

## Article

# Propagation of Native Tree Species to Restore Subtropical Evergreen Broad-Leaved Forests in SW China

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**Abstract:** Subtropical evergreen broad-leaved forest (EBLF) is a widespread vegetation type throughout East Asia that has suffered extensive deforestation and fragmentation. Selection and successful propagation of native tree species are important for improving ecological restoration of these forests. We carried out a series of experiments to study the propagation requirements of indigenous subtropical tree species in Southwest China. Seeds of 21 tree species collected from the natural forest were materials for the experiment. This paper examines the seed germination and seedling growth performance of these species in a nursery environment. Germination percentages ranged from 41% to 96% and were  $\geq 50\%$  for 19 species. The median length of germination time (MLG) ranged from 24 days for *Padus wilsonii* to 144 days for *Ilex polyneura*. Fifteen species can reach the transplant size ( $\geq 15$  cm in height) within 12 months of seed collection. Nursery-grown seedlings for each species were planted in degraded site. Two years after planting, the seedling survival rate was  $>50\%$  in 18 species and  $>80\%$  in 12 species. Based on these results, 17 species were recommended as appropriate species for nursery production in forest restoration projects. Our study contributes additional knowledge regarding the propagation techniques for various native subtropical tree species in nurseries for forest restoration.

**Keywords:** propagation; germination; seedling; nursery; subtropical

## 1. Introduction

Deforestation and forest degradation have led to widespread biodiversity loss and environmental destruction in China [1]. Over the past two decades, China has put considerable effort into forest conservation and restoration, such as the implementation of large-scale state reforestation programs

(e.g., the Natural Forest Conservation Program and the Grain for Green Program) [2]. Nowadays, through nationwide afforestation programs, China has one of the highest percentages of plantation forests anywhere in the world [3]. However, most forest cover increases are a result of the increase in monoculture plantations, such as fruit trees, pine, poplar, rubber, and *Eucalyptus* spp., but not through the recovery of natural forests [4,5]. These new plantation-style forests may only provide short-term economic benefits, with limited ecosystem service and biodiversity conservation benefits in the long term [1,3]. Only a small range of tree species have been used for afforestation, despite the highly variable environmental conditions and diverse forest types in China—likely due to knowledge and database gaps for other species [2]. Therefore, the study of more appropriate native tree species for region-specific and biodiversity-based restoration is urgently needed to move beyond the “one-size-fits-all” approach that China has used thus far [3,6].

Globally, mixtures of native tree species are increasingly used to restore disturbed and degraded areas (e.g., McNamara *et al.* 2006 [7]; Shono *et al.* 2007 [8]; Raman *et al.* 2009 [9]). However, one important factor limiting native species selection and diversification is the availability of quality planting materials [10,11]. Restoration with native species requires the identification of tree species with readily available seed and mature propagation technology that are suitable to the local context [11–14]. Several methods have been developed for improving planting stock. Blakesley *et al.* [15] developed the framework species method, which uses a restricted set of “nurse” species, to reduce demands on the propagation of native species for forest restoration. These nurse species can quickly establish a protective tree canopy, which can shade the weeds and accelerate the natural establishment of native species [16,17]. However, ease of propagation in nurseries is still an important consideration for the framework species [18]. In contrast, the maximum diversity method uses a large number of species, but as a consequence this approach is highly dependent on the capacity of local nurseries to propagate a large number of species [19]. As for large-scale government restoration projects, a lack of high-quality tree germplasm from local nurseries is a major constraint to scale up the use of native species [20,21]. Thus, nursery research that both expands our basic knowledge of native species propagation and selects species suitable for afforestation is essential [22].

