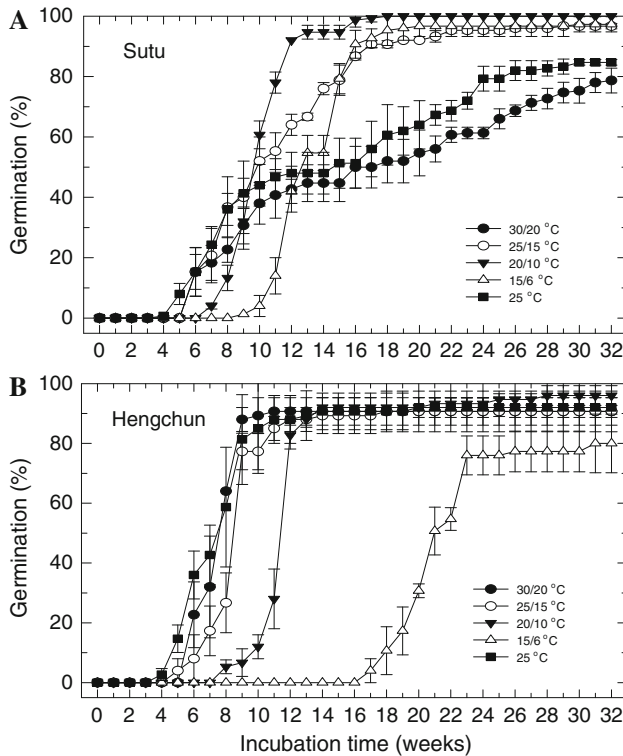
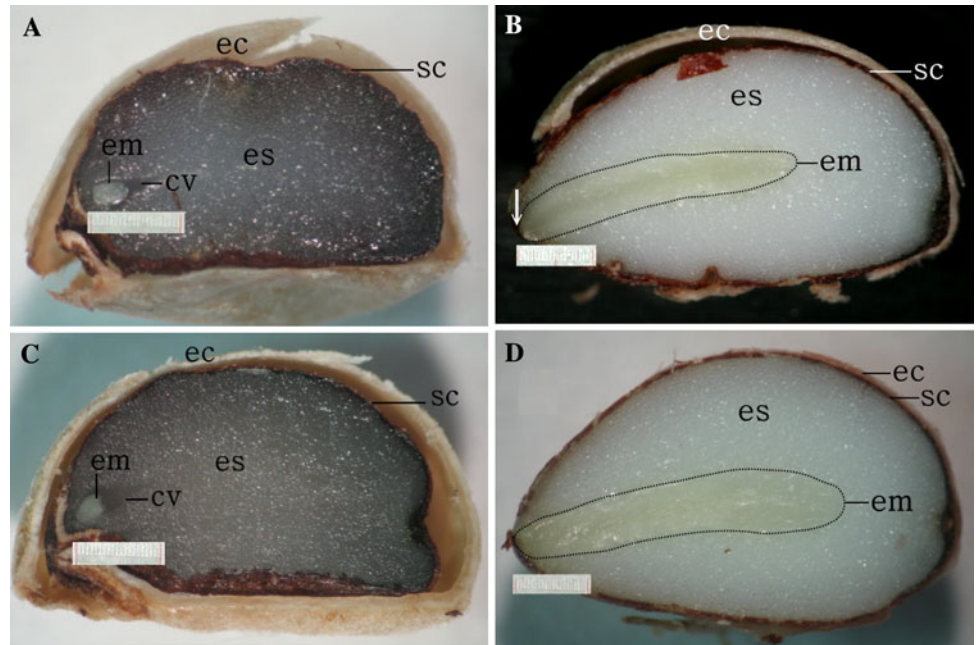


Fig. 2 Effect of temperature on germination of *Ilex maximowicziana* seeds collected at Sutu (**a**) and Hengchun (**b**) and incubated for 32 weeks at various temperature regimes. Vertical bars are ± 1 SE

temperature of the Sutu seeds for maximum germination percentage and rate was 20/10°C.

