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# Germination, Desiccation and Storage of *Ardisia virens* var. *annamensis* Seeds

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## Abstract

*Ardisia virens* var. *annamensis* is a famous ornamental species. Due to deforestation and destructive overexploitation, its natural population size has been greatly reduced in recent years. To provide information for its basic genetic conservation and expanding its population by artificial breeding, the germination, desiccation-tolerance and storage lifespan of the seed were studied. The field investigation found in the natural environment the plant had low seed yield. The indoor seed storage and germination experiment showed that the germination temperature ranged from 4°C to 30°C, and the seeds could germinate in full light and in the dark. At temperatures above 20°C, light had no significant effect on germination, but at temperatures between 10-15°C, light significantly promoted germination. The mature seeds had relatively moisture content and weak desiccation-tolerance, so their storage behaviour should be classified as recalcitrant. The seeds remained viable longer in moist storage than in dry storage. These responses of seed germination to light, temperature and desiccation indicate that this shrub have relatively strong adaptability to the natural tropical and subtropical moist environments in South China, the change environment caused by forest fragmentation may be one of important reasons for decreasing of the population.

## 1. Introduction

The pan-tropical genus *Ardisia* (Myrsinaceae) contains approximately 400–500 species of evergreen shrubs distribute in the subtropical and tropical regions around the world [1]. Some *Ardisia* species have been used for medicinal purposes, because they contain a wide array of biologically active phytochemical constituents such as bergenin and ardisin [2]. The *Ardisia* genus has a stunning appearance, hardy body and clustered berries. It can flourish both indoors as well as outdoors under suitable conditions, but it susceptible to low temperatures and in this regard mulching around the plants helps considerably in its growth. The species is also not drought tolerant.

In China, *Ardisia* plants are used not only for landscaping, but also for traditional medicine. But due to overexploitation of wild *Ardisia* species, it has become increasingly important to conserve the germplasm of this valuable genus. The present article reviews the usage and biological activities of *Ardisia* compounds, as well as recent progress regarding the use of this genus in clinical researches. The information presented here also illustrates the potential of the genus as a source of therapeutic agents [3].

*Ardisia virens* Kurz var. *annamensis* Pitard is an evergreen shrub with attractive bright berries and color petals which are white or light yellow at first but later turn red during the flowering period. It is usually distributed in the sparse woodlands on the top of mountains at an elevation of 900 m in Hainan, Yunnan, Guizhou, Guangxi and Taiwan Province of China and Vietnam. This species is usually regarded as a variant of *Ardisia virens*, because it was significantly different from *Ardisia virens* in that its leaves are blade lanceolate, 19-26 cm in length and 2.5-5 cm in width and have dense margin glandular dots in sepals and sparse smooth dots in fruit. Its inflorescences are terminal compound umbels, glabrous, on specialized lateral branches and flowers are papery, white or pink, 7-8 mm, and fruits are red or blackish red, globose, densely black punctuate [1].

Some *Ardisia* plants thrive in productive, well drained soils with partial to heavy shade in the understory of mesic forests and the growth rates of these shrubs have been positively correlated with soil phosphorus levels [4-5]. These plants are easily propagated by seed or cutting, but only established by seed in the wild. They reach the reproductive maturity around 20cm in height when lateral branches start developing, and vivaciously resprouts after cutting or fire [6].

In germplasm conservation, seeds are critical as they are the major unit of plant reproduction and can be cost-effective for storage, reintroduction and wild population restoration in comparison to preserving genetic material as *ex situ* living collections. Cryopreservation is an efficient and feasible means of long-term storing orthodox seeds. For successful cryopreservation, seeds have to be desiccated to an optimum level and the protocol for cryopreservation needs to be established. The drying rate can significantly impact the success of cryotolerance and this can be species-specific. Flash-drying or rapid drying is known to improve desiccation tolerance of seeds that are desiccation-sensitive compared with slow drying. Wesley-Smith *et al.* (2014) suggested that rapidly dried cells retain a greater ability for cell repair upon rehydration [7]. However, Finch-Savage *et al.* (2006) showed that rapid desiccation could negatively affect germination [8]. More studies are needed to investigate the relationship between desiccation rate and subsequent storage life. Further, little is known about desiccation and storage longevity of seeds of the *Ardisia* genus. The aim for this study was to establish storage protocols for storing the seeds of these species.

