Scientia Horticulturae 124 (2010) 83–87

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Seed dormancy and germination of *Michelia yunnanensis* (Magnoliaceae)

Chun-Yan Han [a,b], Gregory Welbaum [c], Chun-Lin Long [a,d,*]

[a] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100039, China
[c] Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg 24061, USA
[d] College of Life and Environment Sciences, Minzu University of China, Beijing 100081, China

ARTICLE INFO

Article history:
Received 21 June 2009
Received in revised form 16 November 2009
Accepted 24 November 2009

Keywords:
Embryo development
GA_3
Cold stratification
Morphophysiological dormancy

ABSTRACT

Michelia yunnanensis Franch. is a Chinese endemic ornamental shrub with potential for greater utilization as a landscape and medicinal plant if propagation was less difficult. Seed development and breaking of seed dormancy were investigated to improve propagation of *M. yunnanensis*. No fresh seeds germinated when tested at the time of dispersal. Newly matured seeds of *M. yunnanensis* contained differentiated linear underdeveloped embryos that were physiologically dormant. The embryo/seed length ratio of *M. yunnanensis* was 0.15. Warm stratification did not break seed dormancy. Dormancy was broken by cold stratification at 4 °C but not by flowing water or nitrate. Embryos developed grew inside seeds during cold stratification at 4 °C. In newly harvested dormant seeds, embryos were 0.94 mm long and increased in length 139% before radicle emergence (germination). GA_3 substituted for cold stratification to break dormancy in seeds of *M. yunnanensis* incubated at 25 °C or 20/25 °C. Mature *M. yunnanensis* seeds exhibited intermediate complex morphophysiological dormancy. Optimal germination of non-dormant seed in terms of both germination percentage and rate occurred at 20/25 °C.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The genus *Michelia* in the family Magnoliaceae consists of approximately 80 species of trees and shrubs distributed primarily in Asian temperate, subtropical, and tropical zones (Zhang, 2007). There are about 70 *Michelia* species distributed in the evergreen broad-leaved forests of southwest and east China (Liu, 2004; Zhang, 2007). *Michelia yunnanensis* is a 5-m-tall, evergreen, perennial, heliophilic or semi-heliophilic shrub endemic to southern China. It is most commonly found in mountainous thickets, open forests, and forest edges at altitudes ranging from 1100 to 2300 m in Yunnan and Guizhou Province (Liu, 1996, 2004). *M. yunnanensis* is drought tolerant and adapted to a range of soil types and light conditions from full sun to partial shade (Zuo, 1992). *M. yunnanensis* has attractive glossy, dark, blackish-green leaves. The long beautiful inflorescences of *M. yunnanensis* form round clusters of cream-colored 2.5- to 7-cm-wide flowers with yellow stamens and strong cinnamon fragrance. Globally there is potential for greater utilization of *M. yunnanensis* for ornamental planting, medicinal purposes, and manufacture into perfume (Hao, 1999).

Magnolia seeds have morphological and morphophysiological dormancy, although the types of dormancy present among species have not been compared. Seeds of *Michelia* species have underdeveloped embryos at maturity (Martin, 1946; Baskin and Baskin, 1998; Zhou and Hu, 1990). Embryos described as underdeveloped may be dormant at maturity, thus seeds may need warm and/or cold stratification for embryo growth and germination to occur (Nikolaeva, 1977; Baskin and Baskin, 2004).

Limited information is available on dormancy-breaking and germination requirements of *M. yunnanensis* seeds and most of what is known comes from field studies. Seeds stratified for 90 d in wet sand at 8 °C germinated to 91.8% (Zuo, 1994a). Germination was 86.7% in the field after exposure to ambient temperatures and moist storage conditions from October to March (Liang et al., 2006). Germination percentages reached 65.2% following cold stratification in a field study (Zuo, 1994b).

Germination of *M. yunnanensis* seeds has only been partially characterized and detailed information is lacking on the degree of embryo differentiation at maturity, the class of dormancy present, effective dormancy-breaking techniques to accelerate germination for reliable commercial propagation, and the optimum conditions for germination. Thus, the objectives of this study were to characterize seed dormancy in *M. yunnanensis* by: (1) describe seed morphology and embryo development; (2) test effects of flowing water, KNO_3, GA_3, and warm and cold stratification on dormancy-breaking and germination of fresh seeds; (3) determine the optimum temperature for germination.