Site, temperature, site \times temperature, and site \times cold stratification had significant effects on germination percentages and rates, except for site for germination percentage (Table 1). After 32 weeks incubation, >90% of

Hengchun seeds had germinated at 20/10, 25/15, 30/20, and 25°C, but only 80% of them had germinated at 15/6°C (Fig. 2b). By week 12 of incubation, >80% of Hengchun seeds had germinated at 20/10, 25/15, 30/20, and 25°C, but no seeds had done so at 15/6°C (Fig. 2b). MGT of Hengchun seeds at 15/6, 20/10, 25/15, 30/20, and 25°C was 148.2 ± 2.4 , 85.2 ± 2.6 , 62.3 ± 3.2 , 54.1 ± 1.8 , and 54.5 ± 5.2 days, respectively. Thus, the optimum temperatures for maximum germination percentage and rate of Hengchun seeds were 30/20 and 25°C.

At the end of 4 weeks, embryos in Sutu seeds were longer at 20/10 and 25/15°C than at 15/6°C (Fig. 3a). At the end of 5 weeks, Hengchun embryos were longer at 25, 20/10, 25/15, and 30/20 than at 15/6°C, where they began growing between 7 and 9 weeks (Fig. 3b).

For seeds from Sutu incubated in the temperature sequence, germination began at 20/10°C, and germination percentage increased rapidly to 94% at this temperature. Most growth of the embryo occurred at 15/6°C and the rest of it at 20/10°C (Fig. 4).

Effect of cold stratification on embryo growth and seed germination

Site, length of cold stratification period, and interaction between site and length of stratification period had significant effects on germination percentage and MGT, except for site \times 8 weeks stratification and site for 12 weeks cold stratification for germination percentage (Table 2). Seeds of *I. maximowicziana* stratified at 5°C not only retained their original >90% germination, but embryo growth occurred and germination rates increased (decreased days of MGT) (Fig. 5a, b). Seeds from Sutu stratified at 5°C for 4 weeks germinated to 92% in

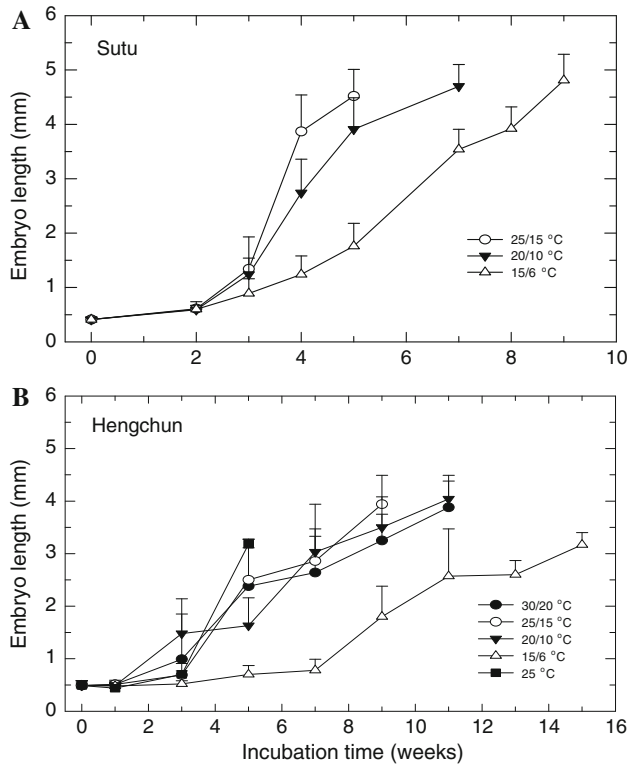
Table 1 Results of two-way ANOVAs showing the effects of seed collection site and incubation temperature on final germination percentage and MGT

