

EFFECTS OF ALTITUDE ON REPRODUCTIVE CHARACTERISTICS OF AN ENDANGERED PLANT *TETRACENTRON SINENSE* OLIV.

QIAN YANG^{1,2}, YANG LI³, SHIBU QUBI⁴, YIHUA GONG⁴, HONGYAN HAN^{1,2*} AND XIAOHONG GAN^{1,2}

¹Key Laboratory of Southwest Wildlife Resources Conservation, Ministry of Education, China West Normal University, Nanchong, Sichuan 637009, China

²Institute of Plant Adaptation and Utilization in Southwest Mountain, China West Normal University, Nanchong 637009, China

³Wanyuan Ecological Environment Bureau, Dazhou City, Sichuan Province, 636300, China

⁴Meigu Dafengding National Nature Reserve Administration, Meigu County, Sichuan Province, 616450, China

*Corresponding author's email: hanhongyan@cwnu.edu.cn; Xiaohong Gan, email: bhgan@cwnu.edu.cn

Abstract

The reproductive characteristics of *Tetracentron sinense* Oliv. an endangered plant, were studied at different altitudes. The effects of climate warming on the future natural regeneration of *T. sinense* were investigated indirectly by simulating climate change with altitude gradient. The results showed that the flowering of *T. sinense* at low altitude started earlier than that at high-altitude. With the increase in altitude, the length of inflorescence and infructescence was decreased, while the single flower area, the length of long stamens, short stamens, and pistil were increased. Analysis of the proportion of components in the infructescence of *T. sinense* showed that the proportion of pericarp biomass was decreased, but the proportion of seed biomass was increased with increasing altitude. The pollen count and P/O value of *T. sinense* were significantly higher at mid-altitude compared to other altitudes. Furthermore, the length, width, and thickness of *T. sinense* seeds were indirectly proportional with the increase in altitude. *T. sinense* at low altitude had lower seed germination and germination potential. These changes will ultimately hinder the reproduction process of *T. sinense* and are not conducive to the regeneration and reproduction of the existing *T. sinense* population.

Key words: *Tetracentron sinense* Oliv., Reproduction, Natural regeneration, Climate change.

Introduction

In recent years, climate warming has become the main trend of global climate change (Parmesan & Yohe, 2003; Hu *et al.*, 2019; Kondrashin *et al.*, 2022). According to the assessment of the Six Assessment Report submitted by the Intergovernmental Panel on Climate Change, global warming would reach 1.5°C in the 2030s, and then exceed the temperature control target by 1.6°C (Wei *et al.*, 2020). To adapt to ever-increasing temperatures, plants have undergone great changes in many aspects, such as geographical distribution, population pattern, morphological structure, physiological process, and reproductive characteristics (Kolanowska *et al.*, 2017; Chen *et al.*, 2019; Zamin *et al.*, 2019; Freimuth *et al.*, 2022). Among these changes, reproduction, as an important part of plant life history, determines whether there is any regeneration of the plant population. Therefore, the impact of climate warming on reproductive characteristics in plants has attracted much attention (Gao *et al.*, 2015).

Numerous studies have shown that environmental factors, especially temperature, will change with the elevation gradients, which provides an ideal condition for studying the ecological adaptability of plants during the warming period (Kim *et al.*, 2013; Julie *et al.*, 2016). For example, Daco *et al.*, (2021) studied populations of *Anthyllis vulneraria* at an altitudinal gradient from 500 m to 2500 m above sea level in the Alps and found that future climate warming may have an impact on the growth and development of mountain plants. Similarly, by comparing the floral phenology of *Primula palinuri* at different altitudes, Aronne *et al.*, (2015) found that *P. palinuri* bloomed earlier and had a longer flowering period in low-altitude areas of the Mediterranean, which is a strategy for

plants to cope with global warming. Therefore, a simple and feasible method is proposed to indirectly predict the effect of climate warming on the plant growth process by using space (altitude) instead of time.

Tetracentron sinense Oliv, a tall deciduous tree of the family Trochodendraceae, is mainly distributed in central and southwest China (Li *et al.*, 2021a) and usually grows at an elevation of 1100-3500 m. Due to its ornamental and medicinal values, *T. sinense* has been over-harvested, resulting in poor regeneration of its natural population (Martyn *et al.*, 2007). Therefore, it has been listed in Appendix III of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Wang *et al.*, 2006). Presently, researches on population regeneration showed that there is a dating phenomenon in *T. sinense* age structure, which is mainly manifested in the few saplings and seedlings in *T. sinense* populations. It is suggested that the reason for this phenomenon might be due to the obstacles in its reproductive process caused by climate warming (Gan *et al.*, 2013; Li *et al.*, 2018; Tian *et al.*, 2018; Li *et al.*, 2020; Lu *et al.*, 2020; Fan *et al.*, 2021). Does climate warming affect the reproduction process of *T. sinense* and lead to changes in its distribution pattern? How would *T. sinense* populations behave in the future during the warming period? Understanding these issues will give us better understanding to evaluate the survival and adaptability of *T. sinense* in the future. In this study, we used altitude gradients to simulate climate changes and analyzed the differences in flowering phenology, floral biology, and fruit and seed characteristics of *T. sinense* at different altitudes. The aims of this study are (1) to reveal the effects of altitude on the reproductive strategies of *T. sinense*, (2) to predict the effects of future climate warming on the population reproduction and natural regeneration of *T. sinense*, and (3) to provide a basis for the protection and rational utilization of *T. sinense*.

Material and Method

Study area: The study was conducted in Dafengding Nature Reserve (103°8'51.3"~103°9'1"E, 28°47'8.3"~28°46'16.6"N) in Meigu County, Liangshan Prefecture, Sichuan Province, China. The study area is located on the southeastern margin of the Qinghai-Tibet Plateau and characterized by a humid climate of the central subtropical monsoon, with the annual average temperature of 9.6-11.4°C, the total annual rainfall is 1100 mm and the average annual relative humidity is 80% (Li *et al.*, 2021b) (Fig. 1).

Selection of samples: *T. sinense* in Dafengding Nature Reserve is mainly distributed at an altitude range of 2000-2500 m. According to the method of Li *et al.*, (2015), three altitude gradients were selected (low-altitude: 2012-2039 m; mid-altitude: 2140-2182 m; high-altitude: 2294-2342 m) and temperature, humidity, light intensity, and canopy density were surveyed at each altitude using Agricultural Environment Monitor (TNHY-5, Zhejiang Top Yunnong Technology Co., Ltd., China) (Table 1). According to the result of Li *et al.*, (2015), the reproductive processes of *T. sinense* were divided into four stages, among which the individuals with a diameter at breast height (DBH) of 35-45 cm are in the reproductive peak stage. Therefore, 6

individuals with a DBH of 35-45 cm at each altitude were selected, and a total of 18 individuals were selected as samples (Table 1).

Floral phenology: Floral phenology of *T. sinense* at three altitudes was continuously observed from April to August 2019. Specifically, the time of buddings flowering initiation (25% flowers open), peak flowering (50% flowers open), final flowering (less than 25% flowers open), and the termination for 18 individuals were observed according to the method of Dafni (1992).

Floral morphology: Thirty inflorescences were randomly selected from the middle layer of the sunny canopy in each individual during the flowering peak stage, and the length of the inflorescences was measured with a ruler. Similarly, thirty flowers were also randomly selected from each individual, and the length of long stamens, short stamens and pistil, as well as the distance from long or short stamen anthers to pistil stigmas, were measured with electronic digital vernier caliper, with an accuracy of 0.01 mm (Guilin Guanglu Digital Measurement and Control Co., LTD. Model: 0-150). The above measurement operations were repeated three times.