* Corresponding author at: Kunming Institute of Botany, Biodiversity and Plant Resources, 132 Lanhei Road, Heilongtan, Kunming 650204, China.
Tel.: +86 871 522 3233; fax: +86 871 522 3233.
E-mail address: long@mail.kib.ac.cn (C.-L. Long).

0304-4238/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2009.11.021

2. Materials and methods

2.1. Seed production

M. yunnanensis plants at the Kunming Botanical Garden, China Kunming bloomed from March to April, and fruits matured and were collected from August to September 2007. At the time of collection, fruits were judged to be mature when their color changed to reddish purple. At full maturity, some fruits dehisced naturally exposing red-oily arillate seeds that either remained in the fruits or were dispersed. After collection, fruits were placed in the shade at ambient temperatures (18–28 °C) to induce dehiscence. When the arils turned from light red to burgundy, seeds were soaked in water, which was changed daily, until the arils softened (Yu et al., 2006). Seeds were cleaned manually to remove the aril and then disinfected in a 0.2% $KMnO_4$ solution for 2 h (Zuo, 1994b). After processing, seeds were dried at room temperature (25 °C), packed in sealed impermeable plastic bags, and stored at 4 °C for later study.

2.2. Embryo morphology and growth

For seed and embryo morphology and dimensional characterizations, five replications of 25 seeds each were examined. Fresh seeds were hydrated by soaking them in water for 24 h at room temperature (25 °C) before embryos were removed and measured. For observing embryo growth, cold-stratified and germinated seeds (25 °C) were cut with a scalpel and soaked in 0.5% 2,3,5-triphenyl tetrazolium chloride ($C_{19}H_{15}ClN_4$) solution for 3 h until embryos stained red (Wang et al., 2005). Embryos were excised from seeds using cuspidal tweezers. Embryo and seed lengths were measured using a dissecting microscope fitted with an ocular micrometer, and the ratio of embryo to seed length (E:S ratio) was calculated. To determine the E:S ratio of seeds at germination, the average ratio of 25 seeds with split seed coats, but no radicle protrusion, (critical E:S ratio) was calculated (Walck et al., 1999; Vandelook et al., 2007).

2.3. Germination testing

Seeds were surface-sterilized in a 0.2% chlorothalonil solution for 20 min (Zhang et al., 2005). Newly matured seeds (i.e. seeds recently removed from arils) were placed on moist filter paper in 150 mm Petri dishes at constant 25 °C or 20/23 °C or 20/25 °C with a 12 h photoperiod (>6000 lx). Water or experimental solutions were replenished to maintain constant hydration for the life of the experiment. Seeds were scored every 3 d for germination for 35 d. Seeds were counted as germinated when radicles protruded at least 1 mm past the seed coat.

To determine whether GA_3 could overcome dormancy in *M. yunnanensis*, seeds were soaked in 0, 200, 500, 1000, 2000, or 2500 mg L^{-1} GA_3 for 48 h at room temperature to obtain full hydration. After 48 h, seeds were transferred to incubators at 20/25 °C or constant 25 °C for germination testing under a 12 h photoperiod.

The effects of imbibition for 48 h in 0.1, 0.5, or 1.0% KNO_3 (w/v) on germination were investigated at 25 °C. Other seeds were treated with 200 mg L^{-1} GA_3 for 48 h following the 0.1% KNO_3 treatment and then incubated at 20/25 °C to assess germination.

Newly harvested *M. yunnanensis* seeds were placed in sealed polyethylene bags containing moist perlite for warm stratification at 20–23 °C for 30, 60, or 100 d. Seeds were cold-stratified at 4 °C in a refrigerator for 100 d, and germination was tested on 4 replications of 26 seeds each at 20 d intervals. Stratified seeds were tested for germination at 20/25 °C. After 14 and 40 d of cold stratification, four replications of 26 seeds each was soaked in 200 mg L^{-1} GA_3 for 48 h and germinated at 20/25 °C as described above.

The optimum temperature for germination was determined using seeds that became non-dormant during 80 d of cold stratification in darkness at 4 °C. Four replications of 26 non-dormant seeds were incubated at 4–30 °C (4, 10, 15, 20, 25 or 30 °C) or 20/25 °C to assess germination.