Source of variation	Germination percentage				MGT		
	df	F	Sig.	R ²	F	Sig.	R ²
Site	1	1.31	0.2661 NS		14.57	0.0011**	
Temperature	4	6.79	0.0013**		74.00	< 0.0001***	
Site × temperature	4	5.03	0.0057**	0.7083	74.52	< 0.0001***	0.9682

R² is the proportion of variation explained by variables

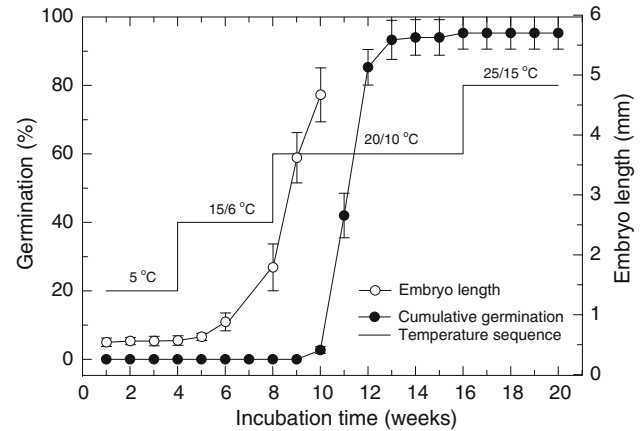
Site × temperature represents the interaction effect

^{NS}*p* > 0.05; ***p* < 0.01; ****p* < 0.001

**Fig. 3** Embryo growth in seeds of *Ilex maximowicziana* from Sutou (a) and Hengchun (b) at various incubation temperatures

10 weeks of incubation, while those stratified at 5°C for 8 weeks or 12 weeks germinated to 92% in 6 weeks of incubation (Fig. 5a). MGT decreased from 78.8 ± 1.7 days in fresh seeds to 42.1 ± 0.3 , 32.3 ± 0.5 , and 27.9 ± 0.3 days in 4, 8, and 12 weeks of cold-stratified seeds, respectively. Meanwhile, embryo length increased from 0.41 ± 0.04 mm to only 0.49 ± 0.07 , 0.60 ± 0.08 , and 0.65 ± 0.10 mm during 4, 8, and 12 weeks cold stratification, respectively.

Cold stratification of seeds from Hengchun for 4, 8, and 12 weeks increased germination rate (Fig. 5b). For example, MGT decreased from 62.3 ± 3.2 days in fresh seeds to 51.4 ± 2.6 , 49.3 ± 2.5 , and 40.5 ± 2.9 days in those cold-stratified for 4, 8, and 12 weeks, respectively. Meanwhile, embryo length increased from 0.49 ± 0.09 mm to only 0.52 ± 0.06 , 0.75 ± 0.16 , and

**Fig. 4** Cumulative embryo length and germination percentage of *Ilex maximowicziana* seeds collected at Sutou and incubated in a cold to warm temperature sequence. Vertical bars are ± 1 SE

0.86 ± 0.13 mm during 4, 8, and 12 weeks cold stratification, respectively.

Effect of GA₃ on seed germination

GA₃ treatment increased germination percentages of the *Ilex* seeds from both collection sites during the first 6–8 weeks of incubation; however, germination percentages of control and treated seeds did not differ significantly at 10 or 12 weeks (Table 3). After 10 or 12 weeks, germination rates of GA₃-treated seeds from both sites were significantly higher than those of controls.

