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Possible causes for the differentiation of *Pinus yunnanensis* and *P. Kesiya* var. Langbianensis in Yunnan, China: Evidence from seed germination

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ABSTRACT

Both *Pinus yunnanensis* and *P. kesiya* var. langbianensis are the main components of forest vegetation in central Yunnan and southern Yunnan, respectively. Previous studies have found that these two species may be differentiated from a common ancestor due to differences in precipitation in their respective habitats. To confirm the possible reasons for the differentiation of the two species in southwest China, we studied the seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis under different temperatures, water potentials and storage conditions. The temperature range of 15℃ to 30℃ was appropriate for seed germination and normal seedling emergence of the two pines. Under the water potential of 0 MPa to −0.6 MPa, germination percentages of *P. yunnanensis* and *P. kesiya* var. langbianensis were 63.2% to 80.8% and 71.2% to 91.6%, respectively. When the water potential decreased to −0.8 MPa to −1 MPa, the germination percentages of the two species decreased below 30%, but the germination percentage of *P. yunnanensis* was higher than that of *P. kesiya* var. langbianensis. After stored for 1, 3, 5 and 7 months at −20, 4, 15 and 25℃, the seed germination percentages of the two species still maintained above 60%. When the seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis were buried at Xishuangbanna in southern Yunnan, China, they lost vigor rapidly due to the highest precipitation. Especially, after buried for 2 months, the germination percentage of *P. yunnanensis* was 0. These results suggested that moisture is a key factor affecting seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis, and the seeds of *P. yunnanensis* preferred lower moisture levels for germination than *P. kesiya* var. langbianensis. Thus, the difference in the response of seed germination and storage of the two species to moisture levels may be an important factor causing the difference in their geographical distribution patterns.

1. Introduction

Pinus Linn. is the largest genus of Pinaceae with about 110 extant species occurring throughout the Northern Hemisphere (Critchfield and Little, 1966; Fu et al., 1999; Xing et al. 2010). The natural distributions of this genus range from the Arctic and subarctic regions of North America and Eurasia to the subtropical and tropical regions of Central America and Asia, except *P. merkusii* Jungh. & de Vriese (Critchfield and Little, 1966; Mirov, 1967; Price et al., 1998; Xing et al., 2010). *Pinus* is often a dominant component of forest vegetation in large portions of the Northern Hemisphere (Richardson and Rundel, 1998; Xing et al 2010). The members of *Pinus* are mostly evergreen trees, and only a few are shrubs. Most of them have high ecological, economic and medicinal value, and play an important role in afforestation, building and medicine.

Geological processes and climate changes of the past play an important role on the geographical distribution of plant species. The uplift of the Qinghai-Tibetan Plateau has greatly influenced the Asian pattern of atmospheric circulation which intensified the Asian monsoon climate (Zhou et al., 2017). Typically, a monsoon climate has an alternation of dry and wet seasons caused by seasonal inversion of atmospheric circulation (Fu and Zeng,1997; Frédéric et al., 2011; Su et al. 2013a, 2013b; Zhou et al., 2017). The uplift of the Himalayas and the tectonics of the Indochina block result in the evolution of Yunnan flora

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(Zhu, 2018). Due to the intensification of monsoon climate, the low precipitation in winter may prevent the survival of *Metasequoia*, leading to the eventual disappearance inYunnan (Wang et al., 2019; Fan et al., 2020). Yunnan Province, southwestern China, harbors more than half of the plant species in China, and is also the most abundant biodiversity area of the same latitude regions in the World (Zhu, 2018). Yunnan with typical monsoon climate receives more than 80% of annual precipitation in summer and autumn (late May to early October), and less than 15% in winter and spring (November to early May) (Wang, 1990; 2006; Zhou et al., 2017). Molecular phylogeny found that *P. yunnanensis* and *P. kesiya* are the closest relatives, and possibly were derived from a common ancestor (Yu et al., 2000; Eckert and Hall, 2006; Gernandt et al., 2005; Leslie et al., 2012). A new species belonging to subsection *Pinus* of subgenus *Pinus*, *P. prekesiya* sp. nov., was found in the fossil from the upper Miocene of central Yunnan, southwestern China (Xing et al., 2010). By comparing the morphological characteristics (such as cone size, shape, apophyses and umbos) with *P. yunnanensis* and *P. kesiya*, this new species shows a combination of characters of *P. yunnanensis* and *P. kesiya*, but has a closer affinity with *P. kesiya* which distributes in the humid region of Yunnan (Xing et al., 2010). Therefore, it suggests that a more humid climate in central Yunnan during the late Miocene than today (Xing et al., 2010). It speculated that with the intensification of the monsoon climate and a decrease in precipitation, the common ancestor distributed in central Yunnan differentiated into two species, *P. yunnanensis* and *P. kesiya* (Xing et al., 2010). The *P. yunnanensis* adapted to drought climate and stayed in central Yunnan; while the *P. kesiya* adapted to humid climate and migrated to southern Yunnan (Xing et al., 2010).