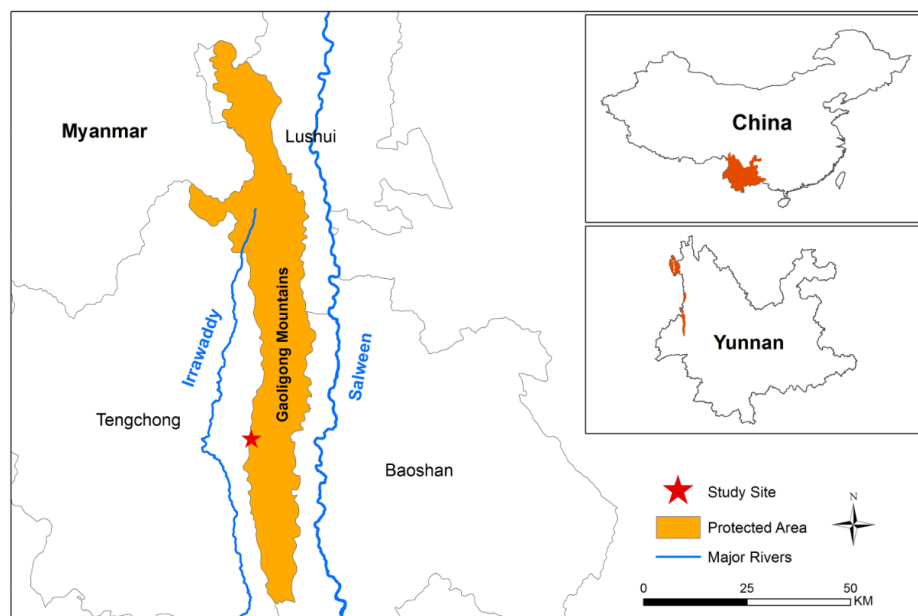
The East Asia subtropical evergreen broad-leaved forests (EBLF) are a key source of biodiversity and provide a wide variety of environmental services, but have been extensively deforested and fragmented [23,24]. In subtropical areas of China, natural EBLF are commonly replaced by coppice woods and fast-growing tree plantations such as *Pinus*, *Cunninghamia*, and *Eucalyptus*, while the remnant natural forests are only found in some nature reserves or isolated areas [25]. Nonetheless, large-scale government restoration programs aimed at protecting soils on slopes and reducing flooding risk (e.g., Grain for Green Program) have created an increasing demand for native planting materials [2,21]. Consequently, there is a need to develop techniques and methods for using EBLF species for forest restoration. Many methods for species selection are available that include spatial distribution of species to ground survey and nursery techniques [21,26,27]. However, existing research on seed germination and seedling growth is limited, and most studies only address certain economic species or some endangered species [28–31]. Integrated knowledge of the seed collection, storage, germination requirements and seedling growth performance of native tree species is vital for region-specific biodiversity conservation and restoration plans [32,33]. Given the costs of nursery operations, species with high germination and fast seedling growth rate are more popular among nursery managers, for which good quality seedlings can be produced within a reasonable timeframe [18,34]. Therefore, quantitative information on species performance through nursery research is necessary for species selection and nursery management.

In this study, we carried out a series of experiments to study the seed germination, seedling growth performance, and seedling early survival of 21 selected indigenous subtropical species. We aim to improve the basic knowledge of propagation techniques for various native subtropical tree species in nurseries and identify suitable species for nursery production in restoration projects.

## 2. Materials and Methods

### 2.1. Study Site

This research was conducted in an experimental nursery site at 1550 meter above sea level, adjacent to the southern part of the Gaoligong Mountains National Nature Reserve ( $24^{\circ}56'$  to  $28^{\circ}22'$  N,  $98^{\circ}08'$  to  $98^{\circ}50'$  E), Yunnan Province in Southwestern China (Figure 1). Under the influence of the Indian Ocean Monsoon, the wet season in this region occurs from May to October, whereas the dry season runs from November until the following April [35]. The average annual rainfall is approximately 1977 mm, nearly 80% of which normally falls during the wet season. The monthly mean maximum and minimum temperatures are  $19.9^{\circ}\text{C}$  and  $7.3^{\circ}\text{C}$ , respectively [36]. The Gaoligong mountain range lies at the intersection of the Eastern Himalayas and the Hengduan Mountains of China, which is recognized as a global biodiversity hotspot [37]. We selected this nature reserve for three main reasons: First, it represents and harbors a typical subtropical evergreen broad-leaved forest community; second, a wide range of native species seeds could be sourced; third, the seedlings produced could be used for restoring degraded land in the reserve's buffer zone.



**Figure 1.** Geographical location of the study site.

### 2.2. Species Selection

A list of 21 native tree species was drawn up from 13 different families and 18 genera based on the regional species pool and the availability of seeds at the time of the study (Table 1). Important families included the Lauraceae, Rosaceae, Magnoliaceae and Betulaceae. The nomenclature follows Wu *et al.* [38]. The classification of successional stages was based on local ecological knowledge and the authors' field experience. For most of these species, seeds are commonly derived from fleshy fruits that are dispersed by wild animals. Three species (*Schima wallichii*, *Betula alnoides* and *Alnus nepalensis*) studied are wind dispersed.

**Table 1.** The characteristics of 21 tree species native to the subtropical evergreen broad-leaved forests in SW China.