Prior to germination testing, four replications of 26 fresh seed samples each were washed in running water for 12 h and stored at 4 °C for 10 d, then germinated in Petri dishes at 20/25 °C. After washing and chilling, other seeds were soaked in 200 mg L^{-1} GA_3 for 48 h and incubated at 20/25 °C to assess germination.

2.4. Statistical analyses

Germination tests were conducted on four replications of 26 seeds each, respectively, unless stated otherwise. Means and standard errors were calculated for germination percentages and embryo lengths. ANOVA with mean separation by protected least significant difference ($p = 0.05$) was used to assess treatment differences. Total germination percentage and time to 50% of total germination (T_{50}) were calculated by linear interpolation from the two germination values closest to median germination.

3. Results

3.1. Embryo morphology and growth

Mature seeds of *M. yunnanensis* were oblate-ovoid shaped with a mean length of 6.10 mm and mass of 60.4 mg (Table 1). At the time of dispersal, embryos were small linear and underdeveloped with only cotyledons and radicles differentiated. Embryos were oval shaped, located basally at the tip of the seeds, and surrounded by endosperm. Embryos in newly matured seeds were 0.94 mm long with an average E:S ratio of 0.15 (Table 1). Embryos grew significantly within the seed to a length of 1.17 mm, and an E:S ratio of 0.18 following cold stratification for 60 d (Table 1). During germination, embryos also grew prior to radicle protrusion. When seed coats split, but prior to radicle protrusion, germinating embryos had increased in length by 139%.

3.2. Germination testing

Newly matured seeds did not germinate at 25, 20/23, or 20/25 °C (Table 2). Compared with the control, seeds did not germinate in any concentration of KNO_3 at 25 °C unless they were also treated with GA_3 (Table 2). GA_3 effectively broke dormancy and promoted germination (Fig. 1). The addition of GA_3 at 25 or 20/

Table 1
Seed length, single seed weight, embryo length and E:S ratio of *Michelia yunnanensis* seeds (means ± SE).

Seeds	Seed length[a] (mm)	Embryo length (mm)	E:S (ratio)	Single seed weight (mg)
Fresh seeds	6.19 ± 0.18	0.94 ± 0.08	0.15 ± 0.01	60.4 ± 0.1
Cold-stratified seeds	6.44 ± 0.15	1.17 ± 0.03	0.18 ± 0.00	–
Germinating[b] seeds	6.05 ± 0.20	2.25 ± 0.17	0.37 ± 0.02	–

[a] Embryo length of cold-stratified seeds was measured after 100-d cold stratification at 4 °C.
[b] Embryo growth during germination at 25 °C was measured as seed coat split prior to radicle protrusion.

Table 2
Effects of temperature, KNO_3, warm stratification, cold stratification combined with GA_3, and washing plus chilling on germination percentage and time to 50% germination (T_{50}) for *M. yunnanensis* seeds (mean ± SE). Germination percentage and time to 50% germination (T_{50}) of all treatments were calculated after 35 d from germination and a 12 h photoperiod. Seeds treated with 0.1, 0.5, and 1.0% KNO_3 (w/v) for 48 h were incubated at 25 °C. Cold stratification results are shown in Fig. 4.

Treatments	Germination percentage (%)	T_{50} (d)
Fresh seeds		
25 °C[a]	0c	–
23/20 °C (alternating)[a]	0c	–
20/25 °C[a]	0c	–
KNO_3 (25 °C)[a]		
0.1%	0c	–
0.5%	0c	–
1.0%	0c	–
0.1% + 200 mg L^{-1} GA_3	21.6 ± 2.0b	26.5 ± 0.5a
Warm stratification (20/25 °C)[a]		
30 d	0c	–
60 d	0c	–
100 d	0c	–
Washing (12 h) + chilling (4 °C) (10 d) (20/25 °C)[a]		
Without GA_3	0c	–
200 mg L^{-1} GA_3	77.5 ± 2.9a	20.5 ± 0.5b
Cold stratification 14 d (20/25 °C)[a]		
200 mg L^{-1} GA_3	73.5 ± 6.9a	21.5 ± 1.5b
Cold stratification 40 d (20/25 °C)[a]		
200 mg L^{-1} GA_3	74.5 ± 3.9a	15.5 ± 1.5c

Means in columns followed by different letter differ significantly at $p < 0.05$ according to LSD test.
[a] Germination temperature.