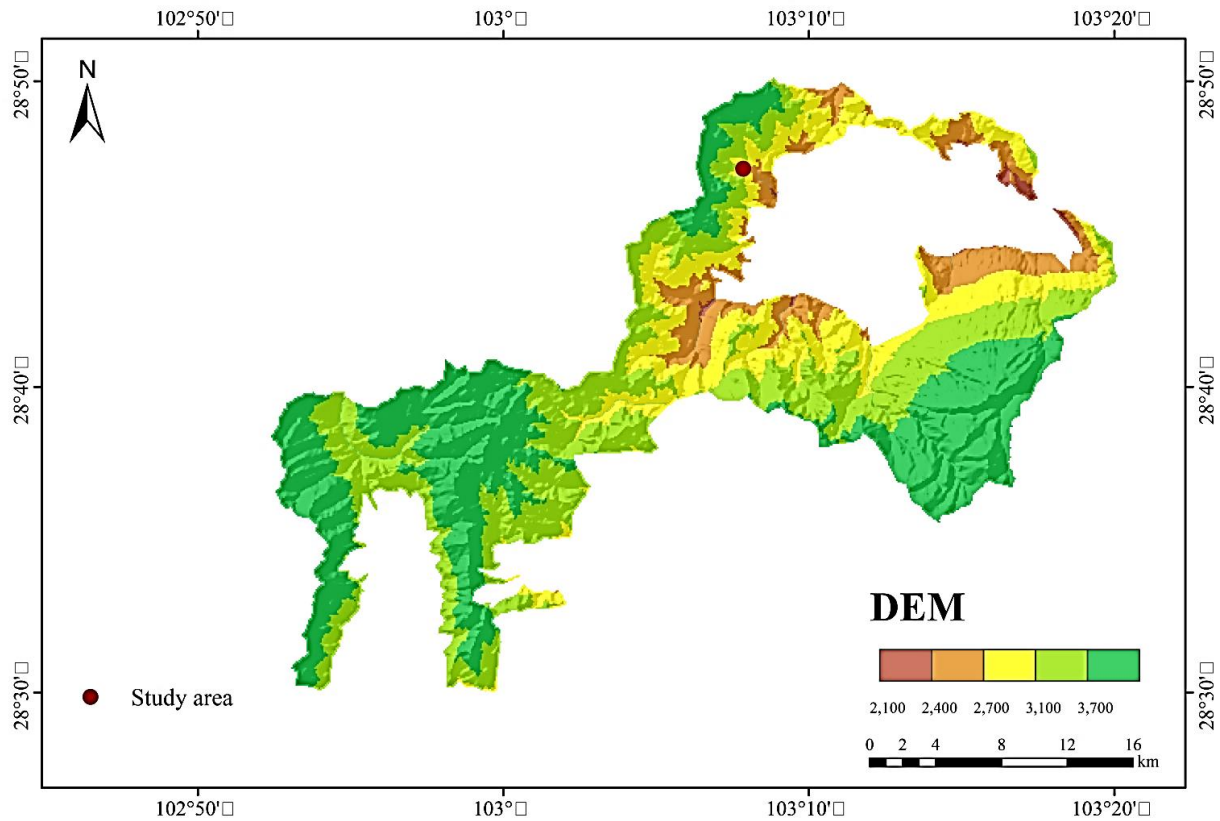


Fig. 1. Schematic diagram of study area.

Table 1. Habitat factors in three altitude areas.

Altitude	I	II	III
Temperature (°C)	21.055 ± 0.406	20.341 ± 0.434	19.492 ± 0.463
Humidity (%)	67.454 ± 0.978	70.254 ± 1.248	73.917 ± 1.339
Light intensity (lx)	58619.833 ± 5310.204	45501.292 ± 3479.641	28574.292 ± 2731.399
Canopy density (%)	>70	>70	>70

Note: I: Low altitude (2012~2039 m), II: Medium altitude (2140~2182 m), III: High altitude (2294~2342 m)

Pollen-ovule ratio: Thirty flowers were randomly selected from each individual. Then, two long anthers of a single flower were mashed with tweezers, and a 10 ml suspension of pollen grain was made with 95% ethanol solution on a blood count plate, and the pollen grains were counted under a microscope (Motic BA410, China). The ovules were counted after ovaries were dissected under Leica stereoscopic microscope (SAPO Stereozoom 1.0x-8.0x, Switzerland). Pollen-ovule ratio (P/O) was calculated according to the criteria of Cruden (1977).

Infructescence characteristics: As previously described, thirty infructescences were randomly selected from each individual before the seeds dispersed, and the length of infructescences was measured with a ruler. The infructescences were placed in the oven (DHG-9140A, China) at 80°C to dry to constant weight, and the biomass proportion of infructescence axis, pericarp, and seeds in infructescences were calculated using the following formulae. Lastly, the number of seeds in each infructescence was calculated with a counter (F60158, China).

$$\text{Biomass proportion of infructescence axis} = \frac{\text{Biomass of infructescence axis}}{\text{Biomass of infructescence}} \times 100 \% \dots\dots\dots (1)$$

$$\text{Biomass proportion of pericarp} = \frac{\text{Biomass of pericarp}}{\text{Biomass of infructescence}} \times 100 \% \dots\dots\dots (2)$$

$$\text{Biomass proportion of seeds} = \frac{\text{Biomass of seeds}}{\text{Biomass of infructescence}} \times 100 \% \dots\dots\dots (3)$$

Seed characteristics: One thousand seeds were randomly selected from each individual and then weighed using an electronic scale (BSA224S-CW, China). Afterwards, the seeds were placed in the oven at 105°C to dry to constant weight. The dry weight and water content were calculated according to the description of Luo *et al.*, (2010). Seed plumpness was evaluated using a stereoscopic microscope (Motic SMZ-168, China) as described by Chen (2018).

In addition, also randomly selected one thousand seeds from each individual and measured the length, width, and thickness of the seeds were measured using the Motic stereo microscopy system (Motic SMZ-168, China). The seeds were then sterilized by soaking in 0.1% NaClO solution and washed with distilled water. The seed germination rate and germination potential were investigated under the conditions of a constant temperature of 25°C, humidity of 80%, the light intensity of 18.5 μmol/m²·s, and light for 8 h/d (Chen, 2018).

Statistical analysis

Statistics and plotting were performed using Excel 2019 and Origin 2018 respectively. One-way ANOVA analysis of variance and Duncan's multiple range test was also used for comparison between different treatments. If the data met the homogeneity of variance, the Duncan test was used for comparative analysis; if the data did not conform the homogeneity of variance, multiple comparative analyses were performed using Dunnett's T3 test.

Results

Flowering phenology of *T. sinense* at different altitudes: Flower buds of *T. sinense* at low-altitude (2012-2039 m) appeared in early April. Flowering began in early June, reached its peak in early July, and ended in mid-July. At mid-altitude (2140-2182 m), *T. sinense* flower buds appeared in mid-April, began to bloom in mid-June, peak was reached in mid-July, and ended at the end of July. At high-altitude (2294-2342 m), the buds appeared at the end

of April, began to bloom at the end of June, peak reached at the end of July, and ended in early August (Fig. 2A-D). Overall, the *T. sinense* at low-altitude had an earlier flowering time of flower bud stage, and initiation, peak, and termination compared with those at higher altitudes.