Seed germination is influenced by temperature, moisture, storage conditions etc. For example, the mismatch between ripening time of the seeds in *Quercus sichourensis* and rainy season leads to the obstruction of seed germination, therefore population reduction and even endangerment (Xia et al., 2012). The response of the seed germination of *P. yunnanensis* and *P. kesiya* to the environments remains unclear. So the speculation lacks evidence of the ecological adaptation of the two species. Thus, we meant to investigate the responses of their seed germination to different conditions, such as temperature and moisture etc. To better understand the seed germination response to the environment, we investigated the seed germination percentages of *P. yunnanensis* and *P. kesiya* var. langbianensis under different temperature, water potential and storage conditions. And we hypothesized that *P. yunnanensis* would outperform *P. kesiya* var. langbianensis at lower moisture levels because the annual precipitation in the natural distribution area of *P. yunnanensis* is lower than that of *P. kesiya* var. langbianensis.

Pinus yunnanensis and *P. kesiya* var. langbianensis are the main components of forest vegetation of the central and south Yunnan, respectively (Wu and Zhu, 1987). *P. yunnanensis* has the characteristics of drought and barren tolerance, wide ecological adaptability and strong natural regeneration ability (Fan et al., 2018). The Yunnan Plateau is the origin and distribution center of *P. yunnanensis*, which is also distributed in Guizhou, Guangxi, Sichuan, Tibet and other regions of China (Cai et al., 2013; Dai et al., 2006). *P. yunnanensis* is mainly distributed at the altitude of 1600 m to 2900 m, and concentrated at the altitude of 2000 m to 2500 m (Chen et al., 2012). *P. kesiya* var. *langbianensis*, a geographical variety of *P. kesiya*. In China, *P. kesiya* var. *Langbianensis* is only found in Yunnan province, and naturally distributed in the southern Yunnan (Liu et al., 2010). And it is concentrated in the mountains with an altitude of about 1000–1700 m.

2. Materials and methods

2.1. Seed collection

The seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis used in

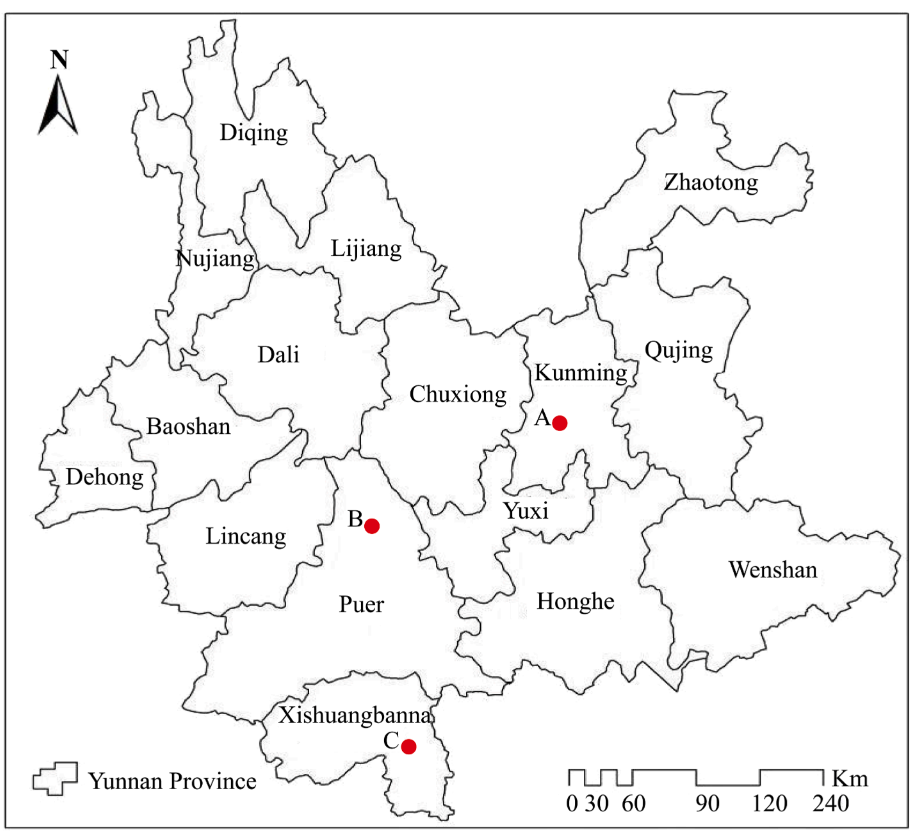

Fig. 1. It is the map of Yunnan pronince, China. The seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis were collected from Kunming and Puer, respectively. Sites A (Kunming), B (Zhenyuan) and C (Mengla) were the seed burial sites.

our study were collected from Kunming and Puer, Yunnan province, respectively, in 2017 (Fig. 1). The seeds were dry-stored at 4℃ prior to testing. The experiments were carried out immediately when the materials were ready.