## 2. Materials and Methods

Most of mature fruits of the shrub were collected manually from more than 30 individual shrubs between June and December 2011 from semi-natural forests, and a small portion of those collected from Xishuangbanna Tropical Botanical Garden in Mengla County, Xishuangbanna Dai Nationality Autonomous Prefecture (21°09'-22°36'N and 99°58'-101°50' E) in Yunnan Province where the average rainfall is 1200-1600 mm; temperature, 18-22°C; and relative humidity,

77-87%; annual sunshine hours, 1700-2300 hours. The seeds were immediately isolated from the fruits, and air-dried at room temperature (18-23°C) for 3 days, then stored in air-permeable plastic bags at 15°C storage room for 1 month. The seeds were randomly divided into 8 sub-lots and used in the various experiments described below.

### 2.1. Observation and Determination of Fruit and Seed Characteristics

Fruit characteristics (shape, size, color) were recorded and moisture content (MC) determined gravimetrically after oven drying at  $103 \pm 2^\circ\text{C}$  for 17h. Similarly, seed characteristics (size, color) were recorded and MC determined after fruit pulp was removed and seeds were manually cleaned (Table 1).

**Table 1.** The traits of mature fruit and seed of *Ardisia virens* var. *annamensis*.

Pericarp color	Red
Fruit Length (mm)	10.45±0.48
Fruit Diameter (mm)	9.86±0.43
Seed Length (mm)	6.98±0.33
Seed Diameter (mm)	6.97±0.27
1000-fruit fresh weight	474.64±8.28
1000-seed fresh weight	195.11±2.34
moisture content (fresh)	50.58±7.59
Flowering period	May - July
Fruit ripe period	October - December or next January

Data in the table are mean  $\pm$  S. D.

### 2.2. Seed Size, Weight and Moisture Content

Quadruplicates of 1000 seeds were weighed and 60 seeds were measured immediately after collection with electronic balances and calipers to ascertain the average seed weight and size. Seed MC (moisture content) tests were conducted according to the Rules of ISTA (1993) [9].

### 2.3. Desiccation Procedure

Initial MC was determined immediately after extraction from fresh fruits. The seeds were desiccated using three methods and each treatment had five durations (12h, 24h, 48h, 72h, and 96h). Firstly, seeds were desiccated by placing them in activated silica gel at  $25 \pm 2^\circ\text{C}$ , and 4% relative humidity in closed jars with the silica to seed ratio of 18:1 by volume (rapid desiccation). The silica was reactivated every 24 h. In the slow desiccation method, seeds were suspended above saturated NaCl solution in closed jars in  $25 \pm 2^\circ\text{C}$  and 75% RH (8d, 16d, 45d, 64d). Lastly, the seeds were air-dried under room conditions at  $27 \pm 2^\circ\text{C}$  and RH approximately 60%. For each desiccation method and duration, a subset of the seeds was used to determine the MC as described above. Eighty seeds for each desiccation method and duration were then germinated in four replicate Petri dishes (each containing 20 seeds) to determine desiccation tolerance. Seeds were germinated in incubators (RXZ-300B, Ningbo Southeastern Equipment Co. Ltd., Ningbo, China) at  $25^\circ\text{C}$  with 24 h

photoperiod (light level of 2800 lx) in 0.8-1.0% agar medium. Germination was monitored for 40 d.