Species	Family	Ecology <sup>†</sup>	Uses <sup>†</sup>	SCM <sup>†</sup>
<i>Lindera communis</i> Hemsley	Lauraceae	ESC	TB, OL, FW	Nov
<i>Lindera thomsonii</i> C. K. Allen	Lauraceae	LSC	TB, OL	Oct
<i>Machilus rufipes</i> H. W. Li	Lauraceae	LC	TB	Oct
<i>Machilus yunnanensis</i> Lecomte	Lauraceae	LC	TB	Oct
<i>Cerasus serrulata</i> Loudon	Rosaceae	ESC	FR, OR	May
<i>Cerasus cerasoides</i> S. Y. Sokolov	Rosaceae	ESC	OR	Mar
<i>Sorbus corymbifera</i> N. T. Kh'ep & G. P. Yakovlev	Rosaceae	ESC	FW	Sep
<i>Padus wilsonii</i> C. K. Schneider	Rosaceae	LSC	TB	Oct
<i>Manglietia hookeri</i> Cubitt & W. W. Smith	Magnoliaceae	LC	TB, OR	Oct
<i>Michelia doltsopa</i> Buchanan-Hamilton ex Candolle	Magnoliaceae	LSC	TB, OR	Oct
<i>Quercus acutissima</i> Carruthers	Fagaceae	EC	TB, FW, FD	Oct
<i>Schima wallichii</i> Korthals	Theaceae	LC	TB	Dec
<i>Ilex polyneura</i> S. Y. Hu	Aquifoliaceae	LC	TB	Nov
<i>Myrsine semiserrata</i> Wallich	Myrsinaceae	ESC	FW	Oct
<i>Diospyros kaki</i> var. <i>silvestris</i> Makino	Ebenaceae	ESC	TB, FR	Nov
<i>Tetradium ruticarpum</i> T. G. Hartley	Rutaceae	LSC	MD	Nov
<i>Heynea trijuga</i> Roxburgh	Meliaceae	LSC	MD	Nov
<i>Betula alnoides</i> Buchanan-Hamilton ex D. Don	Betulaceae	EC	TB	Mar
<i>Alnus nepalensis</i> D. Don	Betulaceae	EC	TB, FW	Dec
<i>Choerospondias axillaris</i> B. L. Burtt & A. W. Hill	Anacardiaceae	LC	TB, FR	Dec
<i>Ligustrum lucidum</i> W. T. Aiton	Oleaceae	ESC	OR, MD	Nov

Ecology: ESC—early-successional subcanopy; EC—early-successional canopy; LSC—late-successional subcanopy; LC—late-successional canopy. Uses: TB—timber; OL—oil; OR—ornamental; FW—firewood; FD—fodder; FR—fruit; MD—medicine. SCM—seed collection month. <sup>†</sup> From [36,38].

### 2.3. Seed Collection

All of the seeds were collected in natural forest areas close to the nursery between elevations of 1600–2500 m. This elevation covers the mid-montane moist evergreen broad-leaved forest areas dominated by species of *Lithocarpus* Blume, *Cyclobalanopsis* Oersted, *Machilus* Rumphius ex Nees, *Litsea* Lamarck, *Schima* Reinwardt ex Blume, and *Michelia* Linnaeus [36]. Seeds were collected from three to six parent trees of each species. To ensure tree genetic diversity, the distance between the same tree species was more than 100 m when we selected parent trees. The parent trees were located by GPS and labeled. The optimal seed collection time was determined by phenological monitoring, where the fruit maturation time of each species was recorded. Eight species were collected in October, six in November, and three in December (Table 1). In agreement with the study conducted by Du *et al.* [39], most species dispersed seeds during the late wet and early dry seasons. Mature fruits were collected from branches or on the ground according to the different fruit or seed types. Subsequently, seeds were treated (extraction, cleaning, and grading) and measured. We recorded the seed source and archived seeds for documentation.