2.2. Climate of seeds burial sites

Seed burial sites were established at Kunming, Zhenyuan county of Puer and Mengla county of Xishuangbanna. The three sites showed in Fig. 1 are A, B and C, respectively. *P. yunnanensis* and *P. kesiya* var. l*angbianensis* distribute naturally in Kunming and Puer, respectively. Thus, site A and B designed meant to study the seed vigor in their parent environment. There is no distribution of the two species in Xishuangbanna, therefore, site C was used as a control (Fig. 1). The data for temperature and precipitation in Kunming, Zhenyuan and Mengla were obtained from the National Meteorological Information Center (http://data.cma.cn) (Fig. 2). The data for Kunming and Mengla are from January 1981 to December 2010, and those for Zhenyuan are from January 1981 to August 1999 and January 2000 to December 2010. As the seed burial sites, we compared the temperature and precipitation of the three places.

Mean annual temperature and precipitation at Kunming are 15.5 ℃ and 81.6 mm, respectively, 19.3 ℃ and 104.5 mm at Zhenyuan, and 21.8 ℃ and 126.1 mm at Xishuangbanna (Fig. 2). The maximum annual temperature of Kunming, Zhenyuan and Xishuangbanna occurr in June, and are 20.3, 23.8 and 25.5 ℃, respectively. The maximum monthly precipitation of the three places are 203.9 (August), 272.2 (July) and 319.5 mm (July), respectively (Fig. 2).

2.3. Temperature requirement for seed germination

To determine the temperature requirements for seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis, the seed germination was tested at 5, 10, 15, 20, 25, 30 and 35℃. Five replicates of 50 seeds per treatment were placed at each temperature. The seeds were sown on the surface of 0.8% agar medium in 90-mm-diameter Petri dishes. Germination was monitored at 2-day interval for 2 months. The criterion for germination was radicle emergence (2 mm), and the seedlings were considered to be fully emerged when they have grown enough for the seed coat to fall off (hereafter normal seedlings). The seeds were viable (Before the formal experiment, we tested the seed germination at 20℃ using 0.8% agar medium in 90-mm-diameter Petri dishes with five replicates of 50 seeds per replicate. And the germination percentages were close to 100%.). The experiment was carried out in the incubators (PQX-430B) with 12 h of light each day and 60% relative humidity. Germination percentage was calculated as number of germinated seed divided by number of sown seed timed 100%. Normal seedling percentage was calculated as number of normal seedling divided by number of sown seed timed 100%.

2.4. Water potential requirement for seed germination

To study the effects of water stress on seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis, the seed germination was tested in light (with 12 h of light each day) at 20℃ (Seeds of the two species have the highest germination and seedling percentages at 20℃.) at the following six water potentials: 0, −0.2, −0.4, −0.6, −0.8 and −1 MPa (In the pretreatment, we determined the six water potential.), using distilled water. Five replicates of 50 seeds were used for each treatment (5 replicates × 50 seeds × 6 treatments × 2 species). Seeds were sown in 90-mm-diameter Petri dishes on the surface of filter paper soaked with the appropriate polyethylene glycol 6000 (PEG-6000) solution. PEG solution always used to simulate drought stress in laboratory experiment. Solutions of PEG-6000 were prepared to obtain the desired levels of water stress using the method of Michel and Kaufmann (1973). Dishes were monitored for germination and presence of normal seedlings at 2-day intervals for 2 months. The Petri dishes were sealed in plastic self-sealing bags and the PEG solution and filter papers were changed every 4 days to make sure the PEG concentration did not increase during the experiment.