## 2.4. Seed Germination Tests

Only full seeds were selected and used for germination tests. Fresh seeds were used to evaluate the effects of temperature, light, MC and storage on seed germination. Quadruplicate samples of 50 seeds were disinfected for 10 min with 0.2% sodium hypochlorite, and then washed with distilled water and finally assigned in 90mm-diameter Petri-dishes with 0.8-1% agar and incubated as above. Germination and emergence was defined as the appearance of a radicle over 0.5 cm in length. The illumination was provided by cool white fluorescent lamps when in photoperiod for each germination treatment. But seed germination was observed every day in the weak indoor natural light, the GP and GI were calculated and recorded and germination experiment lasted 21 d.

**Germination at various temperatures** Seeds were germinated in incubators under two different light conditions (12 h light of 800-1000 lx and 12 h full dark, 24 h in full dark) at 5, 10, 15, 20, 25, 30, 35°C and AT (ambient Temperature). Testa-stripped seeds were germinated at 25°C to evaluate the effect of testa on germination.

**Germination at various light conditions** Seeds were germinated in incubators of 10, 15, 20, 25, 30, 35, 40°C and AT.

## 2.5. Germination and Emergence Percentage, Germination and Emergence Index

GP (%) = number of germinated seeds / number of total sampled seeds × 100.

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

EP (%) = number of emerged seedlings / number of total sampled seeds × 100.

EI (emergence index) =  $\sum (G_t/D_t)$ ,  $G_t$  means numbers of emerged seedlings after t d.

## 2.6. Seed Storage Tests

Seeds were stored by two methods viz. moist storage and dry storage. Fresh seeds in moist storage were firstly mixed in moist sand and then placed into wide-mouth bottles in an incubator of 0-4°C, but those in dry storage were placed onto a plastic screen in an air-ventilated room. After being stored for 90 days, the seeds were taken out to germinate at 25°C with 12 h daily illumination of 800-1000 lx. The freshly harvested seeds were regarded as control.

## 2.7. Determination of Leakage Rate of Electrolyte

Triplicates of 20 seeds per treatment were soaked in 50 ml double-distilled water for 24 h at ambient temperature, boiled at 100°C for 30 min and then cooled to ambient temperature. The leachate conductivity was determined by an electrical conductivity determinator (DDS-11A; Shanghai No. 2 Analytical Instrument Co., China) at 30±2°C. Relative electric conductivity = electric conductivity before boiling / electric conductivity after boiling.

## 2.8. Statistical Analysis

Based on successful germination the interaction between the desiccation method and drying were tested. This was modeled using a generalized linear model with a binomial distribution function using R project [version 2.15.1] and Epicalc package. A separate analysis to examine the effect of the desiccation method and desiccation duration on germination by measuring success in germination on seeds stored for different duration at different temperatures was also conducted. Multiple comparisons were used to test within treatment differences after adjusting for  $\alpha = 0.05$  using Tukey test in the multcomp package of R.

Results of germination experiments are expressed in percentage or index (±SD). GP were transformed into angular values and subjected to ANOVA in Microsoft (R) Excel version 2000 (Microsoft, Inc., 1985–1999) to determine if significant differences were present among means and LSD test was used to determine if significant differences occurred between individual treatments.

## 3. Results

### 3.1. The Characteristics of Fruit and Seed

The characteristics of mature fruits and seeds were described as follows (Table 1). *Ardisia virens* var. *annamensis* flowered from May to July, and the fruits ripened from October to next January, but the flowering and fruit maturity period were slightly different with the different habitats. The vast majority of fruits fell from the mother shrubs until March in next year in South-west China. Field observations showed significant differences in fruit production between different mother shrubs and the different habitats. In organic-rich lowland rain forest valleys, fruit production per mature individual was usually 200 or more, while at higher elevations it was only 50-100 or less, but under the cultivation conditions in gardens, it was 300-400 or more. Our field survey revealed that the fruit production increases with soil fertility and water availability. All the fresh fruits were berry-like drupes. The morphological characteristics of fruits and seeds for this plant are shown in Table 1. After the pericarp broke down, the seeds dehiscence. Most of seeds were flat, oblong or ovate, and the testa of mature seeds was greyish-white with a white stripe.

