

Altitudinal Effects on Osmotic Regulation and Plasma Membrane Permeability of *Lonicera caerulea* Leaves on the Northern Slope of Changbai Mountain, China

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Abstract

Background: *Lonicera caerulea* is a perennial deciduous shrub of medical and edible value that is widely distributed on the northern slope of Changbai Mountain. Soil properties and climate parameters at different elevations affect plant growth, but thus far no studies have been conducted on *Lonicera caerulea* in different elevation gradients on the northern slope of Changbai Mountain. Here, the leaves of *Lonicera caerulea*, collected from different elevations (800–1800 m) on the northern slope of Changbai Mountain in China, were used as test materials. The aim was to determine the changes in the leaves' soluble protein content, soluble sugar content, malondialdehyde (MDA) content, superoxide anion content, and plasma membrane permeability along the altitudinal gradient. The leaf-level data were statistically analyzed with respect to various environmental factors (soil properties and climate parameters) to explore the physiological and biochemical mechanisms of their adaptation to mountainous habitats.

Results: The soluble protein and soluble sugar contents initially increased and then decreased with greater altitude. The soluble protein content reached its maximum value of 1.84 mg/g at 1400 m while soluble sugar content peaked at 37.40 mg/g at 1600 m. The soluble protein content of the leaves was mainly affected by the total K content of the soil, while the total K content of the soil, total P content of the soil, and organic matter were the main factors explaining their soluble sugar content. The MDA content increased at first, then decreased, and then increased with altitude, for which the lowest values of 5.76 mol/g and 6.29 mol/g occurred at 1000 m and 1600 m, respectively; the MDA content was mainly influenced by hydrolysis N in soil. The superoxide anion content initially decreased and then increased with greater elevation, for which the minimum value of 26 µg/g occurred at 1200 m. The superoxide anion content was mainly driven by the total K and total P contents. The leaves' plasma membrane permeability initially decreased and then increased with greater elevation; values were lowest at the intermediate elevations, and the minimum value of 0.30% occurred at 1000 m. Plasma membrane permeability was mainly affected by the total K content. Based on their relationships with the environmental factors (soil properties and climate parameters), the soluble protein, soluble sugar, malondialdehyde (MDA), and superoxide anion contents as well as the plasma membrane permeability of *Lonicera caerulea* leaves are very sensitive to environmental changes. For plants established at differing elevations, the leaf traits appear able to correspondingly adapt to the local habitat.

Conclusions: In general, the growth of *Lonicera caerulea* at low and high altitude areas on the northern slope of Changbai Mountain is easily restricted by various environmental factors, resulting in its poor growth condition there. By contrast, this shrub grows well at mid-elevations; thus, the planting area of *Lonicera caerulea* should be expanded to those areas to increase this shrub's fruit yield and quality.

Introduction

Osmotic regulation substances are essential for plant growth and development, and they take on greater significance under adverse habitat conditions (Bai et al.2020). In recent years, many studies (Liu et al.2021; Tian et al.2017) have investigated the effects of elevation on the physiological and biochemical characteristics of plants caused by changes in their ecological factors. Malondialdehyde (MDA), soluble protein, and soluble sugar contents are often used to explore the physiological and biochemical adaptation mechanisms of plants to changes in certain ecological factors (Fu et al.2018). The MDA content can express the degree of oxidative stress suffered by plants under low temperature stress, so, to a certain extent, the change in the malondialdehyde content can be measured to gauge response and endurance of plants to adverse conditions (Sun et al.2017). Soluble protein and soluble sugar can ensure that cell turgor goes unchanged to maintain normal growth of plants (Zhou et al.2017). Furthermore, the status and structure of the plant cell membrane can be reactive; the plasma membrane is the thin membrane that binds the cytoplasm of a plant cell to the cell wall. In an inhospitable environment, for example, one marked by extreme temperatures or dry conditions, the plasma membrane will incur damage varying in magnitude. If the plasma membrane permeability is too high (i.e., > 50%), irreversible damage to

the plant cells can arise, which could directly lead to cell death (Mansour.2019). The superoxide anion radical is the earliest and most representative reactive oxygen-free radical in organisms. Excessive accumulation of superoxide anions can lead to lipid peroxidation of unsaturated fatty acids, breaking the dynamic balance of free radical production and scavenging in plant cells, thereby initiating membrane lipid peroxidation, which destroys the integrity of the cell membrane (Jung-Hwa et al.2018).