25 °C significantly improved final germination percentages ($p < 0.01$) (Fig. 1). In 200 mg L^{-1} GA_3, germination at 25 or 20/25 °C was 56.8 ± 4.8% (mean ± SE) and 89.3 ± 0.7%, respectively. Alternating temperatures in combination with 200 or 500 mg L^{-1} GA_3 increased germination more than constant temperature (Fig. 1). At higher concentrations of GA_3, no significant differences in germination percentages between alternating and constant temperatures

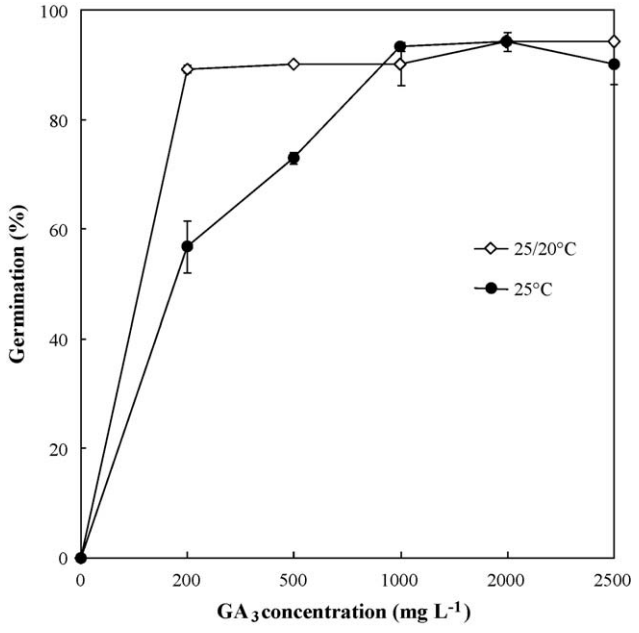

Fig. 1. Germination percentage of *Michelia yunnanensis* seeds treated with GA_3 and incubated at 25 °C or alternating 20/25 °C under a 12 h photoperiod (error bars are ±SE).

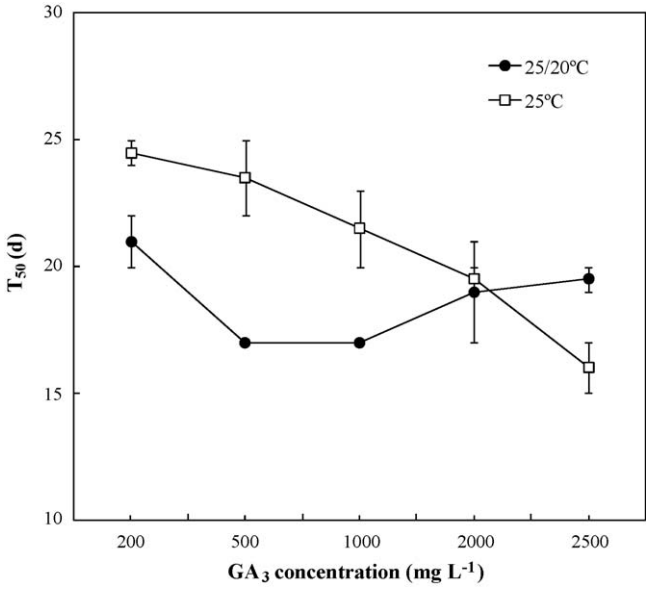

Fig. 2. Time to 50% germination (T_{50}) of *M. yunnanensis* seeds treated with GA_3 and incubated at 25 °C or alternating 20/25 °C under a 12 h photoperiod (error bars are ±SE).

were observed. At 2000 mg L^{-1} GA_3 germination percentages were 94.4 ± 1.8% and 94.3 ± 0.3% for 25 °C or 20/25 °C, respectively (Fig. 1).

At 25 °C, the T_{50} ($p < 0.05$) decreased significantly with increasing GA_3 concentrations (Fig. 2). The T_{50} was 8.5 d less when seeds were treated with 2500 mg L^{-1} GA_3 (16 ± 1.0 d) compared to 200 mg L^{-1} GA_3. At 20/25 °C, T_{50} decreased after treatment with 200 and 500 mg L^{-1} GA_3 and then increased at higher concentrations (Fig. 2). The shortest T_{50} was 17 d following treatment with 500 mg L^{-1} GA_3 and alternating 20/25 °C or 2500 mg L^{-1} GA_3 and constant 25 °C (Fig. 2).