2.5. Seed germination and storage conditions

Among the environmental factors, storage conditions (temperature, humidity, etc.) and time are the important external factors affecting seed germination, and both storage conditions and time can affect seed dormancy, germination and vigor (Shen et al., 2010). In autumn and winter, the seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis mature and disperse, then germinate and produce seedlings in the following spring, during which time the seeds are exposed to the natural environment. In the meantime, the seed viability would be affected by environmental factors. To study the time variation of seed viability and the response of seed viability to environmental factors during this time, in this section, two methods were used for seed storage. One is to store seeds indoors and the other is to bury seeds in soil in the wild (Shen et al., 2010; Lu et al., 2017; Chen et al., 2017). The treatments of the two methods were designed according to the climate of the natural distribution area of the two species and previous studies (Shen et al., 2010; Lu et al., 2017; Chen et al., 2017). The storage time of seeds is 0.5, 1, 2, 3, 5 and 7 months in the experiment of seeds buried in soil in the wild. Probably because the soil moisture was relatively high. The seeds buried in soil could germinate naturally when they were stored over 2 months, which the germination percentages were about 70%. So the date of seeds buried in soil in this paper is for 0.5, 1 and 2 months.

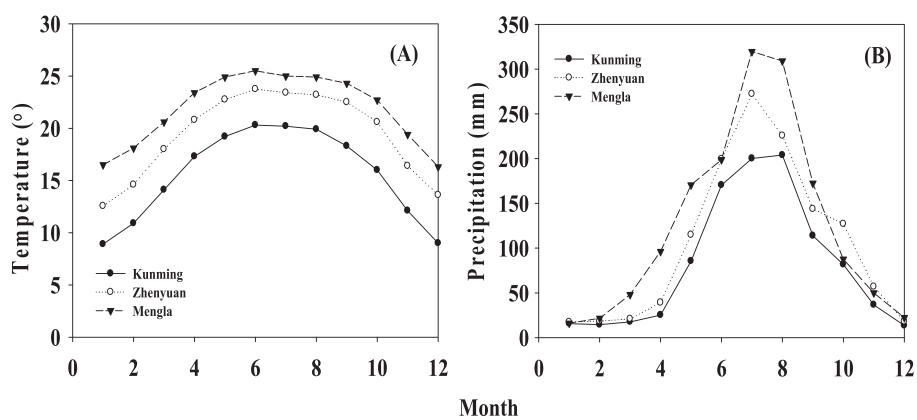

Fig. 2. Mean monthly temperature (A) and precipitation (B) at Kunming, Zhenyuan and Mengla, Yunnan Province. The data for Kunming and Mengla are from January 1981 to December 2010, and those for Zhenyuan are from January 1981 to August 1999 and January 2000 to December 2010.

2.5.1. Germination of seeds stored indoors

To determine the effects of storage temperature and storage time on seed germination of the two species, seeds were sealed in tin foil bags. Then, the seeds were stored at 15, 10, 4 and −20℃. Seeds were tested for germination after they were stored for 1, 3, 5 and 7 months. For the germination tests, five replicates of 50 seeds from each storage condition were sown on the surface of 0.8% agar medium in 90-mm-diameter Petri dishes and incubated at 20℃ in light (with 12 h of light each day). Dishes were monitored for germination at 2-day intervals for 2 months.

2.5.2. Germination of seeds buried in soil

To determine the effect of natural field storage conditions on seed germination of the two species, the seeds were buried in soil. According to the modern distribution of *P. yunnanensis* and *P. kesiya* var. langbianensis, we selected three sites (Kunming, Zhenyuan and Mengla) in Yunnan to store the seeds. Storage time was 0.5, 1 and 2 months. Seeds were sealed in plastic mesh bags, then were buried in soil at a depth of 5–6 cm. The plastic mesh bags were placed horizontally in the soil to ensure that the seeds contacted the soil evenly. Seeds were tested for germination after they reached the storage time. For the germination tests, four replicates of 50 seeds from each storage condition were sown on the surface of 0.8% agar medium in 90-mm-diameter Petri dishes and incubated in light (with 12 h of light each day) at 20℃. Dishes were monitored for germination at 2-day intervals for 2 months.

2.6. Statistical methods

We used SPSS 16.0 to analyze the effects of impact factors on seed germination. And the differences were considered significant when the P value was less than 0.05. Figures were done using SigmaPlot 10.0.