Along altitudinal gradients, changes in local ecological factors are stark. With an increase in elevation, the environmental temperature and local availability of resources will decrease, precipitation and solar radiation will increase, and vegetation will exhibit distinct vertical zonation (Klab et al.2020). Studies have shown that shifts in ecological factors can significantly alter the osmotic regulatory substances and plant cytoplasm membranes, so the gradient formed by changing elevation of mountainous regions is considered a decisive factor (Ortiz-Montiel et al.2020). As a key factor that affects plant growth, elevation drives differential altitude-related environmental factors, including temperature, humidity, illumination, and air pressure, which tend to exhibit stepwise, predictable patterns. The predictable heterogeneity in environmental factors driven by elevation changes can directly affect the growth and development of plants (Cao et al.2020; Sun et al.2018), including their structural, physiological and biochemical characteristics (Bibi et al.2019; Dugasa et al.2019), as well as many other aspects. For some species, growing at too high or too low an altitude can induce stress in plants because of changes in associated ecological factors (temperature and light, to name a few). By analyzing the changes in the substances in plant populations established at different altitude, the most suitable habitats for plant species' growth could be identified. Therefore, it is of great significance to study the effects of altitude upon the above-mentioned physiological and biochemical characteristics of plants.

Lonicera caerulea L. is a perennial deciduous shrub. It is a pure, natural and green edible berry that is globally rare, but its wild resources are abundant in the Changbai Mountain area (Zang et al.2020). The fruit is rich in anthocyanins, amino acids, various vitamins, and various trace elements that the human body must obtain from food, and it has the ability to treat cardiovascular diseases, improve liver detoxification, stabilize blood pressure, treat anemia in the elderly, and resist some viruses and tumors (Luan et al.2021; Zhou et al.2018). It has high commercial, medicinal, and nutritional values, whose prospects for development and utilization are broad and promising (Li et al.2020; Lynne et al.2019). In this study, the osmotic regulating substances and plasma membrane permeability of *Lonicera caerulea* leaves were examined to test two hypotheses. (1) The osmotic regulation substances of *Lonicera caerulea* increased with rising elevation (Wu et al.2019), and (2) plasma membrane permeability of *Lonicera caerulea* declined with elevation (Wu et al.2019). According to our results, the ecological conditions most suitable for *Lonicera caerulea*'s growth were explored, and the relationship between the growth and development of *Lonicera caerulea* and its habitat further investigated. This work thus provides an important theoretical basis for the introduction and domestication of *Lonicera caerulea* and the protection and rational management of its precious natural germplasm gene banks.

Material And Methods

Study site

As one of the key protected forest areas in China, the Changbai Mountain Nature Reserve (41°42'–42°25' N, 127°43'–128°17' E) covers a total area of 196,465 hm², and its main peak reaches 2744 m a.s.l. (Zhao et al.2004; Cao et al.2008). The annual precipitation ranges from 700 to 1400 mm, 60% of which arrives in summer. Since the local temperature is low, there is far less evaporation than precipitation, which leads to the Changbai Mountain area being perennially wetter, with an average annual relative humidity of 70%. The annual average temperature can be as low as 2°C, and the annual average wind speed is 11.7 m/s. The average annual temperature differential along the mountain slope is at least 10°C (Yan et al.2020), accompanied by typical characteristics of a natural vertical landscape in a mountainous area. Here there is little precipitation in winter, more precipitation in summer, a dry spring, and a foggy

autumn (Guo et al.2021). Distinctive vegetation communities are found, which can be clearly divided into five vertical zones (ascending): broadleaved forest (<500 m), mixed coniferous and broadleaved forest (500–1100 m), coniferous forest (1100–1700 m), birch forest (1700–2100 m), and alpine tundra (>2100 m) (Zhang et al.2020).