### 2.4. Species Germination Performance and Seed Storage Experiments

For all the 21 species, fresh seeds were directly used for germination experiments without storage after seed processing. To understand the effect of different seed storage methods on germination rates, we used wet sand storage and conventional dry storage for 100 days. Due to seed availability and practical constraints, only 12 species were tested in seed storage experiments. For wet sand storage, seeds were placed in permeable plastic crates containing moistened sand and stored in the cool and shady environment (10 °C–15 °C). Seeds were air-dried and stored in ventilated mesh bags in the same environment for conventional dry storage [34,40]. Altogether, four 100-seed replications were used for each species and storage method. Seeds were sown in germination trays using a medium of loess and humus mixture and watered daily. The covering depth depended on the seed size of each species. Germination trays were placed in a controlled nursery environment. Conditions

in the nursery were similar to common nursery practice in this region, *i.e.*, ambient temperature (15 °C–25 °C), approximately 50% full sunlight [40]. Seeds with any cotyledon emergence were considered germinated [41]. The number of germinated seeds were counted each day until no additional seeds germinated. The germination percentage and median length of germination time (MLG) were calculated for each species, as informed by Blakesley *et al.* [18] and Sautu *et al.* [42]. The germination percentage used for each species is the maximum one achieved in the above experiments. As diaspores of *Machilus rufipes*, *Machilus yunnanensis*, *Heynea trijuga* and *Choerospondias axillaris* were multi-seeded, multiple germinations in the four species occurred infrequently. Thus, the count was adjusted to a maximum of one per diaspore [42]. For the 12 species, seed germination rates were calculated for each treatment (dry storage and wet storage) and for the control (fresh seed). A total of 1200 seeds (100 seeds  $\times$  4 replicates  $\times$  3 treatments = 1200) for each of the 12 species were used in the experiment. ANOVA was used to test the effect of seed storage treatments on germination rates. Multiple comparisons of the means were tested using Tukey's HSD (Honestly Significant Difference) method ( $p = 0.05$ ). All the statistical analysis were conducted in R (R version 3.1.2) [43].

### 2.5. Seedling Growth

Seedlings were transplanted into individual plastic bags (15 cm  $\times$  25 cm) when the first pair of leaves had expanded. A medium of loess and humus mixture was used and about ten granules of Entec controlled-release fertilizer (NPK 20-10-10) was applied every two months. Seedlings were watered every three days with an automatic water sprinkler system. Weed and pest control was applied as needed for each seedling. The seedlings were protected with shade cloth (about 50% shade) in the nursery. As recorded by a hygrothermograph, air temperatures during the experiment remained between 15 °C to 25 °C, and relative humidity was maintained between 30% and 50% in the nursery. Common nursery cultural regimes in our study region were used to grow these species. The best cultural regime for specific tree species needed more detailed study. The heights of 20 randomly selected seedlings per species were measured every ten days. Monitoring ceased before seedling transplant. As a limitation of this trial, root collar diameter and physiological indexes were not measured. Seedlings were hardened off in direct sunlight, and watering was reduced prior to field planting in August. Given nursery practice, it is hard to produce a crop of seedlings of 21 species with a plantable size, all to be dispatched at the same time of year when knowledge of most propagation characteristics about these native species are still lacking. Thus, seedling height when planting was used for subsequent species ranking in the present study. The total days in the nursery from sowing seeds to planting out were also calculated.

### 2.6. Seedling Early Survival

We evaluated the early survival of seedlings of selected species based on data from the restoration planting trail. The planting site was an abandoned field dominated by dense grasses before seedling transplant in the reserve's buffer zone. Nursery-raised seedlings were planted out randomly with 1230 seedlings of 21 species at a density of nearly 2500 trees per hectare. The site was prepared by clearing shrubs and herbs within the line planting, digging the planting hole (40  $\times$  40  $\times$  40 cm) on a 2  $\times$  2 m spacing before tree planting. Each seedling was watered at the time of planting and three times during the dry season at two month intervals. After planting, hand weeding was carried out three times (May, July, September) a year. Seedlings were fertilized with 100 g of compound fertilizer around the stem before the rainy season after weeding in May. We monitored the seedling survival after planting out in the field in order to assess the effect of nursery practices. Seedling survival rate was calculated for each species after planting two years.

### 2.7. Species Rating System

To evaluate the potential suitability of selected native species for nursery production, we prepared an adapted tree rating system based on existing data and local conditions (Table 2; Elliott *et al.* [44];

Knowles and Parrotta [19]; Meli *et al.* [11]). Depending on nursery practice, criteria and proposed acceptable standards were defined to enable valid comparisons. Standards of seed germination percentage and early seedling survival rate [18,44]: 80% or higher was considered excellent; 50%–80% acceptable; less than 50% unacceptable. MLG < 30 days was considered excellent, 30–90 days good, and >90 days unacceptable [18]. The acceptable planting size of seedling was  $\geq 15$  cm in height according to the local standard. Each species was given a composite rating based on their combined scores for germination percentage, germination time, seedling growth performance, and early survival rate.