Discussion

Effect of temperature on embryo growth and seed germination

Regardless of incubation temperature, little or no germination was recorded for seeds of *I. maximowicziana* until the 5th week. Further, germination at 25/15°C did not reach 50% until about weeks 9 and 7 for Sutou and Hengchun seeds, respectively. Thus, there clearly was a delay in germination, and the seeds were dormant. Part of the delay in germination was due to the time required

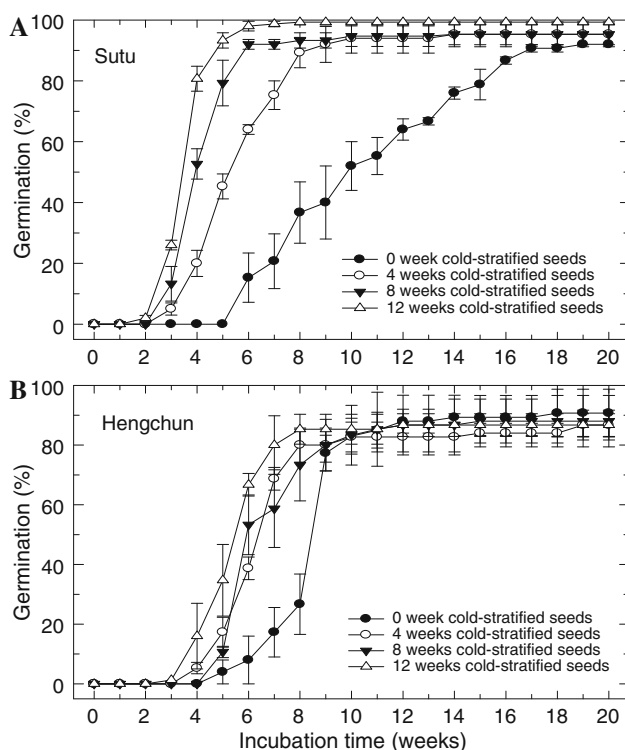
Table 2 Results of two-way ANOVAs showing the effects of seed collection site and cold stratification for 4, 8, and 12 weeks on final germination percentage and MGT

Source of variation	Germination percentage				MGT		
	<i>df</i>	<i>F</i>	Sig.	<i>R</i> ²	<i>F</i>	Sig.	<i>R</i> ²
Site	1	170.28	<0.0001***		2738.00	<0.0001***	
4-week cold stratification	3	81.74	<0.0001***		12513.3	<0.0001***	
Site × 4-week cold stratification	3	33.14	<0.0001***	0.9699	3264.67	<0.0001***	0.9997
Site	1	25.67	0.0001***		74.31	<0.0001***	
8-week cold stratification	3	56.48	<0.0001***		62.30	<0.0001***	
Site × 8-week cold stratification	3	1.63	0.2220 NS	0.9259	14.89	<0.0001***	0.9503
Site	1	4.35	0.0534 NS		87.68	<0.0001***	
12-week cold stratification	3	7.13	0.0029**		134.43	<0.0001***	
Site × 12-week cold stratification	3	8.85	0.0011**	0.7658	12.45	0.0002***	0.9706

*R*² is the proportion of variation explained by variables

Site × cold stratification represents the interaction effect

NS, *p* > 0.05; ***p* < 0.01; ****p* < 0.001

**Fig. 5** Effect of cold stratification at 5°C on germination of *Ilex maximowicziana* seeds collected at Sutu (a) and Hengchun (b) and incubated at 25/15°C for 20 weeks. Vertical bars are ± 1 SE

for embryo growth to occur. Embryos in *I. maximowicziana* seeds must undergo considerable growth prior to radicle emergence, increasing 11.5- and 8.0-fold for Sutu and Hengchun seeds, respectively, before germination. However, embryo growth is not the only requirement for germination. For example, although embryo growth of Sutu and Hengchun seeds at 25/15°C was completed after 5 and 9 weeks, respectively, germination did not reach 90% until about weeks 16 and 14, respectively. We conclude that in addition to a requirement for embryo growth (morphological dor-

mancy) embryos are physiologically dormant (PD); thus, the seeds have morphophysiological dormancy (MPD).