Sampling of plants and soil

Based on a preliminary investigation of *Lonicera caerulea*'s occurrence on the northern slope of Changbai Mountain, a transect line was set along the road in July 2018 that spanned an elevation range of 800–1800 m and bisected a continuous distribution of shrubs. Along this transect, a sampling point was set up for every 200-m elevation rise, for a total of seven sampling sites. At these seven elevations (600, 800, 1000, 1200, 1400, 1600, and 1800 m), in each sampling area, 10-year-old plants (n = 30) at least 10 m apart were randomly selected among the *Lonicera caerulea* population (in the horizontal direction of each population per elevation level). To be included, the plant samples had to be growing normally, with no signs of diseases and insect pest infestations, or obvious structural defects. Three standard branches of each plant were randomly selected among five directions (east, south, west, north, and the top of the shrub), from which the second and third pairs of fully-grown and expanded leaves were collected from each branch tip. These leaves were removed and placed into a plastic self-sealed bag, then immediately taken to the laboratory in a portable refrigerator and stored in an ultra-low temperature refrigerator for later testing. At the same time, soil from a depth of 0–20 cm was collected close to each sampled plant. The collected samples were from 10 shrub individuals at each elevation. Soil samples were mixed to form one composite soil sample. Three replicate soil samples were obtained per elevation level and separately placed into cloth bags and transported to the laboratory for analysis. Finally, at each sampling point location, plant sampling time, surface vegetation status, and meteorological data were recorded.

Soil and leaf variables

All the soil properties were measured according to the national standards. The available P was extracted using sulfuric acid and hydrochloric acid and was determined using the molybdenum-antimony colorimetric method (Chen et al.2000). The available K was extracted using ammonium acetate and was determined using a flame photometer, as was the total K. The hydrolysis N was determined using the alkali diffusion method (Wei et al.2008). The phosphorus in the soil was dissolved using boric acid (sulfuric acid-perchloric acid) and was determined using the molybdenum-antimony colorimetric method. The full N was determined using an automatic Kjeldahl apparatus. The pH of the soil was determined using the potential method. The potassium dichromate oxidation and reheating method was used to determine the soil organic matter. The soil samples were collected using a ring cutter with a volume of 100 cm³ to measure soil bulk density; then they were sealed in cloth bags and transported to the laboratory, where each was weighed to determine its bulk soil density.

The low temperature-preserved leaves to be tested were taken out; each leaf trait was analyzed in triplicate, with 0.3 g per leaf sample used. Each physiological and biochemical index was measured following the methods described by Jing (Shu et al.2021). Specifically, the soluble protein was dyed using Coomassie bright blue; the soluble sugar was colorimetrically analyzed using anthranone; and the malondialdehyde (MDA) was treated using the thiobarbituric acid method. The plasma membrane permeability was determined using the conductivity method; and the superoxide anion content was determined using the pyrocatechol method.

Statistical analysis

Data for the five measured leaf traits (soluble protein, soluble sugar, malondialdehyde (MDA), superoxide anion content, and plasma membrane permeability) were analyzed. First, one-way ANOVA was performed for each leaf trait variable. Each leaf trait variable was compared on a pairwise basis using Duncan's multiple range test in SAS v9.1 software. To

determine whether there was multicollinearity among independent variables, SPSS v19 was used, and any non-significant factors were removed. After this step (confirming no multicollinearity), the data was suitable for multiple stepwise regression analysis, and backward stepwise regression analysis was conducted.

Results

Environmental conditions at different elevations

The soil factor analysis results for the *Lonicera caerulea* at different elevations on the northern slope of Changbai Mountain are presented in Table 1. Evidently, as the altitude increased, the various soil factors of the *Lonicera caerulea* changed significantly. According to the grading standards for the soil available N, P, and K, the soil hydrolysis N content at 800 m altitude was 89.95 g/g, i.e., less than 90 g/g, which indicates a state of deficiency. The available P contents of the soil at 1600 and 1800 m were 8.77 and 5.36 g/g, respectively, i.e., less than 10 g/g, which indicates a state of deficiency. The available K contents of the soil ranged from 71.32–99.52 g/g at 1000–1400 m above sea level, i.e., less than 100 g/g, in which indicates a state of deficiency and imposes a certain limitation upon plant growth.