**Table 2.** Components of the species rating system and their assigned scores.

Aspect	Categories	Score
Germination rate	>80%	3
	50%–80%	2
	<50%	1
Germination time (MLG)	<30 days	3
	30–90 days	2
	>90 days	1
Seedling height when planting	>30 cm	3
	15–30 cm	2
	<15 cm	1
Seedling early survival rate	>80%	3
	50%–80%	2
	<50%	1
Species rating	Excellent	10–12
	Good	7–9
	Poor	4–6

### 3. Results

#### 3.1. Species Germination Performance

Germination percentages ranged from 45% to 96% (Table 3), depending on the species. Nineteen species had a germination rate greater than 50% and ten species had a germination rate greater than 80%. Two species had low germination rates: *Ilex polyneura* (45%) and *Alnus nepalensis* (48%), respectively.

The MLG (median length of germination time) ranged from 24 days for *Padus wilsonii* to 144 days for *Ilex polyneura* (Table 3). Germination was defined as rapid if the MLG was less than 30 days (one month), and slow if the MLG was more than 90 days (three months) based on local conditions. In our experiment, only *Padus wilsonii* had rapid germination. In contrast, *Lindera communis*, *Lindera thomsonii*, *Machilus rufipes*, *Myrsine semiserrata*, and *Ilex polyneura* all had slow germination. The remaining 15 species had MLGs of between 30 days and 90 days. Of the 18 species collected in the late wet and early dry seasons, one species germinated rapidly, five species germinated slowly and intermediate germination was observed in the remaining 12 species. All three species (*Cerasus cerasoides*, *Cerasus serrulata*, and *Betula alnoides*) collected in the late dry season had intermediate MLGs.

**Table 3.** Nursery production characteristics for the 21 selected tree species from a subtropical evergreen broad-leaved forest in SW China.

Species	GR (%)	MLG (Days)	TD (Days)	SH (cm)	SS (%)	Rating
<i>Lindera communis</i>	52 (11.7)	102	202	19.9 (3.6)	91.8	G
<i>Lindera thomsonii</i>	78 (7.4)	111	203	20.5 (3.9)	89.9	G
<i>Machilus rufipes</i>	96 (10.6)	94	204	26.4 (4.4)	71.8	G
<i>Machilus yunnanensis</i>	85 (16.0)	84	205	18.7 (3.1)	83.6	E
<i>Cerasus serrulata</i>	95 (3.1)	30	81	28.1 (5.5)	92.3	E
<i>Cerasus cerasoides</i>	85 (10.2)	45	122	58.9 (6.3)	86.8	E
<i>Sorbus corymbifera</i>	96 (5.9)	46	203	40.3 (8.8)	93.8	E



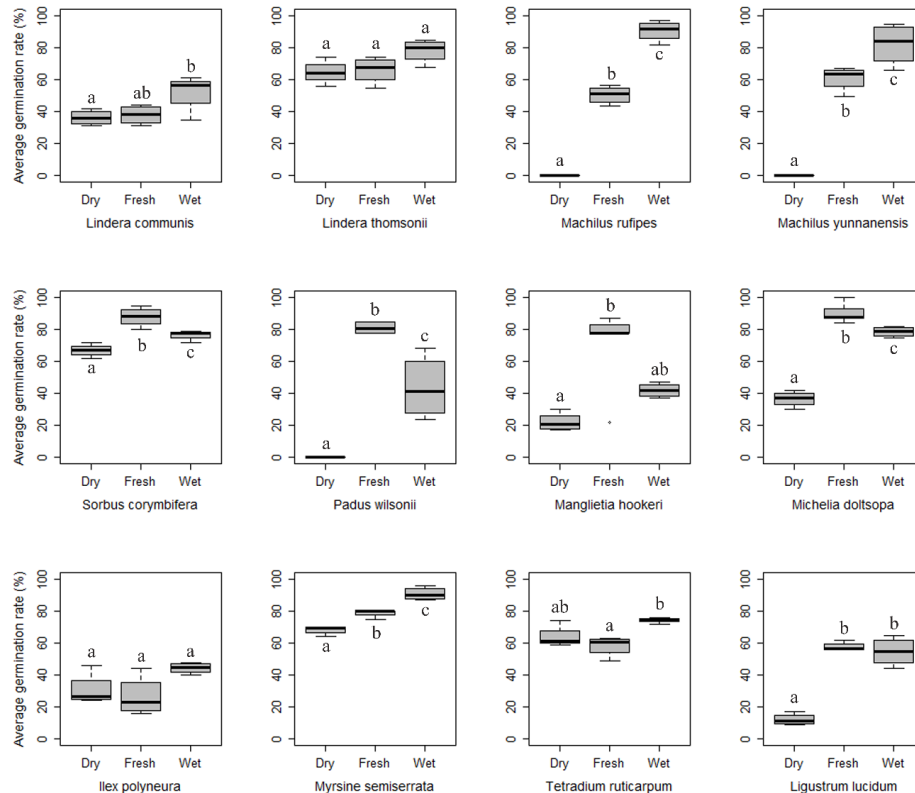
Table 3. Cont.

