

Germination, desiccation, storage and germination-accelerating pretreatment of *Ardisia virens* seeds

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(Accepted February 2011)

Summary

Ardisia virens (Myrsinaceae) is a tropical and subtropical understory shrub in South China. We have studied the effect of various treatments on seed germination and seedling emergence. Seeds were germinated at temperatures ranging from 10 to 35°C and at ambient temperature (15-24°C/24-35°C, night/day), with 25°C, 30°C and ambient temperature being the optimum for germination. Light had no significant effect on germination, compared with darkness. The seeds could not tolerate drying to at least 38% moisture content and had a short lifespan at 15, 25 and 35°C, so they can be classified as recalcitrant. Soaking for 24 hours in 10% potassium nitrate (KNO_3), 100-500 ppm gibberellic acid (GA₃) and 50-200 ppm 6-Benzylaminopurine (6-BA) did not significantly improve germination percentage, but these treatments appeared to accelerate the germination process. Soaking in 20% KNO_3 and 400 ppm 6-BA solutions had no significant effects on germination, while 30% KNO_3 and 5-20% sodium nitrite ($NaNO_2$) significantly inhibited germination. Seeds of *A. virens* therefore have shallow dormancy and an after-ripening period is required; a low storage temperature and moist conditions help maintain seed viability. To propagate this shrub, the seeds should be harvested in the winter, and then stored for about two months in a moist and cool environment to ensure the completion of after-ripening at 15°C-24°C. Application of chemical pretreatments can increase the rate of seed germination.

Introduction

Ardisia virens is native to India, Indonesia, Malaysia, Thailand, Vietnam and China. It grows in dense evergreen broad-leaf forests and hillsides at altitudes of 300-2700 m. It is an ornamental plant cultivated for its very decorative red fruit and glossy foliage, and is commonly planted in gardens and on hillsides in China. Its roots are used as Chinese herb medicine to alleviate fever, detoxify swollen areas, dispel phlegm and relieve coughs (Dixit *et al.*, 2005). Our observations show that it thrives in productive, well drained soils with partial to heavy shade in the understory of mesic forests, and its growth rates are positively correlated with soil phosphorus levels. It can be propagated by seed or cuttings, but it mainly establishes by seed in nature.

Seeds are classified into three categories in relation to longevity, orthodox, recalcitrant and intermediate. These classifications are based on their sensitivity to dehydration and temperature. Orthodox seeds can tolerate drying to as low as 5% moisture content under common conditions and low storage temperatures. Their lifespan is always prolonged with low seed moisture and temperature. In contrast, recalcitrant seeds readily lose germinability if their moisture content decreases below a critical value ranging from 12-30% and generally cannot withstand temperatures lower than 20°C. Intermediate seeds are somewhere in between orthodox and recalcitrant seeds (Ellis *et al.*, 1990; Sershen *et al.*, 2008). Tropical forests have a high number of species with recalcitrant seeds. In moist, relatively aseasonal habitats desiccation sensitivity is more frequent, and in both temperate and tropical habitats the proportion of species with recalcitrant seeds declines as seasonality increases (Tweddle *et al.*, 2003; Berjak and Pammeter, 2008).

In previous experiments, we have found that poor germination of fresh seeds is a major problem for cultivation of *A. virens*. Traditionally seed dormancy prevents germination under optimal environmental conditions and imposes a delay between seed shedding and germination. The delayed germination safeguards some seeds and seedlings from suffering damage or death as a result of short periods of bad weather and also allows some seeds to germinate when competition from other plants for light and water might be less intense (Finch-Savage and Leubner, 2006). This dormancy can be relieved by after-ripening (storage at 15-30°C and ambient temperature of air-dried or low-hydrated seed), scarification, stratification or chemical pretreatments (Schutz *et al.*, 2002; Bove *et al.*, 2005; Rouhi *et al.*, 2010). Various chemicals, such as GA₃, 6-BA, KNO₃, NaNO₂, have been shown to break dormancy or accelerate germination (Fenner and Thompson, 2005; Tang *et al.*, 2010).

Optimal conditions for seed germination and seedling emergence often reflect the optimal conditions for the entire life cycles of plants (Marques and Oliveira, 2008). The survival and invasion of a species under specific conditions are associated mainly with mechanisms of seed dormancy, germination and seedling emergence at a favorable time and place (Fenner and Thompson, 2005). An understanding of how varying environments affect seed germination will enhance our ability to predict fluctuations in population dynamics in natural habitats. To our knowledge, there was no information on seed germination of *A. virens*. Therefore, in this project, we studied seed germination of *A. virens* with varying storage conditions, incubation temperatures, light regimes and chemical pretreatments.