Floral morphology of *T. sinense* at different altitudes:

The inflorescence length of *T. sinense* was decreased with increasing altitude, and the difference was significant among the three altitudes ($p < 0.05$). In addition, there was no significant difference in single flower areas of *T. sinense* between low-altitude and mid-altitude ($p > 0.05$), but the index was increased significantly at high-altitude ($p < 0.05$) (Table 2).

There were no significant differences in the length of long stamens, short stamens, and pistil at low and mid altitudes, however, there was a significant increase at high-altitude ($p < 0.05$). In addition, an interesting result presented that the distance between the anthers of long stamen and stigma was negatively correlated with altitude, while an opposite trend occurred in the distance between the anthers of short stamen and stigma ($p < 0.05$) (Table 2).

Furthermore, pearson correlation and linear model regression analysis showed that inflorescence length was significantly negatively correlated with altitude ($R^2 = 0.4045$, $p < 0.01$) (Fig. 3A), while the single flower area, the length of long or short stamens, and pistil were significantly positively correlated with altitude ($R^2 = 0.0491$, $p < 0.01$; $R^2 = 0.1217$, $p < 0.01$; $R^2 = 0.4806$, $p < 0.01$; $R^2 = 0.3297$, $p < 0.01$; respectively) (Fig. 3B-E).

The pollen-ovule ratio of *T. sinense* at different altitudes:

The number of pollen grains presented a maximum value at mid-altitude, which significantly differed from other altitudes ($p < 0.05$). Moreover, there was no significant difference in the number of ovules among different altitudes ($p > 0.05$). Therefore, *T. sinense* at mid-altitude had a higher ratio of pollen-ovule compared with those at low and high altitudes ($p < 0.05$) (Table 3).

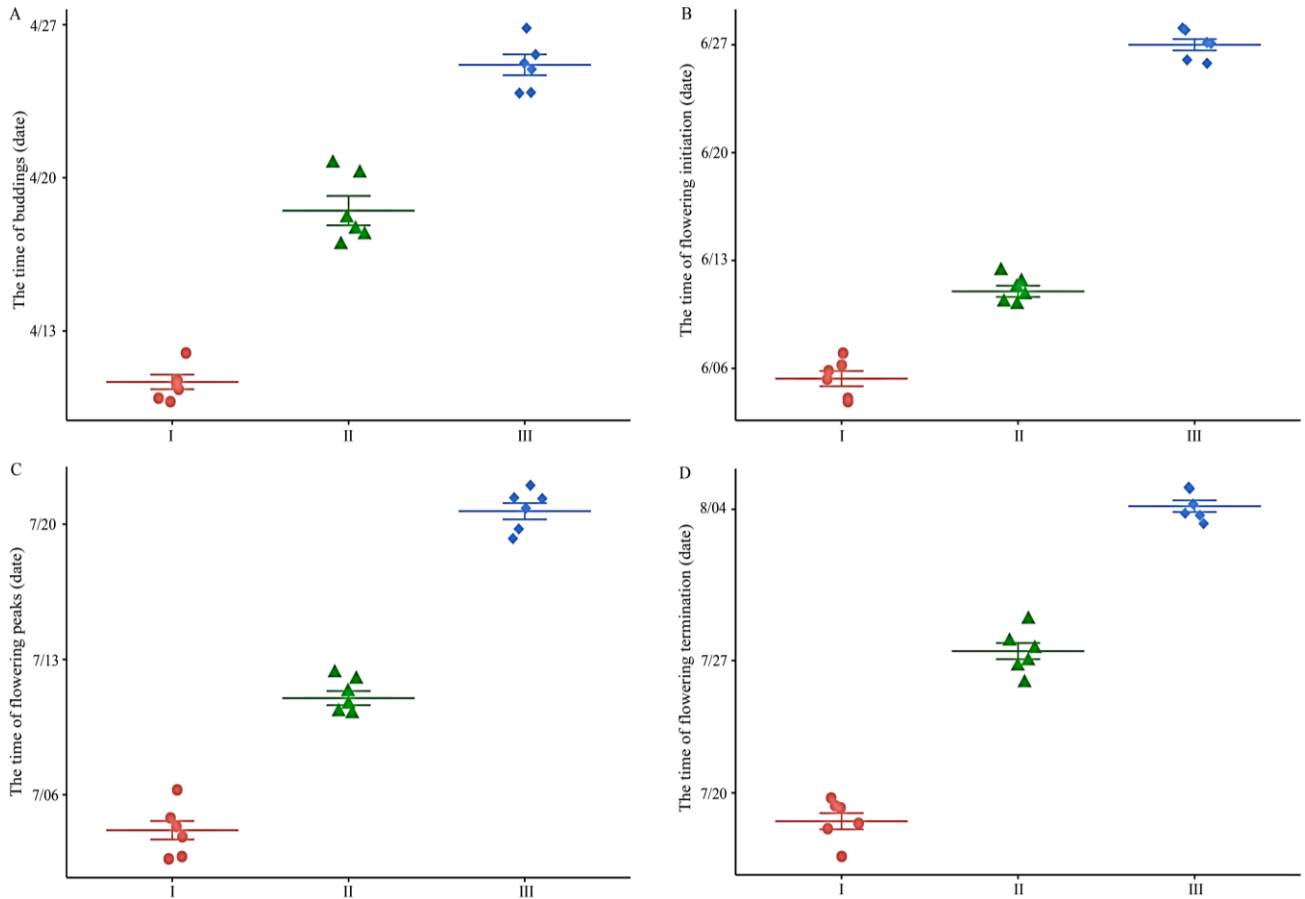


Fig. 2. Flowering phenology of *T. sinense* at different altitudes.

(A) The time of buddings. (B) The time of flowering initiation. (C) The time of flowering peak. (D) The time of flowering termination. The red circle represents six trees at altitude I (2012-2039 m). The green triangle represents six trees at altitude II (2140-2182 m). The blue diamond represents six trees at altitude III (2294-2342 m).