3. Results

3.1. Temperature requirement for seed germination

Temperature had a significant effect on seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis ($P < 0.05$). With the increase of temperature, the percentages of germination and seedling of the two species increased first and then decreased (Fig. 3). At 20℃, the percentages of seed germination and seedling in *P. yunnanensis* and *P. kesiya* var. langbianensis were the highest among treatments, though not statistically different from germination percentages at 25℃. The germination percentage of *P. yunnanensis* was high (67.2–87.6%) in the temperature range from 15℃ to 35℃, but the germination percentage declined dramatically at 10℃, only 13.6% (Fig. 3A). The seed germination percentage (84–93.2%) of *P. kesiya* var. langbianensis was not significantly different in the temperature range from 15℃ to 30℃, but significantly decreased at 10℃ and 35℃ (≤50%). The seeds of both species did not germinate at 5℃ (Fig. 3B). In Fig. 3A, it showed that the seed germination percentages of *P. yunnanensis* were lower than that of *P. kesiya* var. langbianensis at 15℃ to 30℃, however, it's the opposite at 10℃ and 35℃. The seedling establishment of *P. kesiya* var. langbianensis was not as well as that of *P. yunnanensis* (Fig. 3B). From 15℃ to 30℃, the seedling percentage of *P. yunnanensis* was 60.4% to 76%, while the seedling percentage of *P. kesiya* var. langbianensis was 56.4% to 64.8% (Fig. 3B). There was no seedling of the two species at 5℃, 10℃ and 35℃. With the increase of temperature, the initial germination time of seed decreased in the temperature range from 10℃ to 25℃, but did not change from 25℃ to 35℃ (Table 1). This indicated that the seeds of both *P. yunnanensis* and *P. kesiya* var. langbianensis could germinate and form seedling well at 15℃ to 30℃.

3.2. Water potential requirement for seed germination

The effect of water potential on seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis was significant ($P < 0.05$). With the increase of water potential, the seed germination percentages of both species increased first and then decreased (Fig. 4). At −0.2 MPa, there were the highest germination percentages of *P. yunnanensis* (80.8%) and *P. kesiya* var. langbianensis (91.2%), while the lowest germination percentages occurred at −1 MPa. The seed germination percentages of the two species were higher than 60% at water potential of 0 MPa to −0.6 MPa, but was lower than 30% at −0.8 MPa. Although the germination percentages of *P. kesiya* var. langbianensis at water potential of 0 MPa to −0.6 MPa were higher than that of *P. yunnanensis*, the germination percentages of *P. yunnanensis* from −0.8 MPa to −1 MPa were higher than that of *P. kesiya*. In the water potential range from 0 MPa to −0.4 MPa, the initial germination time of the two species was almost the same (Table 2). However, from −0.6 MPa to −1 MPa, the initial germination time of *P. yunnanensis* was longer than that of *P. kesiya* var. langbianensis (Table 2). This indicated that seed germination of *P. yunnanensis* can tolerate more drought stress than that of *P. kesiya* var.

Table 1
The initial germination time (days) of *P. yunnanensis and P. kesiya* var. *langbianensis* seeds under different temperatures.

Species	Temperature						
	5℃	10℃	15℃	20℃	25℃	30℃	35℃
P. yunnanensis	–	38	12	5	3	3	3
P. kesiya var. langbianensis	–	38	12	5	3	3	3

Note. "-" means there was no seed germinated at 5℃ during the experiment.

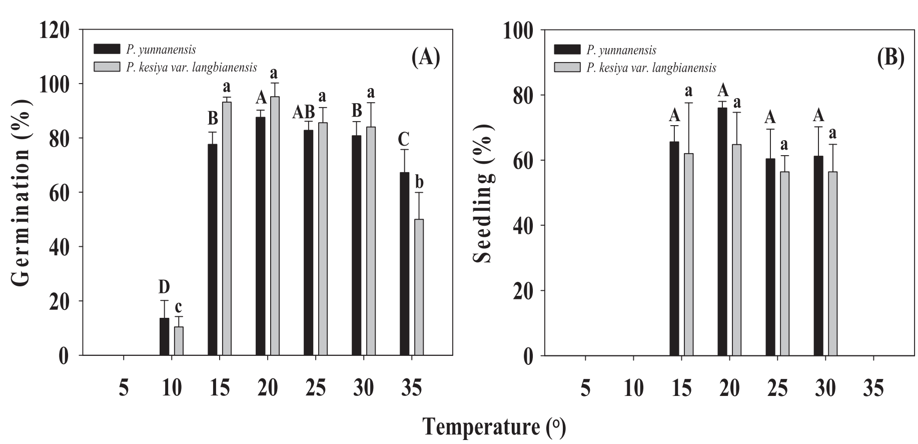

Fig. 3. Percentages of seed germination and normal seedling emergence for *P. yunnanensis* (A) and *P. kesiya* var. langbianensis (B) at different temperatures. Letters represent the results of ANOVA (P-Value = 0.05), and the same letter indicates no significant difference.