The available evidence indicates that the level of PD in seeds of *I. maximowicziana* is nondeep. (1) Dormancy break occurred in a relatively short period of time at high temperatures, i.e., higher than those required for cold stratification. In some cases, deep PD is broken by high temperatures, but extended periods of time are required for the seeds to become nondormant, e.g., >100 weeks to reach 90% germination (Baskin et al. 2005). Seeds of *I. maximowicziana* from both populations had germinated to 90% by 16 weeks. (2) GA₃, which is usually effective in promoting germination of seeds with nondeep PD, increased the rate but not the final percentage of germination.

There are nine levels of MPD based on (1) the warm and/or cold stratification requirements to break PD, (2) temperature (warm vs. cold) at which embryo growth occurs, and (3) response of seeds to GA₃ (Baskin and Baskin 2004; Baskin et al. 2008, 2009). The nine levels of MPD are divided into two categories: simple and complex. In seeds with simple MPD, temperatures >10°C are required for embryo growth, and in those with complex MPD temperatures <10°C are required for embryo growth. The nine levels of MPD are: nondeep simple, intermediate simple, deep simple, nondeep simple epicotyl, deep simple epicotyl, deep simple double, nondeep complex, intermediate complex, and deep complex.

Since embryos of *I. maximowicziana* seeds require relatively high (non-cold stratifying) temperatures for growth, the level of MPD in these seeds fits into the simple category. Embryos grew little while seeds were incubated at 5°C for 4 up to 12 weeks. Among the six simple levels of MPD, only two of them do not have a cold stratification requirement for the breaking of seed dormancy: nondeep simple MPD and nondeep simple epicotyl MPD. Since seeds of *I. maximowicziana* germinated to $\geq 90\%$ when incubated at 25, 20/10, or

Table 3 Effect of GA₃ on germination of *Ilex maximowicziana* seeds collected from Sutu (A) and Hengchun (B) and incubated at a day/night temperature of 25/15°C

Treatment	Germination (%) and MGT in parenthesis (days)				
	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
(A) Sutu					
ddH ₂ O	0 (0)	0.7 ± 0.9h (0h)	52.7 ± 9.3e (53.8 ± 0.7c)	86.7 ± 3.4cd (58.1 ± 1.5ab)	92.7 ± 2.5abc (59.4 ± 1.8a)
26 μM GA ₃	0 (0)	4.7 ± 3.4g (42.0 ± 0.0g)	58.0 ± 1.6e (52.9 ± 0.8c)	87.3 ± 4.1bcd (56.5 ± 1.0b)	94.0 ± 4.9ab (57.9 ± 1.9ab)
260 μM GA ₃	0 (0)	24.0 ± 5.9f (42.0 ± 0.0g)	77.3 ± 0.9d (49.0 ± 1.3e)	89.3 ± 0.9bcd (51.1 ± 0.9d)	94.0 ± 2.8abc (52.9 ± 0.6c)
2600 μM GA ₃	0 (0)	31.3 ± 5.2f (41.7 ± 0.2g)	90.7 ± 3.4abc (47.3 ± 0.4f)	95.3 ± 5.2a (48.1 ± 0.5ef)	95.3 ± 5.2a (48.1 ± 0.5ef)
(B) Hengchun					
ddH ₂ O	0 (0)	8.0 ± 6.8c (38.5 ± 2.9e)	26.7 ± 10.1c (48.8 ± 3.5cd)	77.3 ± 6.1a (57.8 ± 2.3a)	88.0 ± 4.0a (60.9 ± 1.4a)
26 μM GA ₃	0 (0)	17.3 ± 4.6bc (40.3 ± 0.5e)	68.0 ± 6.9ab (49.6 ± 1.4bc)	84.0 ± 4.0a (52.6 ± 1.3b)	84.0 ± 4.0a (52.6 ± 1.3b)
260 μM GA ₃	0 (0)	25.3 ± 10.1b, (38.4 ± 0.2e)	64.0 ± 4.0b (45.8 ± 2.6d)	76.3 ± 4.6a (47.8 ± 3.5cd)	80.0 ± 4.6a (47.8 ± 3.5cd)
2600 μM GA ₃	0 (0)	60.0 ± 14.4a (38.3 ± 1.2e)	80.0 ± 10.6a (41.4 ± 1.2e)	81.3 ± 11.5a (41.7 ± 0.7e)	81.3 ± 11.5a (41.7 ± 0.7e)