### 3.2. Germination at Various Temperatures

The seeds had rod-shaped haustorial cotyledons (0.5-1 mm wide, 2.2-3 mm long) that remained inside the endosperm and showed hypogeal germination. The seeds germinated most rapidly with fluctuating temperatures of 10-25°C, and reached GP (germination percentage) 100% after a week, but at 4°C, 10°C, 25°C, 30°C and 15-30°C, they reached the highest germination percentage after 2-3 weeks. No seed germinate at temperatures above 35°C. The optimal temperature range for germination is therefore 10-25°C. At optimal temperature, the seeds mostly germinated in the first week during germination experiment, i.e., the seeds required less days to germinate at

10~25°C than at other temperatures (Table 2).

**Table 2.** Effect of temperature on germination of *Ardisia virens* var. *annamensis*.

Duration	4°C	10°C	25°C	30°C	35°C	40°C	AT	10~25°C	15~30°C	25~40°C
1W	68.75±6.29c	90.00±7.07b	92.00±5.10b	82.50±8.66b	0d	0d	80.00±12.25b	100a	53.75±16.52c	0d
2W	90.00±8.16b	98.75±2.50ab	95.50±2.50ab	85.00±10.80b	0d	0d	96.25±4.79ab	100a	77.50±15.55c	0d
3W	92.50±6.45b	98.75±2.50ab	95.50±2.50ab	86.25±12.50ab	0d	0d	96.25±4.79ab	100a	92.50±2.89b	0d
4W	92.50±6.45ab	98.75±2.50ab	95.50±2.50ab	88.75±10.31ab	0d	0d	97.50±5.00ab	100a	95.00±4.08ab	0d
5W	95.00±4.08ab	98.75±2.50ab	95.50±2.50ab	88.75±10.31ab	0d	0d	97.50±5.00ab	100a	95.00±4.08ab	0d
6W	96.25±4.79ab	98.75±2.50ab	95.50±2.50ab	88.75±10.31ab	0d	0d	97.50±5.00ab	100a	95.00±4.08ab	0d

GP: Germination percentage; GI: germination index; AT: ambient temperature (15-24°C/24-35°C, night/day). The values marked with the same letter (a-c) in the same line are not significantly different at 5% level.

### 3.3. Germination Percentage and Index After Desiccation and Storage

The MC of freshly harvested mature seeds of *Ardisia virens* var. *annamensis* was about 50% and GP was approximately 100%. After desiccation for 12 and 24 h with the rapid method, seed MC was reduced to about 42% and 39%, the GP was 100% and 96.25% respectively, and was almost equivalent to that of the fresh seeds. But the GP was declined to below 80% when seed MC was reduced to 28.35%, and was 0% when MC was further reduced to below 9%. The GP were all 0% when seed was stored at -20°C for 1, 2 and 4 months. The seeds stored at the higher temperatures (10°C and 15°C) with the higher MCs had higher GP than the control. For the desiccated seeds with

the similar MC, those desiccated by rapid desiccation method had higher GP than those dried by the slow method (Table 3). When at the moisture of about 41%, the seeds dried by the rapid method had a higher germination percentage than those dried by the slow method. The seeds with 50% MC stored at 4-15°C had a relatively long storage life compared with those with MC below 42% and stored at 10°C and 15°C. For the seeds with the similar MC, rapid desiccation is more effective to extend the storage life than slow desiccation. The seeds lose their germinability when the MC was reduced to below 10%, and slight desiccation would significantly shorten the storage life when seed MC was reduced to below 30%, and most of them lose germinability after 3-month storage (Table 3).