Materials and methods

A survey of the distribution of the species, the fruit ripening seasonality and fruit production of the wild and the cultivated populations of *A. virens* was conducted in Southern Yunnan, South-west China. The fruits ripened from October to December, with most remaining on the maternal plants until later spring of the next year. Mature fruits of *A. virens* (their pericarps are blackish red or black) were collected from more than 50 individual trees in February 2009 in a semi-natural forest in Mengla County, Xishuangbanan Dai Nationality

Autonomous Prefecture (21°09'-22°36'N and 99°58'-101°50'E) in Yunnan Province where the ranges of annual rainfall, temperature, relative humidity and sunshine hours are 1200-1600 mm, 18-22°C, 77-87% and 1700-2300, respectively. The fruits were transported in polyethylene bags to Xishuangbanna Tropical Botanical Garden about 5 hours after collection, and the seeds were immediately isolated. The seeds were selected and air-dried at room temperatures (18-24°C) for 3 days, and were considered the freshly-harvested seeds. They were then stored in air-permeable plastic bags at 15°C in a storage room for 30 days, then air-transported to Jiaying University, Meizhou, Guangdong Province. There, they were randomly divided into samples for the various experiments described below, except those used in storage test.

Seed size, weight and moisture content

Four replicates of 1000 freshly-harvested seeds were weighed, and 60 seeds were measured with calipers, to obtain fresh seed weight and seed size. Three replicates of 10 seeds were used for determination of seed moisture content (MC) on a fresh weight basis by the low temperature method according to the Rules of ISTA (1985).

Desiccation procedure

The seeds transported to Jiaying University were placed in airtight glass desiccators containing active silica gel at ambient temperatures (AT, 15-24°C at night and 24-35°C in the day, the weight ratio of seed to silica gel was about 1/10). Samples were taken at intervals 4 h - 7 d to investigate the seed desiccation tolerance.

Seed germination tests

In order to investigate whether the freshly harvested seeds had germinability, the fresh seeds were set to germinate at 25°C in the light. In all the other germination and emergence tests described below, 50 seeds each were disinfected for 10 min with 0.2% sodium hypochlorite, then washed with distilled water, and plated in 90 mm-diameter Petri-dishes containing 0.8-1% agar. Seeds were germinated in incubators at 5°C, 10°C, 20°C, 25°C, 30°C, 35°C and AT (18-30°C) with 12 h photoperiod (light of 800-1000 lx). Light was provided by cool white fluorescent lamps. Germination was defined as the appearance of a radicle over 0.5 cm in length. Seed germination was observed every day and the germination experiment lasted for 28-30 days. For all these tests, four replicates were used per treatment.

The germination percentage (GP), and germination index (GI) was calculated after each germination test:

$$\text{GP (\%)} = \frac{\text{number of germinated seeds}}{\text{total number of seeds per sample}} \times 100, \text{ and}$$

$$\text{GI} = \sum(G_t/D_t), G_t \text{ means numbers of germinated seeds after } t \text{ d.}$$

Effect of light and dark on germination: Seeds were germinated at 25°C at 12 h daily photoperiod from 8:00 to 20:00, or in dark 24 h a day. In the dark, 16 50-seed samples were packed with an air-permeable black cloth, and these bags were then put into a closed closet. Four samples were taken out to germinate every 7 days.

Chemical treatments and seed germination

After maintenance in a glass bottle with a 5-mm diameter vent hole in the lid for 10 days in an air-ventilated room, seeds were soaked for 24h in 80 ml of each of the following test solutions at 25°C in the dark: GA₃ at 100, 300 and 500 ppm; 6-BA at 50, 100, 200 and 400 ppm; KNO₃ at 10%, 20% and 30%; and NaNO₂ at 5%, 15% and 20%. Then, they were rinsed for 20 minutes in running tap water prior to germination tests. The control was the seeds soaked in 80 ml distilled water for 24 hours. The MC of seeds in these treatments was 48-50%.