Warm stratification for 30, 60, or 100 d did not break seed dormancy of *M. yunnanensis* (Table 2). However, cold stratification significantly increased germination to about 50% after cold-stratifying for 60 d (Fig. 3). The germination percentages significantly increased with cold stratification time ($p < 0.01$). After 80 d, germination increased to 89.2 ± 3.0%, before decreasing to 70.9 ± 3.6% after 100 d of cold treatment (Fig. 3).

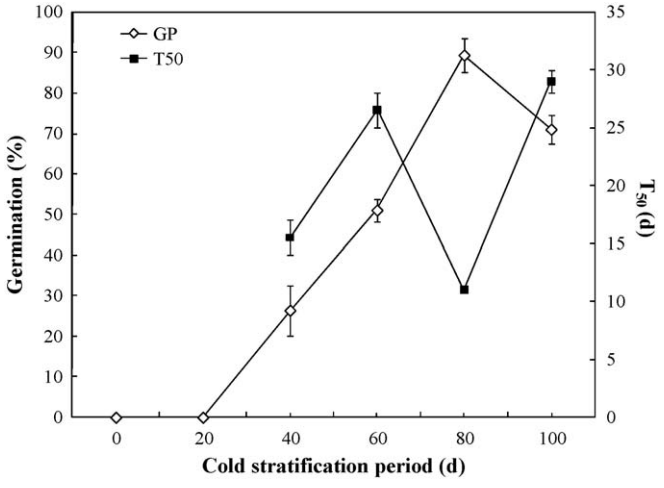

Fig. 3. Germination percentage (GP) and time to 50% germination (T_{50}) of 4 replications of 25 *M. yunnanensis* seeds cold-stratified for 0, 20, 40, 60, 80, or 100 d and incubated at alternating 20/25 °C under a 12 h photoperiod (error bars are ±SE).

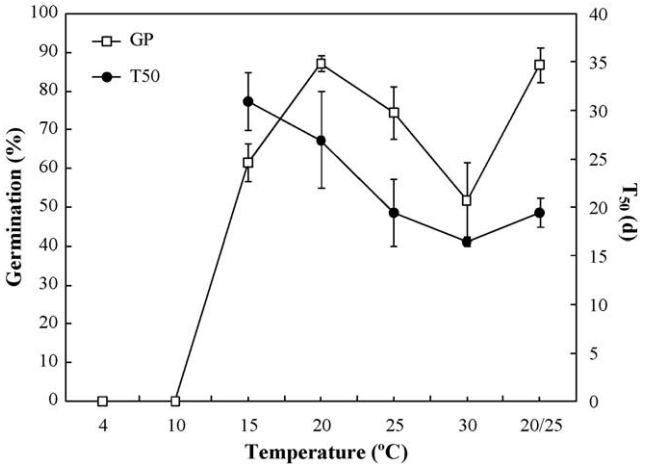

Fig. 4. Germination percentage (GP) and time to 50% germination (T_{50}) of 4 replications of 25 *M. yunnanensis* seeds cold-stratified at 4 °C for 80 d incubated at 4, 10, 15, 20, 25 or 30 °C or alternating 20/25 °C to assess germination at under a 12 h photoperiod (error bars are ±SE).

The duration of cold stratification also significantly increased T_{50} ($p < 0.01$). Initially T_{50} increased between 40 and 60 d before declining at 80 d (Fig. 3). With prolonged cold stratification, T_{50} increased from 11.5 ± 0.6 d at 80 d to 29.0 ± 1.1 d at 100 d as dormancy was broken (Fig. 3). Therefore, cold stratification broke dormancy in *M. yunnanensis* seeds, but generally slowed germination of non-dormant seeds with the exception of seeds stratified for 80 d (Fig. 3). The cold-stratified seeds (14 and 40 d) treated with 200 mg L^{-1} GA_3 each germinated to approximately 74% (Table 2), although the T_{50} was reduced by 6 d following the 40 d treatment.

Washing seeds in water followed by chilling at 4 °C for 10 d did not break dormancy (Table 2). However, treatment with 200 mg L^{-1} GA_3 after washing and chilling increased germination (77.5 ± 2.9%) at 20/25 °C and produced similar results as treatment with 200 mg L^{-1} GA_3 without washing and chilling (Table 2 and Fig. 1). This illustrates that washing and short-term chilling alone could not break dormancy.