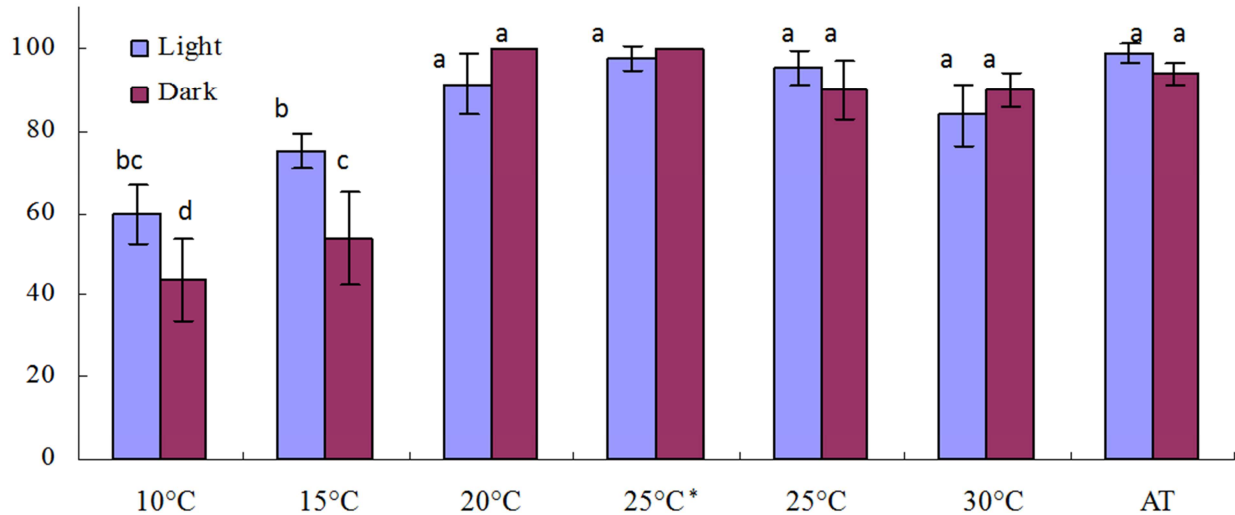
**Table 3.** Germination of *Ardisia virens* var. *annamensis* seeds after storage at different temperatures.

Dry method	MC (%)	Storing temperature	Initial GP	1 month	2 months	4 months
Fresh seeds	50.58 ±0.76	4°C	100.00±0.00a	98.75±2.50a	100.00a	95.00±4.08a
		10°C		97.50±2.89a	100.00a	92.50±5.00a
		15°C		96.25±4.79a	98.75±2.50a	91.25±6.29a
		-20°C		0.00g	0.00g	0.00g
Rapid desiccation	41.96 ±1.15	4°C	100.00±0.00a	99.00±2.00a	15.00±3.83f	0.00g
		10°C		97.00±3.83a	96.00±4.62a	34.00±10.58d
		15°C		96.00±3.27a	98.00±2.31a	49.00±8.25cd
		-20°C		0.00g	0.00g	0.00g
	38.97±2.21	4°C	96.25±22.50a	75.00±16.33bc	40.00±7.07c	8.75±4.79f
		10°C		57.50±6.45cd	48.75±24.62cd	0.00g
		15°C		88.00±6.53b	27.50±10.41ef	21.25±13.15ef
		-20°C		0.00g	0.00g	0.00g
Slow desiccation	28.35 ±4.23	4°C	77.50±14.43b	0.00g	0.00g	0.00g
		10°C		0.00g	0.00g	0.00g
		15°C		0.00g	0.00g	0.00g
		-20°C		0.00g	0.00g	0.00g
	8.95 ±0.70	4°C	0.00	0.00g	0.00g	0.00g
		10°C		0.00g	0.00g	0.00g
		15°C		0.00g	0.00g	0.00g
		-20°C		0.00g	0.00g	0.00g
	41.69 ±0.83	4°C	100.00±0.00a	99.00±2.00a	80.00±13.86bc	0.00g
		10°C		100.00a	60.00±8.00cd	1.00±2.00g
		15°C		84.00±14.24b	22.50±5.25f	1.00g
		-20°C		32.50±14.43def	10.50±2.50f	0.00g
	34.73±1.47	10°C	97.50±2.89a	36.00±14.24e	0.00g	0.00g
		15°C				

Note: MC means moisture content (%); GP means germination percentage.