Seed storage tests

The seeds transported to the university were placed into an air-permeable plastic bag for 30 days in an air-ventilated room (temperature: 18-25°C, relative humidity: 65-75%), and these were considered the indoor-stored seeds. Then, in the Laboratory of the Department of Biology, Jiaying University, four replicates of 50 seeds were stored in glass bottles with a 5-mm diameter vent hole in the lid at 15°C, 25°C and 35°C for 30 days respectively, and as a control, four replicates of 50 seeds were stored in a wide-mouth glass bottle without lid for 180 days in an air-ventilated room (ambient temperature 18-36°C). The MC of seeds stored for 30 days at 15°C, 25°C and 35°C was about 45%, 35% and 25% respectively. Freshly harvested seeds and seeds in the different storage conditions were germinated at 25°C with 12 h daily illumination of 800-1000 lx.

Statistical data analysis

GP was transformed into arcsine of the square root of GP in degrees before analysis in order to obtain an approximately normal distribution. The transformed GP and GI were subjected to one-way analysis of variance using Excel (Microsoft, Inc., 1985-1999, version 2000), and LSD test was used to determine if there were significant ($p < 0.05$) differences among individual treatment means. However, original GP data are presented in our text.

Results

Fruit and seed morphology

In organic-rich lowland rainforest valleys, the number of fruits produced by an individual mature tree was usually 200 or more a year, while at the high elevations of the mountains, it was only 50-100. Under cultivation conditions in Xishuangbanna Botanical Garden, fruit production was greater with 300-400 fruits per year. The fresh fruits were berry-like drupes, 361.68 ± 28.47 g per 1000, and MC was $72.95 \pm 0.16\%$. The pericarp turned from green, to yellowish red, blackish red, and to black during the process of fruit maturation. A typical fruit was oblate spherical, approximately 8.0-9.5 mm in length, 6.0-6.8 mm in diameter with dense glandular spots. The fruits had juicy flesh and each fruit usually contained a seed. A typical seed was flat, oblong or ovate, 6.74 ± 0.03 mm in length, 7.43 ± 0.04 mm in diameter, and the seed coat of a mature seed was brown or black. The weight of 1000 fresh seeds was 252.30 ± 3.21 g, that of 1000 dried seeds was 123.92 ± 0.69 g, and the MC of fresh seeds was $50.88 \pm 0.47\%$.

Germination at various temperatures

GP was significantly higher at 20, 25 and 30°C and AT than at other temperatures, and there were no significant GP differences among these four temperatures. GI was significantly larger at 25 and 30°C and AT than at other temperatures (table 1). The seeds took significantly less time to start germination at 30 and 35°C and AT than at other temperatures (table 1).

Table 1. Seed germination of *Ardisia virens* at different temperatures. GP: Germination percentage; GI: germination index; AT: ambient temperature (15-24°C/24-35°C, night/day).

Temperature	10°C	15°C	20°C	25°C	30°C	35°C	AT
GP	13.33 ±1.44c*	95.83 ±0.04b	100.00 ±0.00a	100.00 ±0.00a	98.33 ±2.89a	91.67 ±0.05b	100.00 ±0.00a
GI	0.22 ±0.03c	4.12 ±0.99b	5.71 ±1.02b	6.39 ±1.01a	6.34 ±1.13a	4.49 ±1.29b	5.91 ±0.92ab
Days to start germination	23.00 ±1.00d	10.33 ±1.53c	8.33 ±1.15bc	7.00 ±0.00b	6.00 ±0.00a	7.00 ±1.00ab	6.33 ±0.58a

* The values (mean ± D, n = 4) marked with the same letter (a–c) in the same row are not significantly different at 5% level by LSD test.

Germination at different light conditions

Most of the seeds had germinated in the light and dark after incubation for 14 days, with the GP being 100% and 94.17% after 21 days, and 100% and 97.5% after 28 days in light and in the dark respectively. There was no significant difference in GP at any time from 14 to 28 days.

Germination percentage and index after desiccation

After being desiccated for 4h, 12h, 2d, 4d and 7d, seed MC was reduced from 50.88% to 48.33%, 48.03%, 46.43%, 38.54% and 36.81%, respectively. GP and GI decreased with decreasing seed MC (figure 1). GP remained above 90% as seed MC decreased from 50.88% to 46.43% and the small decrease in GP was not significant. However, as the MC decreased from 46.43% to 38.54% and then to 36.81%, GP decreased significantly to approximately 60% and 20%, respectively. The un-desiccated seeds with the highest MC had significantly higher GI than any of the desiccated seeds (figure 1) and GI fell as the MC declined.