There were significant differences ($p < 0.001$) among germination percentages at different temperatures for non-dormant seeds. No seeds germinated at 4 or 10 °C, and only 61.8 ± 4.9% germinated at 15 °C (Fig. 4). Germination was highest, 87.3 ± 2.0 or 86.9 ± 4.6%, at 20 °C or 20/25 °C, respectively. At 30 °C, germination percentages declined because approximately half the seeds decayed before germinating (Fig. 4). The base temperature was between 10 and 15 °C, while the ceiling temperature was in excess of 30 °C. The T_{50} declined with increasing germination temperatures (Fig. 4). Constant 25, 30, or 20/25 °C, produced T_{50} values of 20 d or less (Fig. 4). With respect to both germination percentage and rate, the optimal germination of non-dormant seeds occurred at 20/25 °C.

4. Discussion

No newly matured seeds germinated regardless of temperature (Table 2). Therefore, mature seeds of *M. yunnanensis* were dormant at dispersal. Mature seeds contained small embryos that were differentiated into cotyledons and axes that were longer than wide, with an average E:S ratio of 0.15 (Table 1). In the embryo classification system of Martin (1946), embryos were divided into three primary types: basal, peripheral, and axile. Basal types are characterized as having a small embryo that is globular to oval-oblong with cotyledons that are rudimentary or obscure and sometimes evident within seeds that are generally of medium size or larger (Martin, 1946). Previous studies showed that seeds of Magnoliaceae had rudimentary embryos (Martin, 1946; Baskin and Baskin, 1998; Nikolaeva, 2004). Thus, according to the revised system of classification (Baskin and Baskin, 2007), *M. yunnanensis* embryos are linear and underdeveloped.

In the family Magnoliaceae, mature seeds of *M. platypetala*, *Manglietia insignis*, *Magnolia officinalis* subsp. *biloba* and *Liriodendron chinense* also have linear underdeveloped embryos with differentiated cotyledons, while mature seeds of *Magnolia sieboldii* and *Manglietia patungensis* have only undifferentiated embryos (Fang et al., 1990; Zhou and Hu, 1990; Zhou, 1991; Fan et al., 1992; Wang and Tian, 1996; Chen et al., 2007). Nevertheless, after cold stratification, undifferentiated embryos of *M. sieboldii* and *M. patungensi* developed into torpedo-shaped embryos with cotyledons (Wang and Tian, 1996; Chen et al., 2007).

The fact that embryos in mature seeds were linear and underdeveloped implies that *M. yunnanensis* seeds are morphologically or morphophysiologically dormant at maturity. Fresh seeds required cold stratification to overcome dormancy (Fig. 3). Cold but not warm stratification overcame dormancy in *M. yunnanensis* seeds. Up to 89.2% of seeds in *M. yunnanensis* germinated after cold stratification (Fig. 3), similar to results obtained by Zuo (1994a, b), who recorded 91.8% germination of seeds after cold stratification for 90 d. Therefore, the seed dormancy classification for *M. yunnanensis* is morphological and physiological, i.e. morphophysiological.

Considerable embryo growth occurred in seeds of *M. yunnanensis* prior to germination (Table 1). Based on temperatures at the time of pregerminative embryo growth, the eight types of morphophysiological dormancy have been divided into two categories: simple and complex (Baskin and Baskin, 1998). Embryo growth occurs at relatively high temperatures (≥15 °C) in simple morphophysiological dormancy; whereas, in complex morphophysiological dormancy, embryo growth occurs only at low temperatures (0–10 °C) (Nikolaeva, 1977; Baskin and Baskin, 1998; Walck et al., 1999). Since embryos grew within the seeds of *M. yunnanensis* during cold stratification at 4 °C in this study (Table 1), the morpholophysiological dormancy should be considered "complex". Consequently, seeds of *M. yunnanensis* can be characterized as having intermediate or deep complex morphophysiological dormancy for cold stratification is needed for loss of physiological and morphological embryo dormancy (Baskin and Baskin, 1998).

In other species of *Michelia*, cold or winter stratification outdoors successfully overcame seed dormancy for commercial propagation (Shi et al., 1986; Zhou, 1990; Gan and Fan, 2005). After cold stratification for 77 d at 3–15 °C, seed germination of *M. maudiae* reached 99% (Shi et al., 1986). The germination percentage of *M. chapensis* increased to 77% after stratification for 80 d at 5 °C (Zhou, 1990).