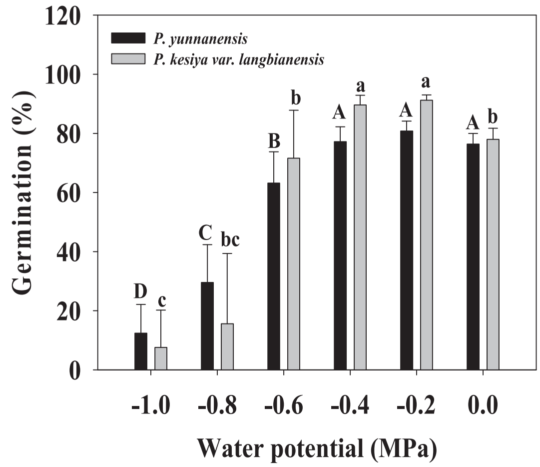

Fig. 4. Percentage of seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis at different water potentials. Letters represent the results of ANOVA (P-Value = 0.05), and the same letter indicates no significant difference.

Table 2
The initial germination time (days) of seed germination under different concentration of PEG6000 solutions.

Species	PEG-6000 solution					
	0 MPa	−0.2 MPa	−0.4 MPa	−0.6 MPa	−0.8 MPa	−1MPa
P. yunnanensis	3	5	5	8	15	19
P. kesiya var. *Langbianensis*	4	5	5	6	10	16

langbianensis.

3.3. Germination of seeds stored indoors

The effects of storage temperature and storage time on seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis were significant ($P < 0.05$). After stored at −20, 4, 15 and 25℃ for 1, 3, 5 and 7 months, the seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis still maintained a high germination percentage (64.4–91.2%) (Fig. 5). The germination percentages of *P. yunnanensis* decreased with the increase of storage temperature for a given storage time (Fig. 5A). In most cases, the higher the storage temperature, the lower germination percentages of *P. kesiya* var. langbianensis were for a given storage time (Fig. 5B). The tested storage time and storage temperature did not dramaticlly decline the seed viability of the two species.

3.4. Germination of seeds buried in soil

With the extension of the storage time, the germination percentages of *P. yunnanensis* and *P. kesiya* var. langbianensis decreased (Fig. 6). The seed germination percentages of the two species stored in the soils at Kunming and Zhenyuan did not decrease sharply (67.5–88.5%), but decreased significantly at Xishuangbanna (0–88.5%). When the storage time increased from 0.5 month to 1 month, the germination percentages of *P. yunnanensis* and *P. kesiya* var. langbianensis seeds stored at Kunming, Zhenyuan and Xishuangbanna were 78.5% to 87% and 80% to 88.5%, respectively. When the storage time was 2 months, although the germination percentages of *P. yunnanensis* seeds stored at Kunming and Zhenyuan were 82% and 67%, respectively, the seed germination percentage of *P. yunnanensis* stored at Xishuangbanna was 0 (Fig. 6A). However, when the storage time was 2 months, the germination percentages of *P. kesiya* var. langbianensis seeds stored at Kunming and Zhenyuan were 84.5% and 73.5%, respectively, and was only 13.3% at Xishuangbanna (Fig. 6B). This suggested that high soil moisture was not conducive to the preservation of seed viability, and the climate of Xishuangbanna was not suitable for maintaining the seed viability of *P. yunnanensis*.

4. Discussion

Seed germination is susceptible to mechanical damage, disease and environmental stress. Thus, it is considered to be the most important and vulnerable stage in plant life cycle (Rajjou et al., 2012). Environmental conditions, such as temperature and moisture, play an important role in seed germination (Zhang et al., 2009; Benvenuti et al., 2010). However, each species has a specific environmental requirement for its seed germination. In this study, we asked what are possible causes for the differentiation of *Pinus yunnanensis* and *P. kesiya* var. langbianensis in Yunnan, and we hypothesized that *P. yunnanensis* will outperform *P. kesiya* var. langbianensis at lower moisture levels.

4.1. Temperature requirement for seed germination

Storage temperature and time are important factors influencing seed vitality and germination (Wu et al., 2007; Beardmore et al., 2008; Shen et al., 2010). In our experiment, after stored at −20, 4, 15 and 25℃ for 1, 3, 5 and 7 months, the seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis still maintained high germination percentage (>60%) (Fig. 5). After the seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis mature around November, they are dispersed and exposed in the field during the period before germinating in the next spring (around April). Kunming, a