Chemical treatments and seed germination

After being indoor-stored for 30 days and stored in an air-permeable glass bottle for 10 days, the seeds had a high GP (99.38%). Soaking them in 100-500 ppm GA₃ and 50-200 ppm 6-BA solutions increased the GI (table 2). The 20% KNO₃ and 400 ppm 6-BA solutions had no effect on the GP or GI, while 30% KNO₃ and 5-20% NaNO₂ significantly reduced germination, and no seed germinated after soaking in 10-20% NaNO₂ solution (table 2).

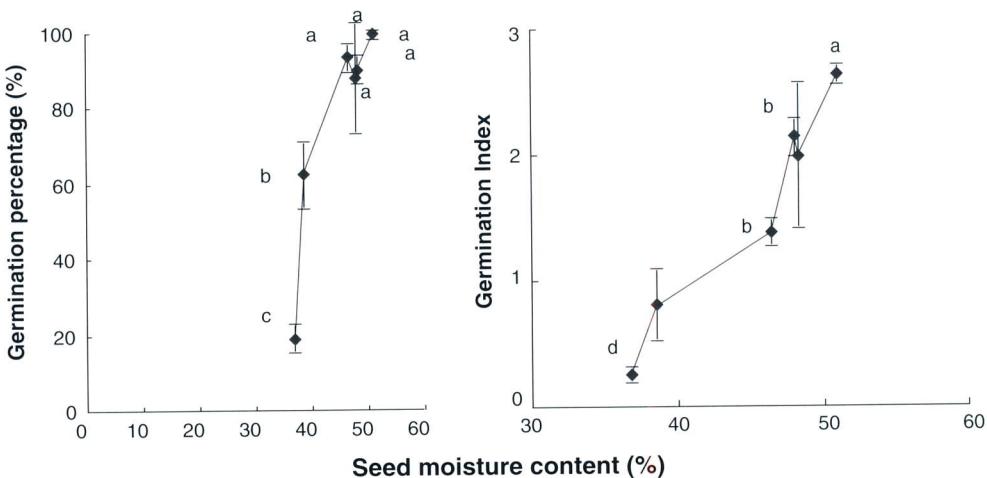


Figure 1. Germination percentage and germination index plotted against seed moisture content. The bars represent SD. The values marked with the same letter are not significantly different at 5% level by LSD test.

Table 2. Germination percentage and germination index of *Ardisia virens* seeds after soaking for 24 h in various chemical pretreatments.

Chemical	Concentration	Germination percentage	Germination index
GA ₃	500ppm	96.88 ± 2.39 ab*	4.01 ± 0.12 b
	300ppm	99.38 ± 1.25 a	4.11 ± 0.14 b
	100ppm	98.13 ± 1.25 ab	4.06 ± 0.12 b
6-BA	400ppm	98.13 ± 3.75 a	3.79 ± 0.18 ab
	200ppm	100.00 ± 0.00 a	4.04 ± 0.09 b
	100ppm	98.75 ± 0.07 ab	4.04 ± 0.30 b
	50ppm	100.00 ± 0.00 a	4.04 ± 0.13 b
KNO ₃	30%	88.13 ± 5.54 b	2.46 ± 0.14 c
	20%	99.38 ± 6.25 a	3.12 ± 0.07 a
	10%	98.75 ± 1.44 a	3.61 ± 0.06 a
NaNO ₂	20%	0.00 ± 0.00 c	0.00 ± 0.00 d
	10%	0.00 ± 0.00 c	0.00 ± 0.00 d
	5%	81.88 ± 9.66 b	2.48 ± 0.28 c
CK		99.38 ± 1.25 a	3.51 ± 0.11 a

* The values (mean ± SD, n=4) marked with the same letter (a-c) in the same column are not significantly different at 5% level by LSD test. The CK was the seeds soaked in distilled water.

Storage and germination

The freshly harvested seeds had the lowest (14.17%) and the indoor-stored seeds (being placed for 30 days at 15°C and for 30 days at ambient temperature) had the highest final GP (100%) (figure 2). When stored at 15, 25 and 35°C final GP decreased from 86.67% to 44.17% and 22.50% respectively (figure 2). Seeds stored in a wide-mouth bottle without a lid for 180 days in the ambient conditions did not germinate.