### 3.4. Effect of Light on Seed Germination

There was no significant difference in germination percentage when germination took place at 20°C and above, while at 10°C and 15°C, the germination percentage in the light was significantly higher than that in dark. The GP of testa-stripped seeds was almost the same as that of intact seeds, so it may be concluded that the testa had no significant effect on GP.



Note: \* the testa-stripped seeds were only used here, and the other seeds were all intact.

Figure 1. Germination of *Ardisia virens* var. *annamensis* in the light and dark.

### 3.5. Electric Conductivity of Seeds After Desiccation

The EC (electric conductivity) of seed leachate rose with increasing desiccation duration whether by the rapid or the slow method. However, the rate of desiccation did have a significant effect on the EC of the seed leachate (Table 4). At similar low moisture contents, the EC of the seed leachate obtained through slow desiccation was higher than that through obtained by rapid desiccation. This may be due to the longer time taken to dry the seed was associated with seed ageing. In this study, the appropriate osmotic regulation treatment was not carried out prior to the EC determination experiment, which suggesting rapid desiccation maybe brought less damage to seed microstructure than slow desiccation.

Table 4. Electric conductivity of seed leachate after desiccation of *Ardisia virens* var. *annamensis*.

Seedlot	Moisture content	Electric conductivity
CK	50.58±0.76a	14.65±2.43e
FD12h	46.15±0.80b	21.11±3.56d
FD1d	43.56±1.29bc	40.75±6.28c
FD2d	38.52±0.80c	66.31±5.84ab
FD3d	39.70±3.30c	78.85±3.35a
SD8d	35.86±1.75c	46.16±3.66c
SD16d	25.18±0.96d	60.89±5.13b
SD45d	9.57±0.03e	73.42±7.37a
SD64d	8.11±0.12f	76.94±4.95a

Table 5. Length and dry weight of seedlings of *Ardisia virens* var. *annamensis*.

	15d		30d	
	Length	Dry weight	Length	Dry weight
RD2T 4°C	4.87±0.72c	0.48±0.29b	6.02±0.61b	0.52±0.09b
RD2T 10°C	4.36±0.99c	0.22±0.03c	6.31±1.49b	0.46±0.14b
RD2T 15°C	2.99±0.69d	0.17±0.05c	6.36±0.52b	0.46±0.10b
OH4°C	7.70±0.29a	0.90±0.06a	8.38±0.25a	0.86±0.18a
OH10°C	7.06±0.99a	0.83±0.15a	8.48±1.66a	1.01±0.28a
OH15°C	7.04±0.78a	0.66±0.14b	8.73±1.10a	1.01±0.11a

Note: RD means rapid desiccation; OH means the non-desiccated seeds.

### 3.6. Length and Dry Weight of Seedlings

There were significant differences in length and dry weight of the seedlings obtained from fresh seeds and desiccated seeds. The length and dry weight of desiccated seeds were significantly lower than those of fresh seeds. The desiccation brought some damage on seedling growth. There was no significant difference of length and dry weight at 30 d in the seedlot at between different temperatures. But at 15 d, for the desiccated seeds by the rapid desiccation, the length and dry weight at 15°C was significantly less than those at 4°C and 10°C (Table 5).

## 4. Discussion

The field survey showed that in the natural or semi-natural forest of Southern Yunnan, China, most *Ardisia* species, including *A. virens* var. *annamensis* populations usually grow in

shaded areas of dense evergreen broad-leaved forests, hillsides, dark damp places and valleys. A significant observation was that few seedlings were found in the natural habitats. Compared with records of previous surveys [10], due to the deforestation of a large number of natural or semi-natural forest and utilization as economic forests through planting rubber, banana, litchi, etc, the habitat area suitable for the growth of this shrub has been shrinking. In addition, the populations have been rapidly shrinking probably because of excessive digging the maternal individuals used as traditional Chinese medicine. Although the adult maternal individuals can produce relatively copious quantities of viable seeds under artificial cultivation conditions, they do not produce many seeds in the natural environments.