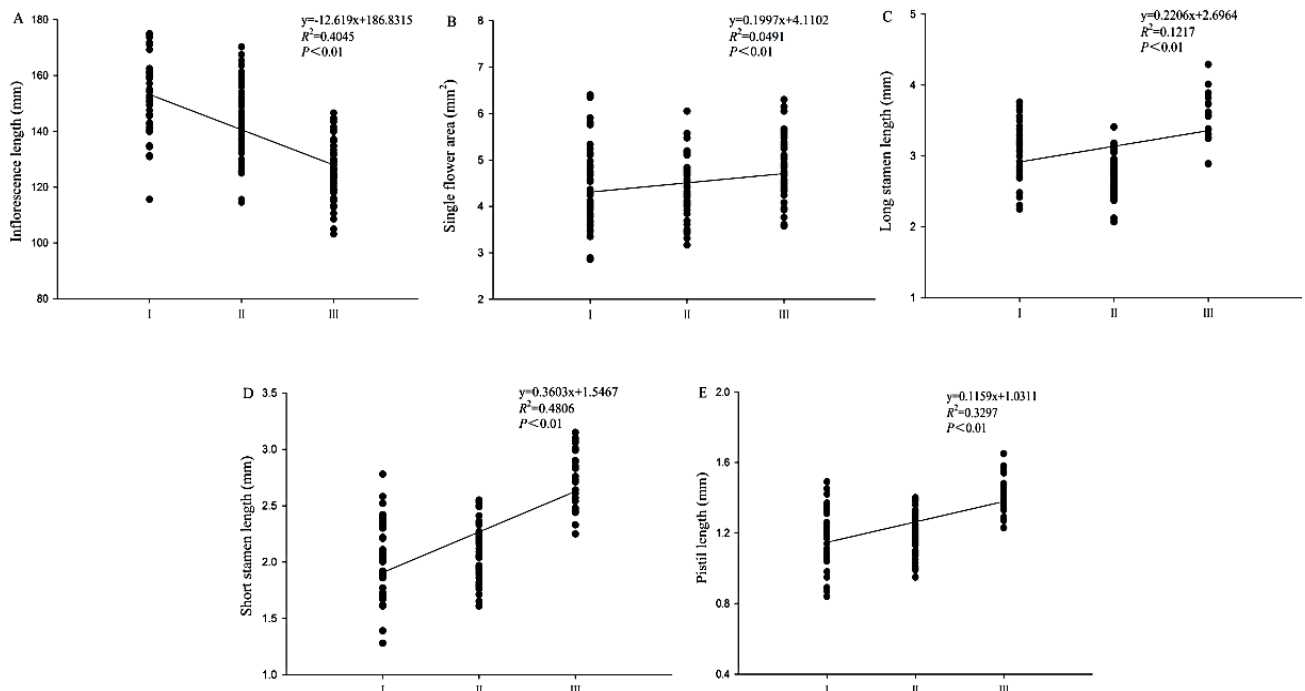


Fig. 3. Trends of floral characteristics at different altitudinal gradients.

(A) The inflorescence length of *T. sinense* at different altitudes ($p < 0.01$). (B) The single flower area of *T. sinense* at different altitudes ($p < 0.01$). (C) The long stamen length of *T. sinense* at different altitudes ($p < 0.01$). (D) The short stamen length of *T. sinense* at different altitudes ($p < 0.01$). (E) The pistil length of *T. sinense* at different altitudes ($p < 0.01$).

Table 2. Characteristics of floral morphology of *T. sinense* at different altitudes.

Characters	I	II	III
Inflorescence length (mm)	151.538 ± 1.821a	143.623 ± 1.626b	126.436 ± 1.266c
Single flower area (mm ²)	4.438 ± 0.114b	4.219 ± 0.085b	4.837 ± 0.090a
Long stamen length (mm)	3.125 ± 0.055b	2.722 ± 0.038b	3.566 ± 0.045a
Short stamen length (mm)	2.006 ± 0.045b	2.070 ± 0.034b	2.727 ± 0.034a
Pistil length (mm)	1.179 ± 0.023b	1.200 ± 0.015b	1.410 ± 0.014a
Inflorescence length (mm)	151.538 ± 1.821a	143.623 ± 1.626b	126.436 ± 1.266c
Distance between long stamen anther and stigma (mm)	1.978 ± 0.032a	1.769 ± 0.026b	1.571 ± 0.020c
Distance between long stamen anther and stigma (mm)	0.862 ± 0.012c	0.975 ± 0.010b	1.064 ± 0.018a

Note: The data in the table are average values ± standard errors; different lowercase letter indicates significant differences between the measured data at different altitudes ($p < 0.05$)

Table 3. Characteristics of pollen-ovule of *T. sinense* at different altitudes.

Characters	I	II	III
Number of pollen grains (P)	20909.091 ± 1760.447b	29318.182 ± 2262.423a	19375.000 ± 1601.418b
Number of ovules (O)	25.136 ± 0.100a	25.227 ± 0.113a	25.227 ± 0.113a
Ratio of pollen grains to ovules (P/O)	831.148 ± 70.350b	1162.471 ± 90.525a	771.074 ± 64.249b

Table 4. Characteristics of reproductive organ of *T. sinense* at different altitudes.

Characters	I	II	III
Infructescence length (mm)	149.883 ± 2.098a	126.210 ± 1.634b	106.539 ± 1.216c
Infructescence weight (g)	0.193 ± 0.004b	0.234 ± 0.008a	0.205 ± 0.006b
Ratio of infructescence axis distribution (%)	19.434 ± 0.760a	19.946 ± 0.583a	18.238 ± 0.482a
Ratio of pericarp distribution (%)	69.180 ± 0.690a	62.044 ± 0.713b	63.323 ± 0.765b
Ratio of seeds distribution (%)	11.386 ± 0.650b	18.010 ± 0.349a	18.439 ± 0.960a
Seed number per fruit	425.600 ± 20.536c	666.211 ± 38.455a	537.539 ± 32.465b

Table 5. Characteristics of seeds of *T. sinense* at different altitudes.

Characters	I	II	III
Weight of 1000 seeds (g)	0.0688 ± 0.0005b	0.0729 ± 0.0004a	0.0681 ± 0.0005b
dry weight (g)	0.0635 ± 0.0004b	0.0683 ± 0.0005a	0.0639 ± 0.0004b
water content (%)	7.6427 ± 0.2161a	6.2759 ± 0.1296b	6.1279 ± 0.2185b
Plumpness (%)	51.9307 ± 0.2955b	60.6522 ± 0.6426a	47.7262 ± 0.3186c
Seed length (mm)	2.978 ± 0.027a	2.779 ± 0.024b	2.240 ± 0.021c
Seed width (mm)	0.561 ± 0.010a	0.488 ± 0.009b	0.496 ± 0.009b
Seed thickness (mm)	0.334 ± 0.004a	0.323 ± 0.004a	0.283 ± 0.005b
germination rate (%)	64.523 ± 0.880b	70.908 ± 0.604a	49.323 ± 1.114c
germination potential (%)	26.750 ± 3.646b	38.083 ± 3.519a	12.917 ± 2.069c

The infructescence characteristics of *T. sinense* at different altitudes: The length of inflorescence was decreased with increasing altitude, and there was a significant difference in the inflorescence among the three altitudes (Table 4). In addition, the infructescence weight of *T. sinense* at mid-altitude had a maximum value, which significantly differed from other altitudes ($p < 0.05$).

There was no significant difference in the biomass proportion of the infructescence axis in *T. sinense* at different altitudes ($p > 0.05$). However, the biomass proportion of pericarp in infructescence was decreased with increasing altitudes, and the biomass proportion of seeds in infructescence was increased with increasing altitude ($p < 0.05$). Moreover, the infructescence at mid-altitude had more seeds compared with other altitudes ($p < 0.05$) (Table 4).

Furthermore, Pearson correlation and linear model regression analysis showed that infructescence length and the biomass proportion of pericarp in infructescence were both significantly negatively correlated with altitude ($R^2=0.5059$, $p < 0.01$; $R^2=0.4524$, $p < 0.01$; respectively) (Fig. 4A-B). Whereas, the biomass proportion of seeds in infructescence and seed number per fruit were increased with increasing altitude ($R^2=0.0239$, $p < 0.01$; $R^2=0.0468$, $p < 0.01$; respectively) (Fig. 4C-D).

The seed characteristics of *T. sinense* at different altitudes: The 1000-grain weight and dry weight of *T. sinense* seeds at mid-altitude were significantly higher than those at low and high altitudes ($p < 0.05$). In addition, the seeds at low-altitude had the highest water content, which significantly differed from other altitudes ($p < 0.05$). As the altitude increased, the plumpness of *T. sinense* increased first and then decreased (Table 5).

There was a significant decrease in seed length with the increase in altitude ($p < 0.05$). Meanwhile, the seed width and seed thickness of *T. sinense* tend to decrease with increasing altitude ($p < 0.05$) (Table 5). Pearson correlation and linear model regression analysis also showed that there was a negative correlation between seed characteristics and altitude ($R^2=0.5881$, $p < 0.01$; $R^2=0.0754$, $p < 0.01$; $R^2=0.1966$, $p < 0.01$; respectively) (Fig. 5A-C).