Any means ($n = 3$) ± SE in (A) or in (B) for germination percentage or MGT (*in parenthesis*) followed by the same letter are not significantly different from each other (LSD, $p = 0.05$)

Any means ($n = 3$) ± SE in (A) or in (B) for germination percentage or MGT (in parenthesis) followed by the same letter are not significantly different from each other (LSD, $p = 0.05$)

25/15°C, we can conclude that they do not require any cold stratification for seed dormancy break. Further, germinated seeds of *I. maximowicziana* do not exhibit a long (several-week) delay between time of radicle and cotyledon emergence; consequently, they do not have nondeep simple epicotyl MPD. Thus, seeds from both populations of *I. maximowicziana* have nondeep simple MPD. Also, it seems reasonable to suggest that seeds of tropical *Ilex* such as *I. amara* (Zamith and Scarano 2004), *I. cymosa* (Ng 1991), and *I. nitida* (Marrero 1949) have nondeep simple MPD.

In contrast to nondeep simple MPD in seeds of *I. maximowicziana*, those of the temperate-zone species *I. aquifolium*, *I. glabra*, *I. montana* (Nikolaeva et al. 1985), *I. opaca* (Ives 1923; Barton and Thornton 1947), *I. verticillata* and *I. vomitoria* (Nikolaeva et al. 1985) have deep simple MPD. To break deep simple MPD, seeds require warm stratification (summer) to break part of the PD, intermediate (autumn) temperatures for embryo growth, cold stratification (winter) to break the second part of PD and intermediate (spring) temperatures for emergence of root and cotyledons (Baskin and Baskin 1998).

Although they have the same class (MPD) and level (nondeep simple) of dormancy, *Ilex* seeds from northern and southern Taiwan differed in their germination response to temperature. Seeds from Sutu (northern) germinated best at 20/10°C, whereas those from Hengchun (southern) did so at 30/20 and 25°C. Clearly, high temperatures were more inhibitory to Sutu than to Hengchun seeds, whereas low temperatures, especially 15/6°C, was more inhibitory to Hengchun than to Sutu seeds.

Why did seeds collected in northern (Sutu) and southern (Hengchun) Taiwan differ in their germination responses, including embryo growth? One possible reason is maternal environmental effects (see Roach and Wulff 1987). Temperature and soil moisture during seed development are well known to affect the germination status of the mature seeds (Baskin and Baskin 1998), and this may have been the reason for the differences in germination responses to temperature of seeds from the two localities. The mean annual temperature in 2005–2009 was 19.4–19.8°C at Sutu and 25.0–26.0°C at Hengchun (Fig. 6). The mean monthly temperature in 2005–2009 was approximately 25.4°C in July and August and 13.8°C in December through February at Sutu, and 28.5°C in July through September and 21.5°C in December through February at Hengchun. Further, precipitation in 2005 was 4,883 and 2,339 mm at Sutu and Hengchun, respectively, and mean annual precipitation in 2006–2009 was 3,412–4,642 mm at Sutu and 1,690–2,139 mm at Hengchun (Fig. 6). Thus, the climate is warmer and drier at Hengchun than it is at Sutu. Another possible reason for the differences in germination between northern and southern populations is genetics (ecotypes) (Baskin and Baskin 1998). Thus, the differences in temperature and rainfall at Sutu and Hengchun may have exerted different selective pressures on the seed germination stage of *I. maximowicziana*.