Species	GR (%)	MLG (Days)	TD (Days)	SH (cm)	SS (%)	Rating
<i>Padus wilsonii</i>	81 (3.5)	24	204	44.7 (11.4)	70.8	E
<i>Manglietia hookeri</i>	70 (26.9)	84	220	9.9 (3.6)	39.2	P
<i>Michelia doltsopa</i>	90 (6.3)	87	218	25.1 (4.3)	90.7	E
<i>Quercus acutissima</i>	88 (5.2)	47	173	46.0 (5.3)	90.2	E
<i>Schima wallichii</i>	69 (4.1)	46	134	9.4 (2.1)	35.8	P
<i>Ilex polyneura</i>	45 (3.4)	144	216	4.4 (1.5)	77.8	P
<i>Myrsine semiserrata</i>	91 (4.1)	118	219	4.9 (1.4)	36.4	P
<i>Diospyros kaki</i> var. <i>silvestris</i>	73 (3.3)	75	206	31.5 (5.0)	96.5	E
<i>Tetradium ruticarpum</i>	75 (1.7)	32	203	66.4 (13.1)	52.5	G
<i>Heynea trijuga</i>	87 (2.6)	83	217	15.5 (3.6)	89.7	E
<i>Betula alnoides</i>	54 (3.3)	41	123	9.1 (3.2)	58.3	G
<i>Alnus nepalensis</i>	48 (21.2)	82	198	14.0 (6.2)	94.3	G
<i>Choerospondias axillaris</i>	68 (17.5)	71	213	81.7 (21.9)	54.9	G
<i>Ligustrum lucidum</i>	61 (4.4)	74	201	50.7 (7.5)	92.7	E

GR—germination rate (Mean  $\pm$  SD); MLG—median length of germination time; TD—total days in nursery; SH—seedling height when planting (Mean  $\pm$  SD); SS—seedling survival after planting two years; E—excellent; G—good; P—poor.

### 3.2. Seed Storage

For all of the 12 tested species, seed germination percentages after wet sand storage were higher than for dry storage and for 8 out of the 12 species the difference was significant ( $p < 0.05$ ) (Figure 2). For *Machilus rufipes*, *M. yunnanensis* and *Padus wilsonii*, no seeds germinated after dry storage. Seed germination percentages of fresh seeds were significantly higher than stored seeds for *Sorbus corymbifera*, *Padus wilsonii* and *Michelia doltsopa*.



**Figure 2.** Average germination rates of seeds under different storage treatments. Dry—dry storage; Fresh—fresh seed; Wet—wet storage. Treatments with the same letter are not significantly different (Tukey HSD test,  $p > 0.05$ ).

### 3.3. Seedling Growth Performance

Seedling growth rates varied considerably among the 21 species (Table 3). Seedlings of *Cerasus cerasoides* and *Cerasus serrulata* grew quickly. *Cerasus cerasoides* reached a mean height of 58.9 cm 61 days after transplanting into containers, and *C. serrulata* reached a mean height of 28.1 cm 40 days after transplanting into containers. *Choerospondias axillaris*, *Tetradium ruticarpum*, *Ligustrum lucidum* and *Quercus acutissima* performed relatively well in the nursery, producing seedlings suitable for planting in less than 132 days. However, *Ilex polyneura*, *Manglietia hookeri*, and *Myrsine semiserrata* grew very slowly. By planting time, they had grown to a mean height of less than 10 cm after growing more than 210 days in the nursery. The other species had moderate growth rates. Seedling height was more than 30 cm for eight species when planting. Seven species had an acceptable seedling height (15–30 cm) while the other six species had an unacceptable seedling height less than 15 cm.