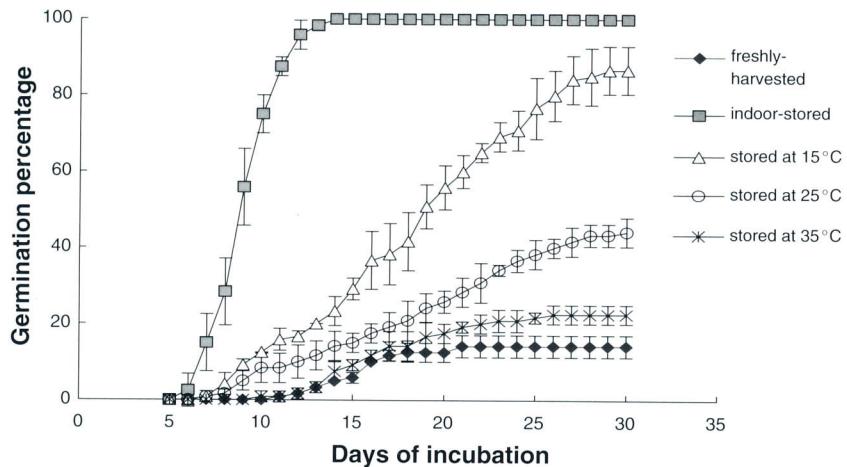


Figure 2. Germination percentage of freshly-harvested seeds, bottle-stored seeds held at 15°C, 25°C and 35°C for 30 days, and indoor-stored seeds. The bars represent SD.

Discussion

We observed that in natural or semi-natural forests in our surveyed areas, *A. virens* populations could only grow in moist habitats of valleys, but not in other habitats. Even in moist valleys, it was difficult to find the seedlings. Compared with records of previous surveys (Li *et al.*, 1996), we found that the populations have shrunk and regeneration appears to be unsuccessful. One reason for this may be excessive harvest of the shrubs as traditional Chinese medicine, and another one may be that the maternal shrub can produce only a small amount of seeds in the natural environments. In the tropical areas of Yunnan, the wild populations of many species have decreased, because of forest fragmentation (Zhao *et al.*, 2006) leading to the warming and drying of local climate. Therefore, for the protection and continued utilization of this shrub it is beneficial to develop effective cultivation techniques based on an understanding of the physiological characteristics of its seed germination.

Effects of temperature and light on seed germination

GP and GI at 25°C and 35°C were significantly higher than at other constant temperatures, so 25°C-35°C may be the optimal temperature range for seed germination of *A. virens*. Temperature fluctuations improve seed germination for some plants (Benvenuti *et al.*, 2001), but in this study AT had no significant effects on GP and GI compared with those

at constant temperatures of 25°C and 30°C. The AT represents the natural alternating day/night temperatures in many tropical and subtropical areas in spring and early summer when seeds of *A. virens* germinate. Thus, it is possible that the seeds could germinate well around the optimal temperature, either constant or alternate.

Many tropical plants need high temperatures (20-35°C) to promote germination (Leite and Takaki, 2001). However in this study, after a month of incubation at 15°C, at least 91% of the *A. virens* seeds germinated at 15-35°C, and 13% did so at 10°C. Germination over a wide temperature range might be one of the reasons that this plant is distributed widely in tropical and southern subtropical areas of South and South-western China.

It has been found that seed germination becomes less dependent on light with increasing seed mass (Milberg *et al.*, 2000). The 1000-seed weight of *A. virens* was over 250 grams and much larger than that of some typical small seed plant, such as Compositae, Gramineae, whose seed germination require light (Milberg *et al.*, 2000). Light did not significantly improve the seed germination of *A. virens* in our study. This may partially explain why this shrub can grow in shady understory.

Effects of desiccation on seed germination

The seed germination of *A. virens* in our study decreased drastically when seed MC decreased below 46%, indicating that the seeds had a weak desiccation-tolerance. This suggests that the seeds should be classified as recalcitrant (Hong and Ellis, 1996), and may be one of the reasons that this shrub prefers a moist habitat in nature. Previous research has shown that seeds of other *Ardisia* species, e.g., *Ardisia crenata*, have similar seed germination characteristics (Farnsworth, 2000; Peng *et al.*, 2006; Yang *et al.*, 2009), but seeds of *Ardisia crenata* and its variety *Ardisia crenata* var. *bicolor* are more tolerant of desiccation than those of *A. virens*. This may explain why *A. virens* distributed in tropical and south subtropical zones, but *A. crenata* and *A. crenata* var. *bicolor* grow not only in these two zones, but also in central subtropical zones in China. In general, among these three different zones in China, the tropical zone has the most annual rainfall, the highest temperature and relative humidity, followed by south subtropical zone, but the central subtropical zone has the least annual rainfall, the lowest temperature and relative humidity. The *Ardisia* species with the more desiccation-tolerant seeds may be more adapted to the drier areas.