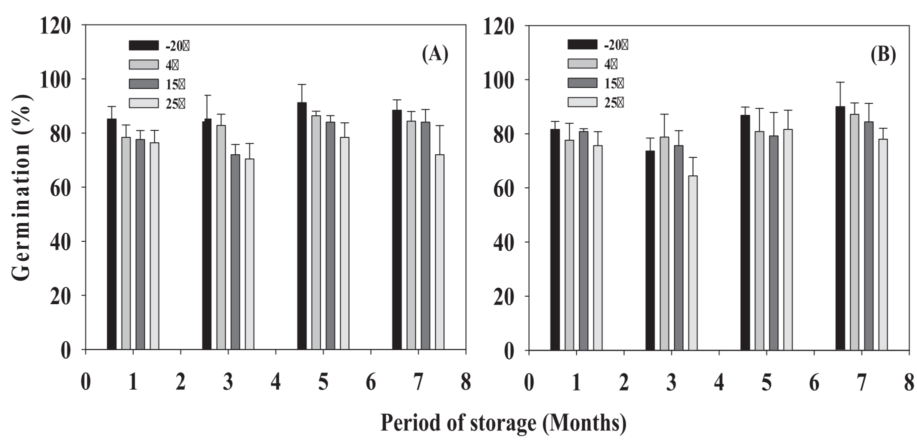

Fig. 5. Seed germination percentage of *P. yunnanensis* (A) and *P. kesiya* var. langbianensis (B) subjected to different storage conditions.

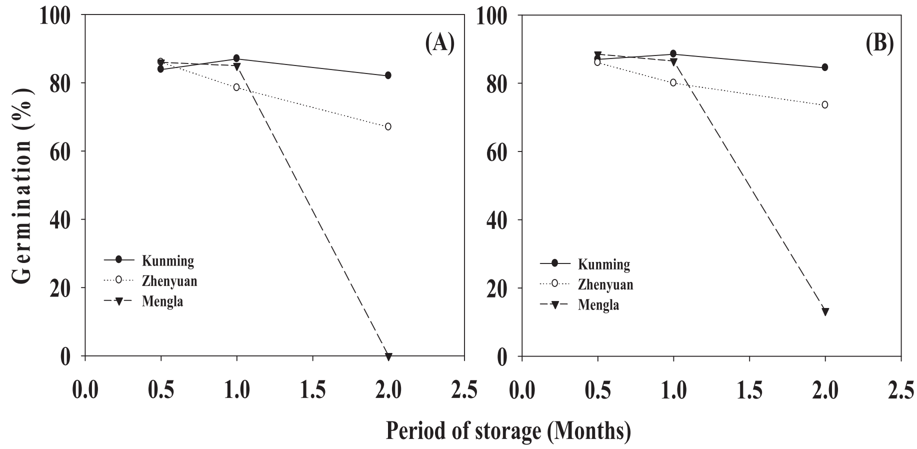

Fig. 6. Seed germination percentages of *P. yunnanensis* (A) and *P. kesiya* var. langbianensis (B) buried in soil of different sites.

natural distribution area of *P. yunnanensis*, has a winter temperature of 8.9–10.9℃, while Zhenyuan, a natural distribution area of *P. kesiya* var. langbianensis, has a winter temperature of 12.6–14.6℃ (Fig. 2A). Thus, we can conclude that the temperature of the duration when the seeds of the two species exposed in the field did not make a dramaticlly decrease for the seed viability. And the seeds of the two species will germinate successfully in spring when the environmental conditions are suitable.

Temperature can directly affect the germination percentages, and indirectly influence the timing of seed germination (Roberts, 1988). Temperature is a primary environmental factor affecting seed germination and determining the seed germination speed, and plant species differ with regard to the temperature range and optimum temperatures for seed germination (Song and Wang, 2009; Liu et al., 2014). Seed germination can be accelerated by an increased temperature within a certain range, but excessively high temperatures inhibit seed germination by destroying the properties of enzymes (Cui et al., 2014). Here, we found that the germination and seedling percentages of the two species were high at the temperature range of 15–30℃, and did not significantly decline until temperatures dropped below 15℃ or increased above 30℃ (Fig. 3). At the temperature range of 15–30℃, although the seed germination percentages of *P. yunnanensis* were lower than that of *P. kesiya* var. langbianensis for a given temperature, the seedling percentages were opposite. At 10–35℃, the germination percentages of *P. yunnanensis* were higher than that of *P. kesiya* var. langbianensis. It showed that the requirement of temperature for seed germination of *P. kesiya* var. langbianensis was more strict than that of *P. yunnanensis*. Seeds germinate in spring naturally. The spring temperatures of Kunming and Zhenyuan were 14.1℃ to 19.2℃ and 18℃ to 22.8℃, respectively (Fig. 2A). Thus, the spring temperatures of Kunming and Zhenyuan were suitable for seed germination of *P. yunnanensis* and *P. kesiya* var. langbianensis.

The seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis maintained high viability after exposed to the field at the duration from seed maturation to germination at the following spring, which ensures the seed ability to germinate into seedlings when the environmental conditions are suitable. And the spring temperature was suitable for seed germination of the two species. Thus, the temperature was not a main reason for the differentiation of *P. yunnanensis* and *P. kesiya* var. langbianensis.