### 3.4. Seedling Early Survival

Of the 21 species, seedling survival rates ranged from 35.8% to 96.5% after two years in the field, with a total survival rate of 75.9% (Table 3). Seedling survival rates in 18 species were >50%, >80% in 12 species. Species from the dominant family Lauraceae (four species) and Rosaceae (four species) had survival rates higher than 70%. The nitrogen-fixing species *Alnus nepalensis* had a survival rate of 94.3%. However, seedlings of *Manglietia hookeri* (39.2%), *Schima wallichii* (35.8%), and *Myrsine semiserrata* (36.4%) had less than 50% survival rates.

### 3.5. Species Performance

Ten species were ranked as excellent species for nursery production and seven as good species. The eight species of Lauraceae and Rosaceae were ideal for nursery production with acceptable germination percentages, nearly synchronous germination, and good seedling performance. The two species from Betulaceae are treated as marginal species in the nursery, although they are traditionally fast-growing timber species. However, *Manglietia hookeri*, *Schima wallichii*, *Ilex polyneura* and *Myrsine semiserrata* had poor nursery performances.

## 4. Discussion

In this study, we carried out a series of experiments to study the seed germination, seedling growth performance and seedling survival of 21 selected indigenous subtropical species from SW China. Based on these data, a tree rating system of native species selection for nursery production for forest restoration purpose was developed. This section presents the implications drawn from our results.

### 4.1. Species Germination Performance

This study demonstrated that most native subtropical tree species can germinate well in nursery conditions. The species with high germination percentages (>80%) such as *Machilus yunnanensis*, *Cerasus cerasoides* and *Quercus acutissima* could be used as candidates for a direct seeding approach, reducing nursery costs [33,45]. As a consequence of their use in timber plantations and agroforestry system, 8 of the 21 species reported here have been studied previously, and our results are similar to those reported in the literature [46–50]. These species include *Lindera communis*, *Machilus yunnanensis*, *Cerasus cerasoides*, *Quercus acutissima*, *Michelia doltsopa*, *Alnus nepalensis*, *Betula alnoides*, and *Choerospondias axillaris*. However, the germination rate of seeds of *A. nepalensis* were higher (85%) than we recorded (48%) [51].

In germination studies, the median length of germination time (MLG) is often used as a measure of germination speed [42,52]. If seeds can germinate rapidly and uniformly, nursery managers can use planting space efficiently and arrange the period of planting and transplanting better [18,34]. In contrast, seeds with a long germination period will increase the technical difficulties and costs in



nurseries. In this study, five species including *Lindera communis*, *Lindera thomsonii*, *Machilus rufipes*, *Myrsine semiserrata*, and *Ilex polyneura* had MLGs of more than 90 days, which may be due to seed dormancy. Lang *et al.* [48] found that the embryo of *Lindera communis* seeds had an after-ripening effect, and cold stratification can effectively break its physiological dormancy. Combinational (physical and physiological) dormancy was also reported for seeds of species from *Ilex* [53]. As compared to tropical rainforests, subtropical evergreen broad-leaved forests have more species with dormant seeds [54]. Unfortunately, the information available on dormancy mechanisms and pre-germination treatment techniques for subtropical seeds is still limited. Further study is necessary to determine dormancy mechanisms and the best pre-sowing treatments, which can improve germination performance of native species.

#### 4.2. Seed Storage Method

Seed source and species fruiting phenology will affect seed availability, especially of various native tree species in ecological restoration [11,55]. Therefore, seed storage is necessary for nursery before sowing, and the proper seed storage conditions for each species need to be identified. Wet sand storage was appropriate for short-term seed storage for most species in this study. In other studies with native species from this region, similar results were reported [46,56]. It appears that seeds of *Machilus rufipes*, *M. yunnanensis*, and *Padus wilsonii* may be drought-sensitive since no seed from these species germinated after dry storage. Seeds of these three species should be stored in humid conditions or planted out immediately based on the results. Wet storage is suitable for species with a high level of seed moisture content, especially some desiccation sensitive recalcitrant seeds [34,52]. Previous studies of *Quercus acutissima* show that wet sand storage can promote germination of its recalcitrant seeds and inhibit the growth of insects (*Curculio* spp.) in the nut [47]. Baskin and Baskin [52] also stated that wet storage can facilitate the development of the embryo for some species with physiological dormancy, which can promote seed germination. Normally, orthodox seed can be stored in a dry and low-temperature environment (in a refrigerator) for a long time [57]. However, such equipment and conditions are not possible for some small-scale nurseries [42]. In nursery practices in China, wet storage is not as commonly used as dry storage. As we demonstrate, it can promote seed germination in many species, and hence wet storage methods should receive more attention.