#### 4.1. Effects of Temperature and Light on Seed Germination

Many tropical plants need high temperatures to promote germination [11]. In this study, most seeds of *A. virens* var. *annamensis* could germinate over a temperature range from 4°C to 30°C, and they showed a higher germination percentage between 10°C and 25°C. However, it has been found that other *Ardisia* species, including *A. crenata* and *A. japonica*, germinated well at a temperature range from 20°C to 30°C [12-13]. *A. virens* var. *annamensis* seed germination differs from other typical tropical plants [11], in that the majority of seed germinated after 3-4 weeks even at 4°C and 15°C, but at 30°C or 40°C, no seed could germinate. In other *Ardisia* species, the germination was always better at 30°C or 35°C than at the temperatures below 20°C [12-13]. This suggests that the seed germination of *A. virens* var. *annamensis* is more adapted to shade and warm habitats than other *Ardisia* species.

The effect of light on seed germination was relative to temperature. In lower temperature (10-15°C), light could significantly promote seed germination, but in higher temperature above 20°C, light had hardly any significant effect. In low temperature, the seeds buried in soil had low germination percentage, it is helpful for most of these seeds germinate in higher temperature and develop into seedlings. But in this study, so the germination was not in full darkness, because in the observation intervals, the seeds were exposed to a very short duration of weak indoor illumination which may be essential for germination for seeds of some plants. This may partially explain why most of the shrubs can grow in shady understory areas of dense evergreen broad-leaved forests. It has been found that seed germination become less dependent on light with increasing seed mass [14]. The 1000-seed weight of *A. virens* var. *annamensis* was over 300 grams and much larger than that of some typical small seed plant, such as Compositae, Gramineae, whose seed germination require light. But light did not significantly improve the seed germination of *Ardisia crenata* and *A. virens* at 10-35°C [12-13].

#### 4.2. Effects of Desiccation and Storage on Seed Germination

The germination of desiccated seeds responded differently depending on the desiccation method [15-16]. The seed

germination of *A. virens* var. *annamensis* declined when seed MC was dropped below 40% whether dried by the rapid dehydration or the slow dehydration method. When stored at 15°C and -20°C the seeds lost germinability more rapidly than at 4°C and 10°C. When seed MC was decreased to below 30%, all of the seeds lost their germinability. This indicates that the seeds of *A. virens* var. *annamensis* have a weak desiccation-tolerance. Therefore, the seeds should be classified as recalcitrant [16], which may be one of the reasons that this shrub prefers moist natural habitats. Some researches have shown that seeds of other *Ardisia* species, e.g., *Ardisia crenata*, *Ardisia japonica*, have similar seed germination characteristics [12, 17-18], but the seeds of *Ardisia crenata* and its variety *Ardisia crenata* var. *bicolor* are more tolerant of desiccation than those of *A. virens* var. *annamensis*. The seeds of *Ardisia crenata* collected in late September or later months showed higher germination percentage, and germinated more rapidly after longer periods of storage at low temperature (approximately 5°C) [12]. This may partially explain why *A. virens* var. *annamensis* distributes in tropical and south subtropical zones, but *A. crenata* and *A. crenata* var. *bicolor* have wider distributions, not only in these two zones, but also in central subtropical zones in China. Other researchers have investigated the feasibility and methods of preservation for long-term seed storage in other plants by experimentally testing whether rapid desiccation can increase desiccation tolerance and storage longevity [15, 17-19]. They also obtained some contradictory conclusions: For example Yao et al. found that for *Ardisia elliptica*, *A. brunnescens* and *A. virens* desiccation method and duration significantly affected cryotolerance, but rapid desiccation did not improve germination compared to slow desiccation and desiccation duration significantly affected germination percentage in the three species, especially after 48 h [19]. But in this study, rapidly desiccated seeds had somewhat more storage longevity than the slow desiccated seeds.