Exogenous GA_3 also broke seed dormancy and substituted for cold stratification in *M. yunnanensis*, *M. chapensis*, and *M. platypetala* seeds (Zhou, 1990; Zhou and Hu, 1990) (Fig. 2, 3). Thus, seeds of *M. yunnanensis* have intermediate complex MPD (Nikolaeva, 1977; Baskin and Baskin, 1998). In *M. yunnanensis*, seed dormancy was broken by 2000 mg L^{-1} GA_3 (Fig. 1). The germination and dormancy-breaking requirements of *M. chapensis* and *M. platypetala* were similar to those of *M. yunnanensis* (Zhou, 1990; Zhou and Hu, 1990), because maturing seeds of *M. chapensis* and *M. platypetala* germinated at alternating 30/20 °C after 42 and 35 d, respectively (Zhou, 1990; Zhou and Hu, 1990). GA_3 is widely used to break dormancy of Magnoliaceae seeds, e.g. *M. chapensis*, *M. platypetala* and *Manglietia insignis* (Zhou, 1990; Zhou and Hu, 1990; Zhou, 1991). GA_3 effectively broke dormancy of *M. chapensis* and *M. platypetala* increasing germination percentages to 76.5 and 76.7%, respectively (Zhou, 1990; Zhou and Hu, 1990). Moreover, GA_3 increased embryo growth in *M. chapensis* and shortened the germination time from 18 to 7 d (Zhou, 1990). Nitrate is a naturally

occurring inorganic plant nutrient that stimulates seed germination in some species (Hilhorst and Karssen, 2000). However, in this study nitrate alone did not break dormancy. Only in combination with GA_3 did nitrate effectively break dormancy (Table 2).

Once morphological dormancy in *M. yunnanensis* seeds is broken, germination occurs over a wide range of temperatures (Fig. 4). However, alternating temperature (20/25 °C) produced optimum germination in terms of both percentage and rate (Fig. 4).

5. Conclusion

Seeds of *M. yunnanensis* have linear underdeveloped embryos at maturity, and exhibit intermediate complex morphophysiological dormancy. Pretreatments with GA_3 and cold stratification effectively overcame dormancy, and accelerated seed germination of *M. yunnanensis* and could be used for commercial propagation. Embryonic growth and development occurred during chilling at 4 °C. Alternating 20/25 °C was optimum for seed germination in terms of both germination percentage and rate.

Acknowledgments

This study was supported by the Ministry of Science and Technology of China (2008FY110400-2-2 and 2005DKA21006), the Chinese Ministry of Education (projects B08044 and MUC 985-3-3), and the Knowledge Innovation Program of the Chinese Academy of Sciences. The authors thank Dr. An-Jun Tang, Prof. Zhi-Ying Cheng and Mrs. Ji-Feng Luo for helpful research suggestions.

References

Baskin, C.C., Baskin, J.M., 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.

Baskin, J.M., Baskin, C.C., 2004. A classification system for seed dormancy. Seed Sci. Res. 14, 1–16.

Baskin, C.C., Baskin, J.M., 2007. A revision of Martin's seed classification system, with particular reference to his dwarf-seed type. Seed Sci. Res. 17, 11–20.

Chen, F.J., Liang, H.W., Wang, X., He, Z.Q., Li, F.L., 2007. Seed dormancy and germination characteristics of *Manglietia patungensis*, an endangered plant endemic to China. Biodivers. Sci. 5, 492–499 (in Chinese, English abstract).

Fan, R.W., Ye, J.G., Yin, Z.F., 1992. Studies on seed development and embryogenesis in *Liriodendron Chinense* (Hemsl.) Sarg. Acta. Bot. Sin. 6, 437–442 (in Chinese, English abstract).

Fang, J., Xu, X.Y., Sun, C.K., Zheng, J.B., 1990. Study of seed physiology of medicinal plant effects of stratification on dormancy and germination in *Magnolia biloba*. Chin. J. Modern Appl. Phar. 6, 5–9 (in Chinese, English abstract).

Gan, X.W., Fan, G.S., 2005. Experiments on the germination of *Michelia sphaerantha* seed under different treatments. J. West. Chin. For. Sci. 1, 40–47 (in Chinese, English abstract).