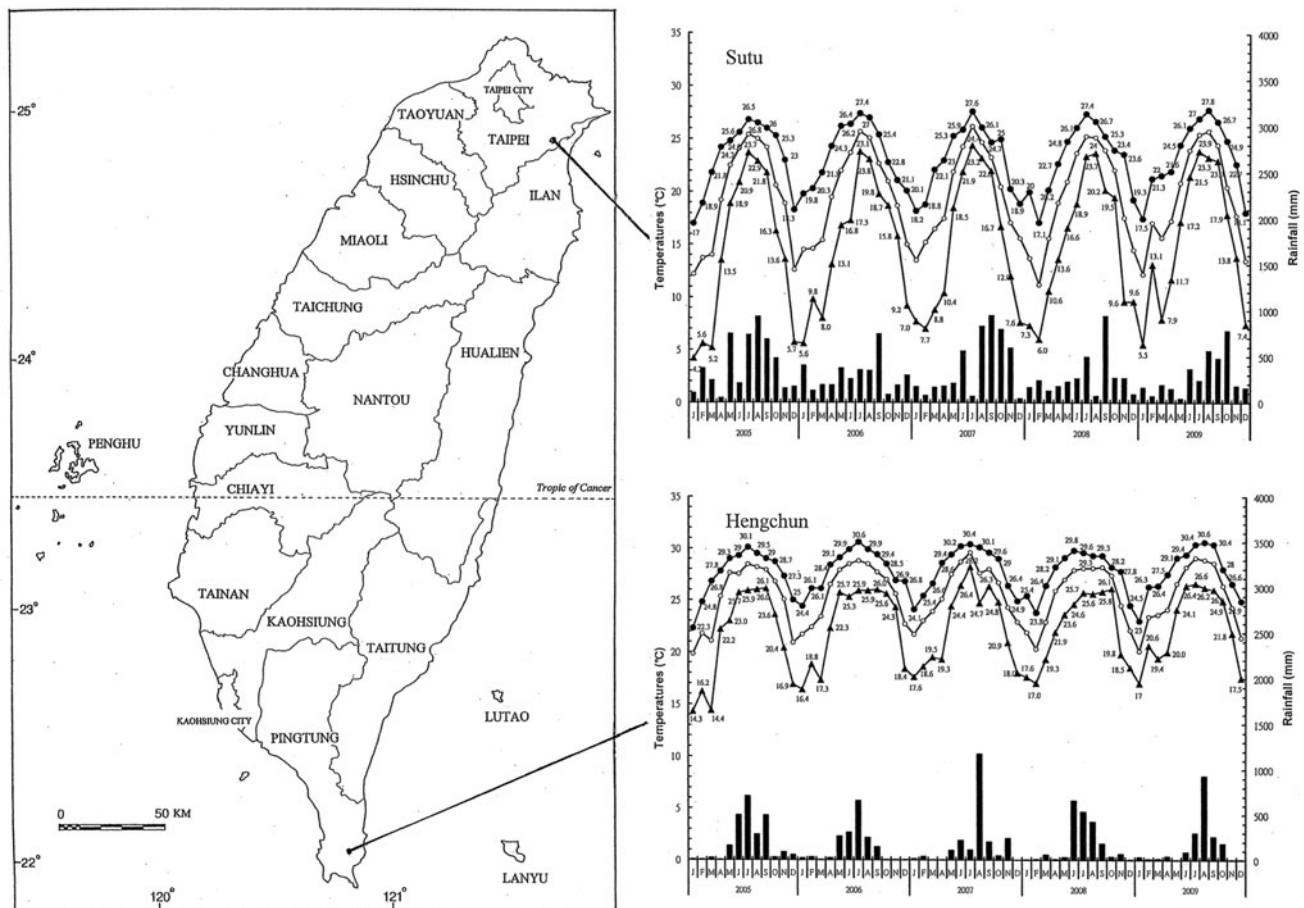


Fig. 6 Monthly mean temperatures (*open circles*) and monthly maximum (*filled circles*) and minimum (*filled triangles*) mean daily temperatures at Sutou and Hengchun (Data purchased from Central Weather Bureau of Taiwan)