The germination rate and germination potential of seeds were significantly different among different altitudes, with the maximum at mid-altitude ($p < 0.05$). Instead, the two indices both reached the minimum at high-altitude, indicating the low fitness of seeds at high-altitude. Pearson correlation and linear model regression analysis also exhibited that the germination rate and germination potential were both negatively correlated with altitude ($R^2=0.2900$, $p < 0.01$; $R^2=0.1800$, $p < 0.01$; respectively) (Fig. 5D-E).

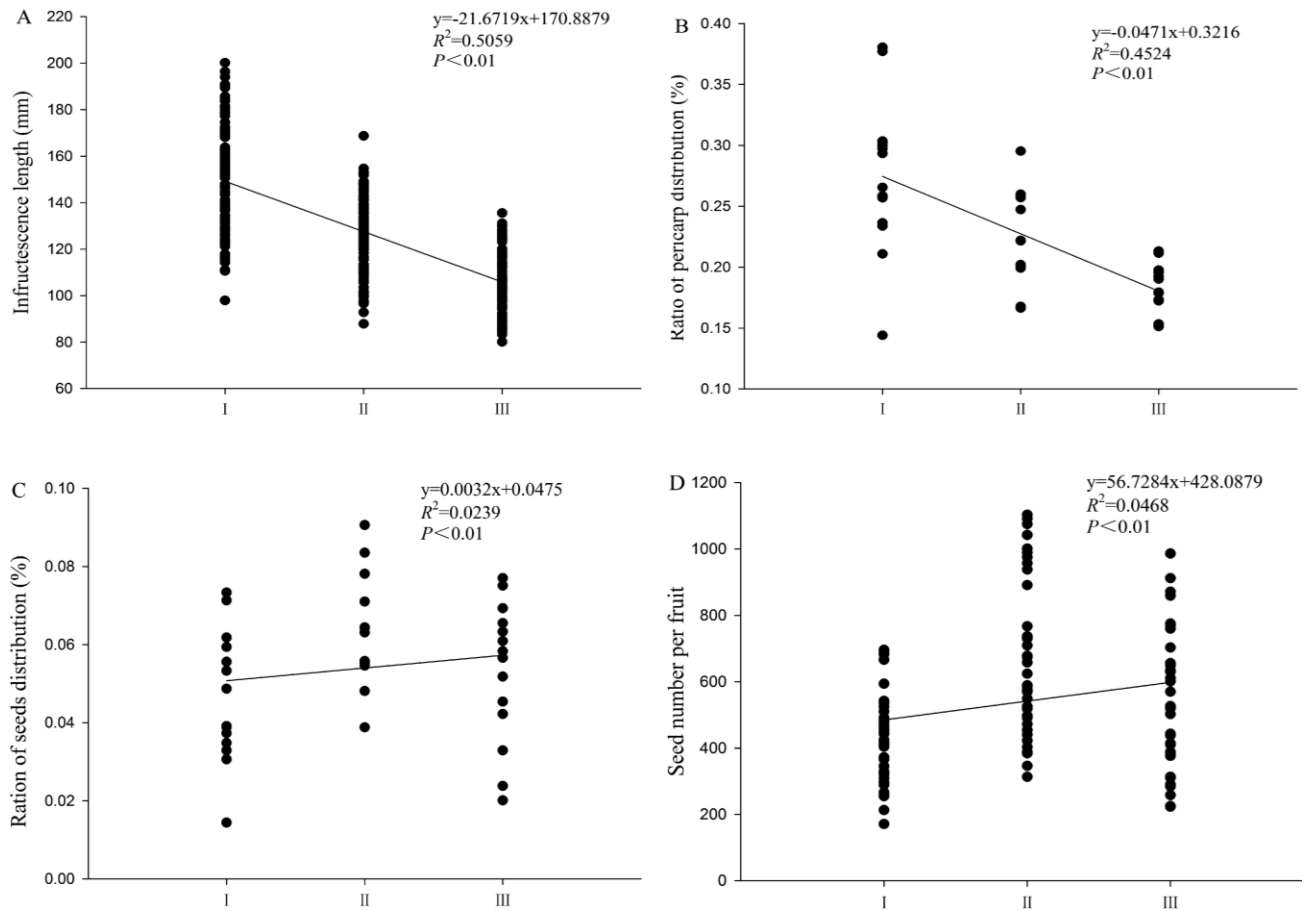


Fig. 4. Trends in reproductive characteristics of fruiting stages at different altitudinal gradients.

(A) The infructescence length of *T. sinense* at different altitudes ($p < 0.01$). (B) The ratio of pericarp distribution of *T. sinense* at different altitudes ($p < 0.01$). (C) The ratio of seeds distribution of *T. sinense* at different altitudes ($p < 0.01$). (D) The seed number per fruit of *T. sinense* at different altitudes ($p < 0.01$).

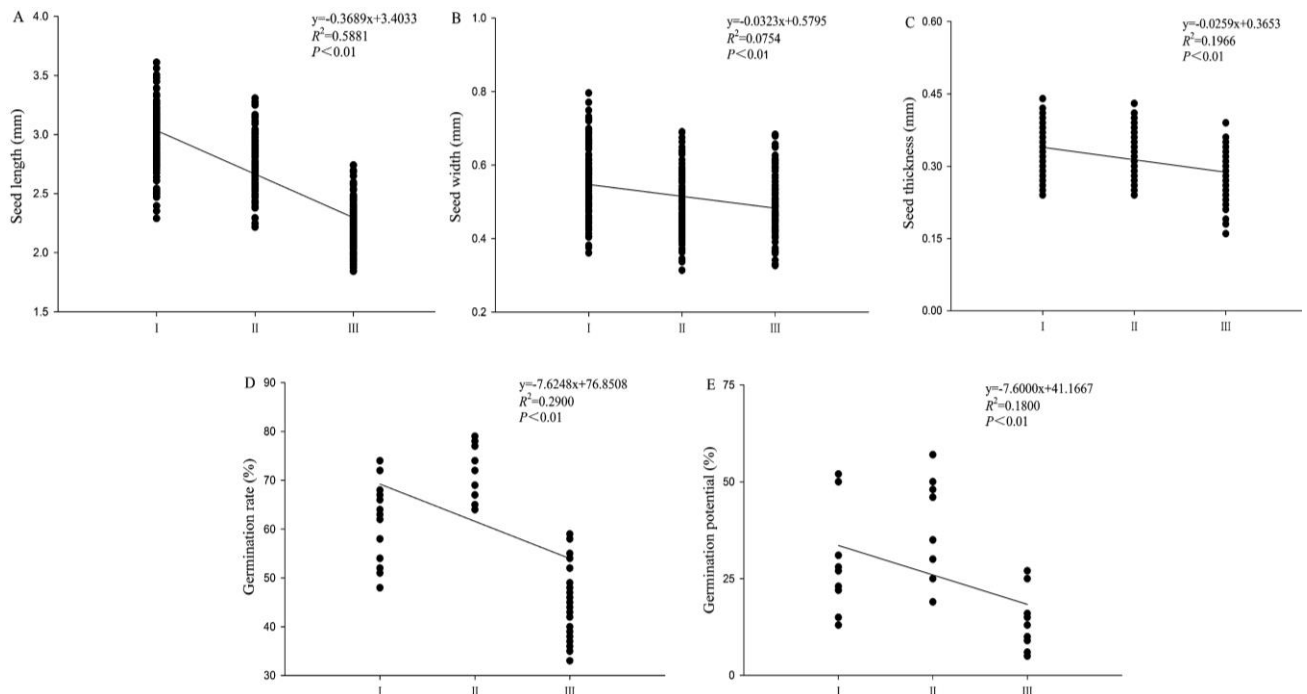


Fig. 5. Trends in seed characteristics at different altitudinal gradients.