Effect of chemicals on seed germination

It is well known that GA₃ and 6-BA increase and synchronize seed germination of many plants; however, their high cost limits their use in agriculture and horticulture (Bewley and Black, 1994; Yang *et al.*, 2009). GA most effectively minimized the difference in onset and final germination which has been attributed to increased activity of hydrolytic enzymes (Manjkhola *et al.*, 2003) or mobilization of nutrients in dormant seeds (Pradhan and Badola, 2010). In our study, soaking seeds in 100-500 ppm GA₃ and 50-200 ppm 6-BA hastened germination, compared with the control. Some studies have shown that the germination accelerating effects of GA₃ are usually associated with its concentrations. For example, 5 or 10 mM GA₃ did not significantly stimulate germination of *Podophyllum hexandrum* seeds (Choudhary *et al.*, 1996), whereas 250 µM GA₃ did (Nadeem *et al.*,

2000). The number of our treatment concentrations of GA₃ and 6-BA and duration were limited. Thus, further refinement at other concentrations and durations might be possible for the rate of seed germination of *A. virens*.

Since both chemicals KNO₃ and NaNO₂ are inexpensive, compared with GA₃ and 6-BA, they can be widely used in practice. It has been reported that they stimulate seed germination of some plant species, especially, some cultivated plants, such as cotton, *Lepidium virginicum*, *Eragrostis curvula*, *Polypogon monspeliensis*, various *Agrostis* species and *Sorghum halepense* (Bewley and Black, 1994; Pandey *et al.*, 2000; Pérez-Fernández and Rodríguez-Echeverría, 2003). However, in our study, 10% and 20% KNO₃ solutions did not increase GP and GI seeds of *A. virens*; while 30% KNO₃ and 5-20% NaNO₂ significantly reduced GP and GI, and 10% and 20% NaNO₂ completely prevented seed germination. Since the highest GI among KNO₃ treatments was significantly lower than some of the other chemical treatments, further studies might find the optimum concentrations of KNO₃ treatment.

Effects of storage on seed germination

In this study, we observed that the freshly harvested seeds had very low GP; storage at 15°C for 30 days resulted in GP above 90%; but further storage at the same temperature for another 30 days reduced GP and GI; and GA₃ and 6-BA treatments increased GI. The tested seeds in this study were detached from matured (blackish red or black) fruits, and anatomical observations revealed that the *A. virens* seeds were nearly or completely morphologically mature and had intact embryos. This suggested that the freshly harvested seeds of *A. virens* had a shallow dormancy and after-ripening at 15°C for 30 days was sufficient to remove the dormancy, but not completely. This dormancy is likely to be a physiological dormancy, and also an endogenous dormancy, associated with the physiological maturity of seed embryo and thought to be caused by a physiological inhibiting mechanism (Bewley and Black, 1994; Schutz *et al.*, 2002). Berjak *et al.* (1989) also reported that for some recalcitrant-seeded species, dormancy was most likely a consequence of seeds being shed relatively immature and continuing development before germination was initiated after being stored for more extended periods. Whether this immaturity is morphological or physiological needs further investigation.

After-ripening usually refers to storage at approximately 10-30°C of air-dried or low-hydrated seeds. After-ripening in cold stratification or fluctuating-temperature stratification is found to accelerate seed germination of other plants (Kaye and Kuykendall, 2001; Romero *et al.*, 2005). However Yang *et al.* (2009) reported that the fresh seeds of *A. crenata* had the highest final GP, and their GP could reach over 90% at the optimal temperature. These differences between their germination of fresh seeds and ours may be caused by the inter-specific genetic and maternal growing environmental differences. The two shrubs are distributed tropical and subtropical forest, but *A. crenata* more widely grow in the understory of subtropical forest, *A. virens* more widely grow in the understory of tropical forest.