Hao, X.Y., 1999. Studies on the chemical constituents of the essential oil of *Tsoongiodendron odorum* and *Michelia yunnanensis*. Guizhou Sci. 4, 287–290 (in Chinese, English abstract).

Hilhorst, H.W.M., Karssen, C.M., 2000. Effect of chemical environment on seed germination. In: Fenner, M. (Ed.), Seeds: The Ecology of Regeneration in Plant Communities. CABI Publishing, New York, pp. 293–309.

Liang, N., Shi, L., Yang, Y., 2006. An experimental study on germination rate and seedling-forming rate of eight species of Magnoliaceae. J. West. Chin. For. Sci. 3, 72–75 (in Chinese, English abstract).

Liu, Y.H., 1996. Magnoliaceae. Flora of China, vol. 30. Science Press, Beijing, pp. 82–199 (in Chinese).

Liu, Y.H., 2004. Magnolias of China. Beijing Science and Technology Press, Beijing, pp. 6–378 (in Chinese).

Martin, A.C., 1946. The comparative internal morphology of seeds. Am. Midl. Nat. 36, 513–660.

Nikolaeva, M.G., 1977. Factors controlling the seed dormancy pattern. In: Khan, A.A. (Ed.), The Physiology and Biochemistry of Seed Dormancy and Germination. North-Holland, Amsterdam, pp. 51–74.

Nikolaeva, M.G., 2004. On criteria to use in studies of seed evolution. Seed Sci. Res. 14, 315–320.

Shi, X.H., Yu, Z.L., Jin, S.Q., Shi, Z.L., Zhou, J.H., 1986. Research on the physiology of seed dormancy of *Michelia maudiae*. Plant. Physiol. Commun. 3, 17–19 (in Chinese).

Vandelook, F., Bolle, N., Assche, J.A.V., 2007. Seed dormancy and germination of the European *Chaerophyllum temulum* (Apiaceae), a member of a trans-Atlantic genus. Ann. Bot. 100, 233–239.

Walck, J.L., Baskin, J.M., Baskin, C.C., 1999. Seeds of *Thalictrum mirabile* (Ranunculaceae) require cold stratification for loss of nondeep simple morphophysiologycal dormancy. Can. J. Bot. 77, 1769–1776.

Wang ZJ., Tian BN., 1996. Preliminary experiment on pregermination method of *Magnolia sieboldii* seeds. 3–4, 195–199 (in Chinese, English abstract).

Wang, L.L., Wang, G.L., Liu, D.Y., 2005. Preliminary studies on seed of the endangered *Magnolia sieboldii*. J. Anhui Normal Univ. 1, 72–75 (in Chinese, English abstract).

Yu, G.Y., Zhou, G.Z., Liu, M.G., Dong, S.J., 2006. Experiments on germination acceleration of seeds of *Magnolia obovata*. Chin. For. Sci. Technol. 5, 50–52 (in Chinese, English abstract).

Zhang, X.H., 2007. Advances in research of *Michelia* Linn. Guihaia 5, 712–719 (in Chinese, English abstract).

Zhang, Z.Y., Luo, Y.L., Guan, C.Y., 2005. Detecting and sterilizing endophyte in rape seeds. Hubei Agric. Sci. 1, 52–55 (in Chinese, English abstract).

Zhou, Y.X., 1990. Preliminary study on the characteristics of dormancy and germination of *Michelia chapensis*. J. Central-South. For. Coll. 2, 165–170 (in Chinese).

Zhou, Y.X., 1991. Preliminary study on the characteristics of dormancy and germination of *Manglietia insignis* seed. Seed 6, 10–13 (in Chinese, English abstract).

Zhou, Y.X., Hu, C.Z., 1990. Preliminary study on the characteristics of dormancy and germination of *Michelia platypetala*. For. Sci. Technol. 8, 7–11 (in Chinese).

Zuo, J.B., 1992. The preliminary study of morphological taxology, ecological environment, and origin of *Michelia yunnanensis*. Guizhou For. Sci. Technol. 4, 16–22 (in Chinese).

Zuo, J.B., 1994a. The analysis of seed characteristic of *Michelia yunnanensis*. Guizhou For. Sci. Technol. 2, 44–46 (in Chinese).

Zuo, J.B., 1994b. The propagation test of *Michelia yunnanensis*. Guizhou For. Sci. Technol. 3, 52–54 (in Chinese).