(A) The seed length of *T. sinense* at different altitudes ($p < 0.01$). (B) The seed width of *T. sinense* at different altitudes ($p < 0.01$). (C) The seed thickness of *T. sinense* at different altitudes ($p < 0.01$). (D) The germination rate of *T. sinense* at different altitudes ($p < 0.01$). (E) The germination potential of *T. sinense* at different altitudes ($p < 0.01$).

Discussion

Effects of altitude on flowering phenology of *T. sinense*:

Generally, altitude has an important effect on flowering phenology (Wang *et al.*, 2022). For example, based on phenological observations of park rangers for 21 plant species at 24 sites from 680 to 1425 m above sea level, Cornelius *et al.*, (2013) showed that phenological periods and species were highly dependent on inter-annual temperature changes and altitude. Similarly, Wang *et al.*, (2022) found that in the flowering phenology of herbaceous in the Qilian Mountains, the flowering time of low-altitude communities were generally earlier than that of high-altitude communities. Likewise, our study showed that the flowering time of the flower bud stage, and initiation, peak, and ending of *T. sinense* at low-altitude were earlier than those of the high-altitude population, which was somewhat consistent with the previous work obtained by Gan *et al.*, (2013). Studies have shown that the temperature at low altitudes is higher than that at high-altitude, and the suitable temperature can accelerate the opening of flower buds and advance the flowering and fruiting stage (Wang *et al.*, 2014; Gugger *et al.*, 2015). Our monitoring of temperature changes also showed as the altitude increased, the temperature gradually decreased (Fig. 6). Therefore, we proposed that the temperature difference caused by the altitude gradients was the main reason for this phenomenon. Through long-term monitoring, we also found that the flowering time of *T. sinense* at the same altitude in 2021 was 15-20 days earlier than that in 2013, which was presumed to be related to global warming.

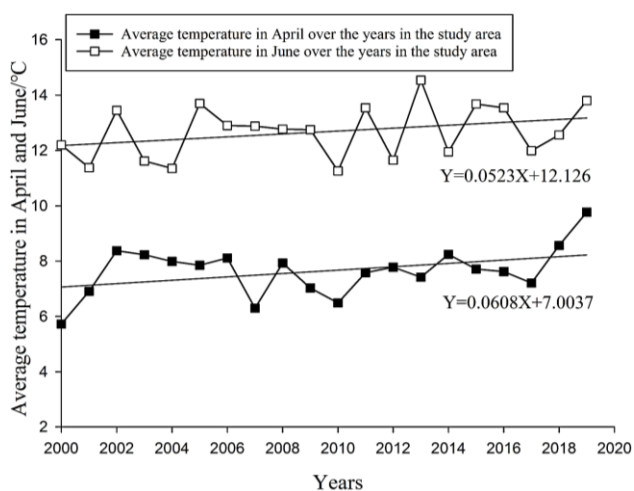


Fig. 6. The annual change trend of the average temperature in April and June in the study area Effects of altitude on floral characteristics of *T. sinense*.

In different environments, plants are differently affected by environmental factors, resulting in specific geographical variations in floral characteristics (Paiaro *et al.*, 2012). In this study, the inflorescence length of *T. sinense* decreased, while the single flower area, the length of long stamens, short stamens, and pistil increased with increasing altitude, suggesting a significant impact of

altitude on the floral characteristics of *T. sinense*. The result was consistent with the results of Malo *et al.*, (2010), in which the flower size of *Cytisus scoparius* in the Guadarrama Mountains was increased with altitude. Likewise, Madd *et al.*, (2013) found that the flower size of *Campanula rotundifolia* also increased with increasing altitude. We proposed that a larger single-flower area of *T. sinense* at higher altitudes could enhance the attraction to pollinators, longer stamens facilitate pollen dispersal, and longer pistils are more conducive to pollen reception, reducing the barrier of perianth segments from pollen dispersal. These plastic adjustments in floral characteristics enable *T. sinense* to efficiently complete pollination in high-altitude harsh environments, thereby compensating for the adverse effects on pollination (Wang *et al.*, 2012), thus improving pollination efficiency and ensuring the successful reproduction of the species.

The previous study has shown that *T. sinense* tends to live in an environment with high humidity and low temperature, and the flowering time of *T. sinense* coincides with the rainy season (Gan *et al.*, 2013). To adapt to this unfavorable environment, *T. sinense* has evolved two types of stamens, long and short. Long stamens are the main pollination structure, while short stamens are complementary to pollination, which begins to shed pollen after long stamens have finished shed pollen after long stamens have completed their dispersion. In this study, an interesting phenomenon was found that the distance between the anthers of long or stamen and stigma was greatly affected by the altitude. At the higher altitude the long stamens were close to the stigma, and the short stamens were away from the stigma. The distance of long or short stamens to stigma showed opposite trends along the elevation gradient, which may be related to the efficiency of pollination. As the altitude decreases, the ambient temperature increases and the wind speed slows down, and the species and numbers of pollinators are larger, the flowering phenology of *T. sinense* is earlier. As a result, its pollination mode preferred cross-pollination. Therefore, compared with high altitude, the long stamen anthers were farther from the stigma in low altitude, showing the adaptive characteristics of cross-pollination, while the short stamen anther closer to the stigma was a reproductive guarantee for *T. sinense* when cross-pollination was unsuccessful (ensuring reproductive success by self-pollination). The trade-off between long and short stamens anthers and stigma spacing with elevation is designed to ensure the efficiency and quality of pollination.

The pollen-ovule ratio of *T. sinense* in the three altitudes all ranged from 771.07 to 1162.47, indicating a breeding system with facultative hybridization, which is consistent with our previous result (Gan *et al.*, 2013). The pollen quantity and pollen-ovule ratio in a flower of *T. sinense* were significantly higher at mid-altitude than those at low and high altitudes. Combined with the actual field survey, the result may be due to the more open terrain environment at mid-altitude, where increasing the pollen quantity is conducive to the success of wind pollination.

Effects of altitude on infructescence characteristics of *T. sinense*:

Plants in different periods of life history

maintain the physiological function of different organs according to the needs of life activities, and then reasonably allocate resources, leading to the different allocation strategies of plant resources in different phenological periods (Liang, 2008). In this study, the length of *T. sinense* infructescence was decreased with the increase of altitude, our findings are consistent with the results from *Betula* in Qingling Mountains, *Oryza sativa* subsp. *japonica* and *Zea mays* (Ren, 2014; Zheng *et al.*, 2020; Yang, 2020). The dry weight and seed yield of infructescence were significantly greater than those at the other two altitudes, which may be related to the higher pollen yield during the flowering period. Some studies have also found that there is a significant correlation between seed number and pollen number of plants at different elevations (Qi *et al.*, 2019). Besides, the proportion of pericarp biomass and seed biomass in an infructescence showed an opposite trend with changing altitude. With increasing altitude, the proportion of pericarp biomass decreased, and the proportion of seed biomass increased, indicating a trade-off relationship. In general, seed biomass represents the energy of seed storage, which is the embodiment of seed fitness, and can reflect the colonization ability of seeds to some extent (Lokesh, 1992; Boulli *et al.*, 2001; Alexander *et al.*, 2009). The higher the altitude, the harsher the environmental conditions are, and the seed investment of *T. sinense* reproductive components (infructescence) increase with increasing elevation, which helps to ensure the high fitness of seeds for successful colonization at high altitude.