Storage conditions affect seed longevity. In this study, *A. virens* seeds stored in a wide-mouth bottle lost germinability completely after 180 days. Similarly Peng (2006) found the *A. crenata* seeds stored at room temperature under ventilation lost germinability

completely, but those stored with humid sand at 5°C for 80 days reached 97% GP. Yang *et al.* (2009) also found *A. crenata* var. *crenata* seeds stored in an air-ventilated room for 90 days lost germinability completely, but moist storage could lengthen the seed life span. These data indicate that low temperatures and moist conditions are necessary for the seeds of *A. crenata* to survive in storage. For orthodox seeds, dry and cold environment is necessary for storage, while recalcitrant seeds can not be dried before storage, and they should be stored in moisture-proof conditions. However a moderate or low-temperature environment is still necessary to maintain seed longevity because a lower storage temperature can lower the metabolism intensity of seeds (Hong and Ellis, 1996). Thus, in our study, *A. virens* seeds had higher germinability at 15°C than at 25°C and 35°C. Peng *et al.* (2003) and Yang *et al.* (2009) reported that *A. crenata* and its variety *A. crenata* var. *bicolor* seeds had greater viability after wet storage at 4°C and 2-5°C than those in dry storage at the ambient temperatures.

In conclusion, the responses of seed germination to light, temperature and storage indicate that *A. virens* is well adapted to the tropical and subtropical moist environments in South and Southern-western China. When artificially cultivating this plant, the timely seed collection, proper storage and timely sowing are helpful for improving seed germination and seedling emergence. To produce seedlings of this shrub in artificial cultivation, the seeds should be harvested in the winter and spring, and then stored for about two months in a moist and cool environment at 15°C-24°C to ensure the completion of after-ripening. Appropriate chemical pretreatment can increase seed germination.

Acknowledgements

This study was funded by Knowledge Innovation Projection of Chinese Academy of Sciences (118500KP01), Ministry of Science and Technology of China (06GK021001), Strategy Resources of Chinese Academy of Sciences (08ZK121B01) and Natural Science Foundation of Guangdong Province (8151401501000003). We thank Dr. Wang M.Z. for his comments on the manuscript and help with the English.

References

- Benvenuti, S., Macchia, M. and Miele, S. (2001). Light, temperature and burial depth effects on *Rumex obtusifolius* seed germination and emergence. *Weed Research*, **41**, 177-186.
- Berjak, P. and Pammerer, N.W. (2008). From *Avicennia* to *Zizania*: Seed recalcitrance in perspective. *Annals of Botany*, **101**, 213-228.
- Bewley, J.D. and Black, M. (1994). Physiology of development and germination. Plenum Press, New York. 445 pp.
- Bove, J., Lucas, P., Godin, B., Ogé, L., Jullien, M., Grappin, P. (2005). Gene expression analysis by cDNA-AFLP highlights a set of new signaling networks and translational control during seed dormancy breaking in *Nicotiana plumbaginifolia*. *Plant Molecular Biology*, **57**, 593-612.
- Choudhary, D.K., Kaul, B.L., Khan, S. (1996). Breaking seed dormancy of *Podophyllum hexandrum* Royle ex Camb. (syn. *P. emodi* Wall. ex Honigberger). *Journal of Non-Timber Forest Product*, **3**, 10-12.
- Dixit, A.K., Chen J.J., Ishikawa, T., Tsai, I.L. and Chen, T.S. (2005). Alkyl phenols from the leaves of Formosan *Ardisia virens*. *The Chinese Pharmaceutical Journal*, **57**, 81-88.