Desiccation of recalcitrant seeds is associated with damage and causes their germination percentage to decrease [12, 16] and this is associated with an increase electrical conductivity of the leachate from the seed (Table 4). The longer was the duration of dehydration, the more serious was the damage. Slower drying means that more time is spent in the stage of dehydration where metabolic damage can occur [20]. Most of the freshly harvested *A. crenata* and *Ardisia crenata* var. *bicolor* seeds exhibited a low germination percentage, but could germinate after being placed for 30 d at 15°C, so the plant seeds had after-ripening period [12]. Whether *A. virens* var. *annamensis* seeds had the similar phenomenon needs further investigation, because in the present study, the seeds were stored in air-permeable plastic bags at 15°C storage room for 1 month after germination experiment, and the germination tests of freshly harvested seeds were not carried out. After-ripening in cold stratification or fluctuating-temperature stratification is found to accelerate seed germination of other plants [21]. But Yang et al. reported that the fresh seeds of *A. crenata* had the highest final germination [12]. The differences between theirs and ours in germination of fresh seeds may be caused by harvesting time

and short-term storing methods before germination test.

Storage conditions affect seed longevity. Peng (2006) found the *A. crenata* seeds stored at room temperature under ventilation lost germinability, but those stored within humid sand at 5°C for 80 days reached 97% GP [22]. Yang et al. (2009) also found *A. crenata* var. *crenata* seeds stored in an air-ventilated room for 90 days fully lost germinability, but moist storage lengthened the seed life span. Collectively these data indicate that low temperatures and moist conditions are necessary for the seeds of *A. crenata* to survive in storage [12]. For orthodox seeds, dry and cold environment is necessary for seed storage, while recalcitrant seeds can not be dried before storage, and they should be stored in moist conditions, but moderate or low-temperatures are necessary to maintain seed longevity because a lower storage temperature can lower the metabolism intensity of seeds [16].

The fruits and seeds of *A. virens* var. *annamensis* remain on the maternal plants until late next spring, and the seeds remain alive in the late next spring and early summer. Therefore, we believe that the maternal individuals must be able to provide enough water to the undetached fruits to prevent dehydration damage to the seeds before the fruits drop. When the fully matured fruits drop from maternal shrubs in late spring and early summer, the warm and wet environment is suitable for seed germination and seedling establishment. Therefore, it seems that the fruit and seed maturation seasonality, and the characteristics of fruit retention on maternal plants in late spring are good adaptation mechanisms to avoid dehydration in the dry season for the seed survival of the species. To produce seedlings of this shrub in artificial cultivation, the seeds should be harvested in the winter and spring first, and then stored for about a month in a moist and cool environment to ensure the completion of probable after-ripening, then finally sowed in moist and aerated soil.

Seeds of *Ardisia* are considered recalcitrant and recalcitrant seeds are most commonly found in moist regions with an invariant climate [18]. In the native regions of *A. virens* var. *annamensis* in Yunnan and Southeast Asia, the wild populations have decreased because of forest fragmentation leading to the warming and drying of local climate [23] which may inhibit seed germination and seedling establishment of this shrub. For example, Xishuangbanna is the most abundant region for *A. virens* var. *annamensis*. In the recent two to three decades, the destruction of the tropical forests (cutting down the original natural forest and semi-natural forests and replanting rubber trees) in Xishuangbanna has increased fragmentation, and the local forest environment has become warmer and dryer [23], one of the evidences is that recent studies have shown the local annual rainfall and foggy days have been decreasing (Fan & Thomas, 2013).

## 5. Conclusion

The responses of seed germination to light, temperature and storage indicate that *A. virens* var. *annamensis* is well adapted to the tropical and subtropical moist environments in South and Southern-western China. From the results of field survey

and characteristics of seed germination and storage, it is not hard to see that the climate change caused by human activities in this region may affect not only the fruit production of maternal trees, but also the seed germination and seedling establishment of this species. When artificially cultivating this plant, the timely seed collection, proper storage and timely sowing are helpful for improving seed germination and seedling emergence.

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