Effects of altitude on seed characteristics of *T. sinense*:

To adapt to different selection pressures for the reproduction of the species, plants exhibit certain altitudinal patterns in their strategies for seed adjustment (Chen *et al.*, 2020). The present study showed that the length, width, and thickness of *T. sinense* seeds continuously decreased with the increase in altitude, which was consistent with the findings of Holtmeier (1994). Higher altitudes tend to have a higher velocity of wind, so the small-size seeds of the *T. sinense* can drop and grow faster, which is a cost-effective method of survival adaptation.

Our results showed that with the increase of altitude, the thousand-grain weight, dry weight, and plumpness of *T. sinense* seeds first increased and then decreased, and the seed biomass of mid-altitude was the largest, which was consistent with the results of Li *et al.*, (2015). The greatest seed biomass of *T. sinense* appeared at mid-altitude, indicating that the seeds at mid-altitude were more suitable for colonization and had stronger survival ability. Additionally, the seeds at mid-altitude had the highest germination rate and germination potential, which might be related to the seed biomass. The seed biomass of *T. sinense* at the mid-altitude region was significantly higher than that in other altitudes, resulting in a higher seed germination rate and germination potential at mid-altitude, which was consistent with the results of Yao *et al.*, (2017).

Conclusion

In conclusion, there was a significant impact of altitude on reproductive characteristics in *T. sinense*. Along the elevation gradient, there are obvious differences in the reproductive characteristics of *T. sinense* due to the temperature variation, and its reproductive strategies change with the elevation variation by using space (altitude) instead of time. We predict that climate change will affect the reproduction of *T. sinense* in the protected area. Increasing temperature will lead to early flowering, longer flowering period, longer maturity time for seeds, smaller flower-size, shorter stamen and pistil, reducing the flower fitness, and then affect the pollination process; however, the input ratio of infructescence to seed biomass will decrease, *T. sinense* will produce seeds with large size and light biomass, resulting in lower germination rate and germination potential, and then decreased seed fitness, which will affect the growth of seedlings. Therefore, we propose that under the background of global warming, the reproduction process of *T. sinense* will be hindered and its reproductive fitness will be reduced, which is not conducive to the regeneration and reproduction of the existing *T. sinense* populations.

In view of the living conditions of *T. sinense* populations in the experimental area, the following suggestions are proposed based on the experimental analysis and results: (1) strengthen the protection of *T. sinense* in situ, especially for the distribution of *T. sinense* in the middle altitude area; (2) to increase ex-situ protection of *T. sinense* appropriately, and choose suitable habitats to help the sustainable survival and propagation of *T. sinense* population by artificial sowing or cutting.

Acknowledgments

We thank all staff in Meigu Dafengding National Nature Reserve who help to collecting data. This study was supported by National Natural Science Foundation of China (No. 32070371), the Innovation Team Funds of China West Normal University (No. KCXTD2022-4), the fund of Sichuan Meigu Dafengding National Nature Reserve (No. mgdfd2022-13), the Sichuan Micang Mountain National Nature Reserve (No. N5108212022000043), and the Natural Science Foundation of Sichuan Province (No.23NSFSC1272).

References

- Alexander, J.M., P.J. Edwards, M. Poll, C.G. Parks and H. Dietz. 2009. Establishment of parallel altitudinal lines in traits of native and introduced forbs. *Ecology*, 90(3): 612-22.
- Aronne, G., M. Buonanno and V.D. Micco. 2015. Reproducing under a warming climate: long winter flowering and extended flower longevity in the only Mediterranean and maritime *Primula*. *Plant Biol.*, 17(2): 535-544.
- Boulli, A., M. Baaziz and O. M'Hirit. 2001. Polymorphism of natural populations of *Pinus halepensis* Mill. in Morocco as revealed by morphological characters. *Euphytica*, 119(3): 309-316.
- Chen, K., Y. Yang, L. Xu and X. Liu. 2020. Comparison of seed trade-off strategies among *Cardiocrinum giganteum* populations along an elevational gradient in Gaoligong Mountains. *Chinese J. Ecol.*, 39(10): 3231-3236.