Effect of cold stratification on embryo growth and seed germination

Cold stratification greatly increased germination rate of seeds incubated at 25/15°C, especially for those collected from Sutou (Fig. 5). Cold stratification also was more effective in shortening germination time at 25/15°C in Sutou than in Hengchun seeds. In the Sutou region, winter temperatures are lower than they are at Hengchun (Fig. 6). During 2005–2009, there were 11–19 days per year with temperatures below 10°C in winter (November through March) in Sutou and none in Hengchun, showing that seeds received more cold stratifying temperatures (i.e., between 0 and 10°C) in the north than in the south. Thus, seeds from both regions have the same level of MPD, but they may be exposed to different conditions for dormancy break and germination after seed dispersal, i.e., warm-plus-cold stratification in the northern region and only warm stratification in the southern region. However, only warm stratification is actually required for dormancy break and germination of seeds from both locations.

Cold stratification at 5°C shortened the incubation time to reach higher germination percentages at 25/15°C.

However, we need to ask: is the effect of cold stratification (incubation at 5°C) on germination due to the cold temperature treatment per se or to the extra incubation time? Considering the data in Fig. 5a, seeds cold-stratified at 5°C for 0, 4, 8, and 12 weeks reached >80% germination at 25/15°C in 16, 8, 6, and 5 weeks, respectively. The total number of weeks required to reach 80% germination was 0 (cold stratification) + 16 (incubation at 25/15°C), 4 + 8, 8 + 6, and 12 + 5 weeks. Thus, we can conclude that cold stratification of Sutou (northern Taiwan) seeds for 4 and 8 weeks, but not for 12 weeks, had a positive effect on germination at 25/15°C. That is, an incubation period at 5°C shortened the time to 80% germination from 16 weeks (no cold stratification) to 12 weeks (4 weeks cold stratification) and 8 weeks (8 weeks cold stratification). The amount of embryo growth in Sutou and Hengchun seeds that occurred during 12 weeks at 5°C was only 5.6 and 10.7%, respectively, of the growth required for radicle emergence. Perhaps, cold stratification mainly promoted the breaking of PD. In nondeep simple MPD, PD can be broken by either warm or cold stratification, depending on the species, but full embryo growth occurs at temperatures higher than those favorable for cold stratification (Baskin and Baskin 1998). In comparison,

seeds from Hengchun stratified at 5°C for 0, 4, 8, and 12 weeks reached >80% germination at 25/15°C in 10, 8, 9, and 7 weeks, respectively. Thus, cold stratification decreased the time to germination of seeds from Hengchun. However, summing the number of weeks required to reach 80% germination at 25/15°C and number of weeks of cold stratification at 5°C, i.e., 0 + 10, 4 + 8, 8 + 9, and 12 + 7, we can conclude that cold stratification was not effective in promoting germination of Hengchun seeds. That is, seeds receiving no incubation time at 5°C germinated at a faster rate than those cold stratified for 4, 8, or 12 weeks and then incubated at 25/15°C. Thus, seeds collected from northern Taiwan responded differently to low temperature than those from southern Taiwan.

Seed dispersal in *I. maximowicziana* occurs from October to December in northern Taiwan and from December to February in southern Taiwan (personal observations). The newly dispersed seeds are dormant, i.e., MPD, and hence they do not germinate until the following spring. Based on the responses of Sutu seeds exposed to the simulated sequence of winter to spring temperatures (Fig. 4), embryos would not be expected to grow during the coldest part of the year but to grow rapidly when temperature increase in early spring. After embryos have grown to full size, germination would occur. Although we do not have data for responses of Hengchun seeds to simulated habitat temperatures, it seems reasonable that embryo growth and germination would occur in southern Taiwan during the wet season beginning from May or June (Fig. 6).

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