#### 4.3. Seedling Growth Performance

Seedling growth studied in the nursery demonstrated that 15 species grew quickly, and could be planted out within 12 months of seed collection. However, the growth of some species, such as *Manglietia hookeri*, *Schima wallichii*, *Ilex polyneura* and *Myrsine semiserrata*, were much slower and require two years growth in the nursery before reaching a suitable size for planting out. The optimum seedling size for planting will vary with the species and the site [57], and need to be considered carefully during restoration activity. We accepted a seedling height of 15 cm or taller for restoration use, and the ideal time to transplant nursery seedlings was during the wet season (June to August). It is a huge challenge for nursery managers to have large numbers of seedlings of diverse species planted at a specified future date [58]. Time and economic limitations are key concerns in the restoration projects. Most of the forest restoration methods involve tree planting, and species that require more growth time in the nursery will increase the management investment. At the same time, the growth of seedlings depends not only on the genetic properties of the species but also to a large extent on the substrate quality [57]. The application of fertilizer can accelerate the seedling growth of many native species [59]. Appropriate substrates and fertilizers according to the requirements of different species can enhance the seedling growth. Some research reported that specific mycorrhiza and other symbionts may need to be added to substrate to enhance the growth [60]. Our study has examined seedling height, which are important criteria of measurement of seedling growth. Beside seedling height, seedling root collar diameter and root system, not included in this study, are considered good measurements of seedling growth and quality [34,61], and that is a limitation of this study.

#### 4.4. Seedling Early Survival

For restoration projects, the potential of field survival is an important consideration for selecting species and seedlings [44]. Therefore, establishing connections between nursery production and the anticipated field site is an important step in producing desirable seedlings. However, the feedback from field trials is often overlooked in nursery research [57]. The results of this study indicated that seedlings of most selected native species had acceptable survival rates in the planting site, except *Manglietia hookeri*, *Schima wallichii*, and *Myrsine semiserrata*. For the three species with low survival, the seedling height when planting was less than 10 cm. However, seedlings of *Ilex polyneura*, *Betula alnoides*, and *Alnus nepalensis* showed acceptable survival with low planting size. In general, high-quality seedling can adapt to different environmental conditions and establish well when planted out in the field [34,57]. However, we have to point out that other factors such as species characteristics, site conditions, tree planting and tending techniques can also influence the survival of planted seedlings in restoration project [9]. Despite this, our study can provide the survival information of seedlings for selected species under local conditions and encourage local farmers to use more native tree species in restoration projects.

#### 4.5. Species Selection Criteria and Species Selected

From the perspective of ecological restoration, the candidate species for nursery propagation should have several of the following characteristics: high seed germination rates, rapid germination, high seedling growth rates and high survival. A combination of these criteria is necessary to give a more comprehensive evaluation of candidate species. Rapid germination and high seedling growth rate are also important to shorten the time of propagation and reduce the management investment [55]. However, the scores assigned to the rating of species have some level of uncertainty [62]. For example, some late-successional species with slower germination or a slower seedling growth rate might be tolerated to accelerate forest succession processes and improve forest quality [63,64]. The perspectives of different stakeholders should also be considered in recommending species for nursery production [65]. Therefore, the rating system and acceptable performance standards proposed in this paper can be applied flexibly elsewhere according to nursery management objectives and local conditions.

Based on seed germination traits and the seedling growth performance, 17 of the 21 selected species can be regarded as appropriate for restoration projects in this study. This result indicates that native tree species have a high potential for nursery production. The best practices for the remaining four species (*Manglietia hookeri*, *Schima wallichii*, *Ilex polyneura* and *Myrsine semiserrata*) will require more research. Continued research is necessary to identify additional suitable species from local species pool for nursery production. Further research should also pay attention to the maintenance of tree genetic diversity and adaptability in the context of changing environmental conditions [58].

### 5. Concluding Remarks

The supply of high-quality planting stock for diverse native tree species is vital to the success of forest ecological restoration [66,67]. We proposed a procedure to identify a preliminary list of suitable native tree species for nursery production in restoration programs. A series of criteria related to nursery propagation were defined, which can be applied flexibly elsewhere. This paper focuses on the technical aspect of propagation of native tree species. Furthermore, social and economic criteria should be included in nursery research. While most studies of native species propagation for forest restoration have been concentrated in the highly diverse tropical area [11,18,19,42], few studies have been done in the subtropical area. Our study will expand the database for nursery propagation of native tree species in the subtropics. Nevertheless, further studies are required to identify the propagation characteristics of other subtropical species for restoration purposes in these very diverse forests.

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