- Chen, Y. 2018. Effects of seed genetic quality on fate of seed and seedlings of *Tetracentron sinense* Oliv. China West Normal University, China. Master Thesis.
- Chen, Y.F., L.W. Zhang, X. Shi, Y. Ban, H.L. Liu and D.Y. Zhang. 2019. Life history responses of spring-and autumn-germinated ephemeral plants to increased nitrogen and precipitation in the Gurbantunggut Desert. *Sci. Total Environ.*, 659: 756-763.
- Cornelius, C., N. Estrella, H. Franz and A. Menzel. 2013. Linking altitudinal gradients and temperature responses of plant phenology in the Bavarian Alps. *Plant Biol.*, 1: 57-69.
- Cruden, R.W. 1977. Pollen-Ovule Ratios: A conservative indicator of breeding systems in flowering plants. *Evolution*, 31(1): 32-46.
- Daco, L., G. Colling and D. Matthies. 2021. Altitude and latitude have different effects on population characteristics of the widespread plant *Anthyllis vulneraria*. *Oecologia*, 197(2): 537-549.
- Dafni, A. 1992. *Pollination Ecology*. Oxford University Press, New York. pp1-57.
- Fan, W.Q., W.Y. Li, X.M. Zhang and X.H. Gan. 2021. Photosynthetic Physiological Characteristics of *Tetracentron sinense* Oliv in Different DBH Classes and the Factors Restricting Regeneration. *J. Plant Growth Regul.*, 1-10.
- Freimuth, J., O. Bossdorf, J.F. Scheepens and F.M. Willems. 2022. Climate warming changes synchrony of plants and pollinators. *P Roy Soc. Biol. Sci.*, 289(1971): 2021-2142.
- Gan, X.H., L.L. Cao, X. Zhang and H.C. Li. 2013. Floral biology, breeding system and pollination ecology of an endangered tree *Tetracentron sinense* Oliv. (Trochodendraceae). *Bot. Stud.*, 54(1): 1-9.
- Gao, X., C.N. Jin, A. Camargo and Y.M. Li. 2015. Allocation trade-off under climate warming in experimental amphibian populations. *Peer J.*, 3: e1326.
- Gugger, S., H. Kesselring, J. Stöcklin and E. Hamann. 2015. Lower plasticity exhibited by high-versus mid-elevation species in their phenological responses to manipulated temperature and drought. *Ann. Bot. London*, 116(6): 953-62.
- Holtmeier, F.K. 1994. *Ecological aspects of climatically-caused timberline fluctuations*. In: Beniston M ed. *Mountain Environment in Changing Climates*. Routledge Press, London, 220-233.
- Hu, H.X., X.Y. Ye, H. Wang and R. Ji. 2019. Selection of reference genes for normalization of real-Time PCR data in *Calliptamus italicus* (Orthoptera: Acrididae) under different temperature conditions. *J. Insect Sci.*, 19: 7.
- Julie, R.E. and J.M. Susan. 2016. How climate change affects plants' sex lives. *Science*, 353(6294): 32-33.
- Kim, E. and K. Donohue. 2013. Local adaptation and plasticity of *Erysimum capitatum* to altitude: its implications for responses to climate change. *J. Ecol.*, 101(3): 796-805.
- Kolanowska, M., M. Kras, M. Lipińska, K. Mystkowska, D.L. Szlachetko and A.M. Naczk. 2017. Global warming not so harmful for all plants- response of holomycotrophic orchid species for the future climate change. *Sci. Rep.*, 7(1): 12704.
- Kondrashin, A.V., L.F. Morozova, E.V. Stepanova, N.A. Turbabinina, M.S. Maksimova, A.E. Morozov, A.S. Anikina and E.N. Morozov. 2022. Global climate change and human dirofilariasis in Russia. *Int. J. Environ. Res. Public Health*, 19(5): 3096.
- Li, H.C., X.H. Gan, Z.P. Zhang, C.X. Zhang and L. Song. 2015. Effects of different altitude and mother tree size on biological characteristics of *Tetracentron sinense* seeds. *Plant Div. Resour.*, 37(2): 177-183.
- Li, M.J., Y.Z. Yang, R.P. Xu, W.J. Mu, Y. Li, X.X. Mao, Z.Y. Zheng, H. Bi, G.Q. Hao, X.J. Li, X.T. Xu, Z.X. Xi, N. Shrestha and J.Q. Liu. 2021a. A chromosome-level genome assembly for the tertiary relict plant *Tetracentron sinense* Oliv. (trochodendraceae). *Mol. Ecol. Resour.*, 21: 1186-1199.
- Li, S., X.H. Gan, H.Y. Han, X.M. Zhang and Z.Q. Tian. 2018. Low within-population genetic diversity and high genetic differentiation among populations of the endangered plant *Tetracentron sinense* Oliver revealed by inter-simple sequence repeat analysis. *Ann. Forest Sci.*, 75(3): 1-11.
- Li, W.Y., H.C. Li, X.H. Gan, X.M. Zhang and Z.L. Fan. 2020. Population structure and dynamics of the endangered tree *Tetracentron sinense* Oliver. *Pak. J. Bot.*, 52(2): 613-619.
- Li, Y., S. Li, X.H. Lu, Q.Q. Wang, H.Y. Han, X.M. Zhang, Y.H. Ma and X.H. Gan. 2021b. Leaf phenotypic variation of endangered plant *Tetracentron sinense* Oliv. and influence of geographical and climatic factors. *J. Forest. Res.*, 32(2): 623-636.
- Liang, Y., X.C. Zhang and X.L. Chen. 2008. Individual size and resource allocation in perennial *Gentiana*. *Acta Botanica Boreali-Occidentalia Sinica.*, 28(12): 2400-2407.
- Lokeshia, R., S.G. Hegde and R. Ganeshiah. 1992. Dispersal mode as a selective force in shaping the chemical composition of seeds. *Amer. Nat.*, 140(3): 520-525.
- Lu, X.H., N. Xu, Y. Chen, Y. Li and X.H. Gan. 2020. Effects of light intensity and ground cover on seedling regeneration of *Tetracentron sinense* Oliv. *J. Plant Growth Regul.*, 40: 1-13.
- Luo, J.D., X.H. Gan, X.J. Jia, D. Xie and L. Zhang. 2010. Biological characteristics of the seeds of endangered plant *Tetracentron sinense*. *Acta Bot. Yunnanica.*, 32: 204-210.
- Maad, J., W.S. Armbruster and C.B. Fenster. 2013. Floral size variation in *Campanula rotundifolia* (Campanulaceae) along altitudinal gradients: Patterns and possible selective mechanisms. *Nord. J. Bot.*, 31: 361-371.
- Malo, J.E. and J. Baonza. 2010. Are there predictable clines in plant-pollinator interactions along altitudinal gradients? The example of *Cytisus scoparius* (L.) Link in the Sierra de Guadarrama (Central Spain). *Diver. Distrib.*, 8(6): 365-371.
- Martyn, R. and C. Peter. 2007. *Tetracentron sinense*. The Board of Trustees of the Royal Botanic Gardens. *Kew Bull.*, 168-173.
- Paiano, V., G. Oliva, A. Cocucci and A. Sersic. 2012. Geographic patterns and environmental drivers of flower and leaf variation in an endemic legume of Southern Patagonia. *Plant Ecol. Divers.*, 5(1): 13-25.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918): 37-42.
- Qi, R.L., W.M. Ma, B.Y. Qi, J.H. Cao, Y.J. Yang, H.R. Ma and Y.F. Wang. 2019. Altitudinal variation of floral organs in *Saussurea przewalskii* and its relationship with the number and mass of seeds. *Chinese J. Appl. Ecol.*, 30: 2647-2653.
- Ren, J.Y. 2014. The study of variation in seed and seedling adaptive strategies of *Betula* and *Quercus* with elevation in Qinling Mountains. Northwest University, China. Masters Thesis.
- Tian, Z.Q., H.C. Li, X.H. Gan, X.M. Zhang and Z.L. Fan. 2018. Structure characteristic and niche of dominant arbor populations in *Tetracentron sinense* communities: implication for conservation. *Bot. Sci.*, 96(2): 157-167.
- Wang, S.P., F.D. Meng, J.C. Duan, Y.F. Wang, X.Y. Cui, S.L. Piao, H.S. Niu, G.P. Xu, C.Y. Luo, Z.H. Zhang, X.X. Zhu, M.G. Shen, Y.N. Li, M.Y. Du, Y.H. Tang, X.Q. Zhao, P. Ciais, B. Kimball, J. Peñuelas, I. A. Janssens, S.J. Cui, L. Zhao and F.W. Zhang. 2014. Asymmetric sensitivity of first flowering date to warming and cooling in alpine plants. *Ecology*, 95(12): 3387-3398.

- Wang, W., Z.B. He, J. Du, D.K. Ma and P. Zhao. 2022. Altitudinal patterns of species richness and flowering phenology in herbaceous community in Qilian Mountains of China. *Int. J. Biometeorol.*, 66(4): 741-751.
- Wang, Y.F., G.F. Lai, Efferth, Thomas., J.X. Cao and S.D. Luo. 2006. New glycosides from *Tetracentron sinense* and their cytotoxic activity. *Chem Biodiv.*, 3: 1023-1030.
- Wang, Y.F., M. Li, S.X. Li, J. Guo, Y.P. Chen and R.X. Wang. 2012. Variation of reproductive allocation along elevations in *Saussurea stella* on East Qinghai-Xizang Plateau. *Chinese J. Plant Ecol.*, 36: 1145-1153.
- Wei, Y.M., R. Han, C. Wang, B.Y. Yu, Q.M. Liang, X.C. Yuan, J.J. Chang, Q.Y. Zhao, H. Liao, B.J. Tian, J.Y. Yan, L.J. Cheng and Z.L. Yang. 2020. Self-preservation strategy for approaching global warming targets in the post-Paris Agreement era. *Nat Comm.*, 11(1): 1624.
- Yang, J.C. 2020. Effects of different elevations on maize yield and related characters in mountainous areas Yunnan. Southwest University, China. Masters Thesis.
- Yao, G.N. 2017. Effects of altitude on seed germination and seedling growth of *Platycladus orientalis* in Mountain Tai. Shandong Agricultural University, China. Masters Thesis.
- Zamin, M., A.M. Khattak, A.M. Salim, K.B. Marcum, M. Shakur, S. Shah, L. Jan and S. Fahad. 2019. Performance of *Aeluropus lagopoides* (mangrove grass) ecotypes, a potential turfgrass, under high saline conditions. *Environ. Sci. Pollut. R.*, 26(13): 13410-13421.
- Zheng, D., C.Y. Wen, X.H. Shen, B.L. Hu, J.Q. Che, Y.H. Xiong, Z.Q. Wang and Y.S. Wu. 2020. Analysis on variation in rice yield components and quality at different altitudes in Tibet. *Crops.*, 05: 199-203.

(Received for publication 17 October 2023)