4.2. Moisture requirement for seed germination

Soil seed bank is the sum of all the surviving seeds that exist on the soil surface and in the soil (Roberts, 1981). It carries the memory of the past vegetation and may store the original, existing and other species that moved into the community, providing the material basis for community renewal, succession and restoration of degraded vegetation (Fenner, 1992). The viable seeds in the soil seed bank is the key to determine whether the seed bank can transform from potential population to actual population (Chen et al., 2005). The seeds were buried in soil at a depth of 5–6 cm, therefore, we think that soil moisture is the main factor affecting seed viability. After buried in soil for 2 months, the seeds of *P. yunnanensis* and *P. kesiya* var. langbianensis at Kunming and Zhenyuan maintained high germination percentages (>65%). However, after buried in soil for 2 months, the seed germination percentages of *P. kesiya* var. *langbianensis* at Xishuangbanna only was 13.3%, and that of *P. yunnanensis* at Xishuangbanna was 0. Seeds vitality lost easier at Xishuangbanna than that at Kunming and Zhenyuan (Fig. 6). We speculated that two possible causes are responsible for these results: (1) The higher precipitation at Xishuangbanna than at Kunming and Zhenyuan made the seeds lose vigor during storage (Fig. 2); (2) There are many animals eating the seeds during storage, which causes the seeds to lose vigor. From November to February of the next year, there was little difference in precipitation between Kunming and Zhenyuan (Fig. 2B). According to the results, we can learn that the soil moisture of the duration when the seeds of the two species exposed in the field did not make a dramaticlly decrease for the seed viability. And the seeds of the two species will germinate successfully in spring when the environmental conditions are suitable.

Precipitation is a main factor affecting seed germination, and insufficient soil moisture can inhibit seed germination And it plays an important role on the distribution of plants (Zhu et al., 2005; Duan et al., 2011). Precipitation is a most important variable to explain the distribution of relict genera (Huang et al., 2015). The intensification of a drier climate in winter and spring because of the uplift of Qinghai-Tibet Plateau may prevent the survival of *Cedrus* and *Sequoia*, leading to the eventual disappearance in Yunnan (Su et al., 2013b, Zhang et al., 2015). In our experiment, the germination percentages of *P. kesiya* var. langbianensis were higher than that of *P. yunnanensis* at 0 MPa to −0.6 MPa (Fig. 4). However, at −0.8 MPa to −1 MPa, it was opposite. It indicated that the seed germination of *P. yunnanensis* showed stronger tolerance to drought than *P. kesiya* var. langbianensis. Therefore, *P. yunnanensis* can adapt to the drought stress caused by the intensification of the monsoon climate. In spring, sufficient moisture is very important for seed germination. The precipitation from March to May is 17.6 mm to 85.5 mm at Kunming, and is 21 mm to 114.9 mm at Zhenyuan (Fig. 2B). It obviously showed that there was more precipitation in Zhenyuan than that of Kunming in spring, which supported our result that *P. yunnanensis* showed stronger drought tolerance than *P. kesiya* var. langbianensis. In order to survive, it became possible for *P. kesiya* var. langbianensis to migrate to areas where the precipitation conditions were more favorable.

Thus, we can conclude that the precipitation in spring of central Yunnan cannot meet the moisture requirement of *P. kesiya* var.

langbianensis for seed germination, which makes it become possible for *P. kesiya* var. langbianensis migrating to areas where it is more humid than central Yunnan. Therefore, the lower precipitation in central Yunnan may be a main reason for the differentiation of *P. yunnanensis* and *P. kesiya* var. langbianensi. And the results are consistent with the study of Xing et al. (Xing et al., 2010).

5. Conclusions

We found that the temperature requirements for *P. yunnanensis* and *P. kesiya* var. langbianensis were similar, but the moisture requirements for the two species were showed significantly different. And the distribution of *P. yunnanensis* and *P. kesiya* var. langbianensis was closely related to precipitation. *P. yunnanensis* is distributed in the relatively arid central Yunnan, and *P. kesiya* var. langbianensis is distributed in the relatively humid southern Yunnan, which is consistent with our results. Thus, *P. yunnanensis* can outperform *P. kesiya* var. langbianensis at lower moisture levels, and precipitation was a main cause for the differentiation of *P. yunnanensis* and *P. kesiya* var. langbianensis. Because the seed germination of *P. kesiya* requires high moisture level, it can only be distributed in southern Yunnan where is more humid than central Yunnan; while *P. yunnanensis* still stay in central Yunnan because it can adapt well to drought climate (Xing et al., 2010). Our findings indicate that the geographical distribution patterns of *P. yunnanensis* and *P. kesiya* var. langbianensis are relevant to their ecological and evolutionary history.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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