- Ellis, R.H., Hong, T.D. and Roberts, E.H. (1990). An intermediate category of seed storage behaviour? I. Coffee. *Journal of Experimental Botany*, **41**, 1167-1174.
- Farnsworth, E. (2000). The ecology and physiology of viviparous and recalcitrant seeds. *Annual Review of Ecology and Systematics*, **31**, 107-138.
- Fenner, M. and Thompson, K. (2005). The ecology of seeds. Cambridge University Press.
- Finch-Savage, W.E., Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytol.*, **171**, 501-23.
- Hong, T.D. and Ellis, R.H. (1996). A protocol to determine seed storage behaviour. IPGRI Technical Bulletin No.1, Pp. 1-51 in Engels, J.M. and Toll, J. (Eds.) International Plant Genetics Resource Institute, Rome, Italy. 51pp.
- ISTA (International Seed Testing Association) (1985). International rules for seed testing. *Seed Science and Technology*, **13**, 299-355.
- Kaye, T.N. and Kuykendall, K. (2001). Effects of scarification and cold stratification on seed germination of *Lupinus sulphureus* ssp. *kincaidii*. *Seed Science and Technology*, **29**, 663-668.
- Leite, I.T.A. and Takaki, M. (2001). Phytochrome and temperature control of seed germination in *Muntingia calabura* L. (Elaeocarpaceae). *Brazilian Archives of Biology and Technology*, **44**, 297-302.
- Li, Y.H., Pei, S.J. and Xu, Z.F. (1996). List of Higher Plants in Xishuangbanna (2nd edition). Yunnan Nationalities Press, Kunming. 509 pp.
- Manjkhola, S., Dhar, U. and Rawal, R. (2003). Treatments to improve seed germination in *Arnebia benthami*: An endangered medicinal herb of high altitude Himalaya. *Seed Science and Technology*, **31**, 571-577.
- Marques, M.C.M. and Oliveira, P.E.A.M. (2008). Seasonal rhythms of seed rain and seedling emergence in two tropical rain forests in southern Brazil. *Plant Biology*, **10**, 596-603.
- Milberg, P., Andersson, L. and Thompson, K. (2000). Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research*, **10**, 99-104.
- Nadeem, M., Palni, L.M.S., Purohit, A.N., Pandey, H. and Nandi, S.K. (2000). Propagation and conservation of *Podophyllum hexandrum* Royle: An important medicinal herb. *Biological Conservation*, **92**, 121-129.
- Pandey, H., Nandi, S.K., Nadeem, M. and Palni, L.M.S. (2000). Chemical stimulation of seed germination in *Aconitum heterophyllum* Wall. and *A. balfourii* Stapf.: important Himalayan species of medicinal value. *Seed Science and Technology*, **28**, 39-48.
- Peng, G.T., Huang, S.Z. and Fu, J.R. (2003). The preliminary studies of storage and germination characters of seeds of *Ardisia* spp. (Myrsinaceae). *Acta Scientiarum Naturalium Universitatis Sunyatseni*, **42**, 79-83.
- Pérez-Fernández, M.A. and Rodríguez-Echeverría, S. (2003). Effect of smoke, charred wood, and nitrogenous compounds on seed germination of ten species from woodland in central-western Spain. *Journal of Chemical Ecology*, **29**, 237-251.
- Pradhan, B.K. and Badola, H.K. (2010). Chemical stimulation of seed germination in ex situ produced seeds in *Swertia chirayita*, a critically endangered medicinal herb. *Research Journal of Seed Science*, **3**, 139-149.
- Romero, F.R., Delate, K. and Hannapel, D.J. (2005). The effect of seed source, light during germination, and cold-moist stratification on seed germination in three species of *Echinacea* for organic production. *HortScience*, **40**, 1751-1754.
- Rouhi, H.R., Afshari, R.T., Shakarami, K. (2010). Seed treatments to overcome dormancy of waterlily tulip (*Tulipa kaufmanniana* Regel.). *Australian Journal of Crop Science*, **4**, 718-721.
- Schutz, W., Milberg, P. and Lamont, B.B. (2002). Seed dormancy, after-ripening and light requirements of four annual Asteraceae in south-western Australia. *Annals of Botany*, **90**, 707-14.
- Sershen, Berjak, P. and Pammenter, N.W. (2008). Desiccation sensitivity of excised embryonic axes of selected amaryllid species. *Seed Science Research*, **18**, 1-11.
- Tang, D.S., Hamayun, M., Khan, A.L., Shinwari, Z.K., Kim, Y.H., Kang, S.M., Lee, J.H., Na, C.I., Nawaz, Y., Kang, K.K. and Lee, I.J. (2010). Germination of some important weeds influenced by red light and nitrogenous compounds. *Pakistan Journal of Botany*, **42**, 3739-3745.
- Tweddle, J.C., Dickie, J.B., Baskin, C.C. and Baskin, J.M. (2003). Ecological aspects of seed desiccation sensitivity. *Journal of Ecology*, **91**, 294-304.
- Yang, Q.H., Ye, W.H., Wang, Z.M. and Yin, X.J. (2009). Seed germination physiology of *Ardisia crenata* var. *bicolor*. *Seed Science and Technology*, **37**, 291-302.
- Zhao, A.L., Chen, X.Y., Zhang, X. and Zhang, D. (2006). Effects of fragmentation of ever-green broad-leaved forests on genetic diversity of *Ardisia crenata* var. *bicolor* (Myrsinaceae). *Biodiversity and Conservation*, **15**, 1339-1351.