Table 1 Environmental factors that might influence the leaf traits of *Lonicera caerulea* at different elevations

Elevation (m)	Organic matter (%)	Hydrolysis N (μg/g)	Available K (μg/g)	Available P (μg/g)	Total N (%)	Total K (%)	Total P (%)
800	1.945	89.952	310.118	24.336	0.117	2.236	0.021
1000	7.262	130.322	88.848	13.848	0.231	1.811	0.027
1200	6.370	184.516	71.324	14.303	0.105	1.484	0.010
1400	10.970	450.461	99.515	24.712	0.407	1.180	0.020
1600	8.525	187.444	146.453	8.774	0.173	1.327	0.022
1800	9.975	396.219	248.515	5.364	0.436	1.269	0.029

Data values are the mean ± s.d. of three independent determinations; the same below.

Continued Table 1

Elevation (m)	Mean annual precipitation (mm)	>5°C accumulated temperature (°C)	Salt leaching pH	precipitation from June to September (mm)	Moisture index	Bulk soil density (g/cm ³)
800	703.62	2285.25	4.630	500.4	2.21	1.039
1000	755.19	1972.49	3.960	537.07	2.82	0.811
1200	810.53	1702.53	3.300	576.43	3.43	0.846
1400	869.92	1469.52	3.290	618.67	4.04	0.548
1600	933.67	1268.4	3.320	664.01	4.65	0.981
1800	1002.09	1094.81	4.010	712.67	5.26	0.689

Continued Table 1

Elevation (m)	Average annual temperature (°C)	Days with snow cover (d)	Drying index	Average temperature in January (°C)	Average temperature in July (°C)	Annual frost-free period
800	2.32	137.58	0.63	-17.64	19.07	116.5
1000	1.29	151.16	0.56	-18.27	17.95	108.12
1200	0.27	164.73	0.5	-18.89	16.84	100.31
1400	-0.75	178.31	0.44	-19.52	15.73	93.06
1600	-1.78	191.88	0.39	-20.15	14.61	86.34
1800	-2.8	205.46	0.35	-20.77	13.50	80.10

Effects of the altitude gradient on the physiological and biochemical indicator traits of the *Lonicera caerulea* leaves

Soluble protein content

The changes in the soluble protein content with altitudes are shown in Fig. 1. As can be seen from Fig. 1, the soluble protein content initially increased and then decreased with increasing altitude. The soluble protein content was lowest at 800 m and was highest at 1400 m. Although the soluble protein content at 800 m was not significantly different from that at 1800 m, it was significantly different from those at other altitudes. Compared with the content at 800 m above sea level, the soluble protein contents at 1000 m, 1200 m, and 1400 m increased by 13.3%, 26.5%, and 37.1%, respectively. Above an altitude of 1400 m, the soluble protein content gradually decreased with increasing altitude. Compared with the content at 1400 m above sea level, the contents at 1600 m and 1800 m were 6.1% and 22.7% lower, respectively.

Soluble sugar content

As shown in Fig. 1, the soluble sugar content increased with increasing elevation, but decreased at 1800 m. The soluble sugar content reached the maximum value at 1600 m and the minimum value at 800 m. The soluble sugar content at 1600 m was significantly correlated with those at other altitudes. Compared with the content at 800 m, the soluble sugar contents at 1000 m, 1200 m, 1400 m, 1600 m, and 1800 m increased by 26.3%, 32.7%, 51.9%, 100.1%, and 57.1%, respectively.

Malondialdehyde (MDA) content

As can be seen from Fig. 1, the MDA content initially increased, then decreased, and finally increased with increasing altitude. The MDA content of the leaves of the *Lonicera caerulea* growing at an altitude of 1000 m was the lowest. Compared with the content at 1000 m, the MDA contents at 800 m, 1200 m, 1400 m, 1600 m, and 1800 m increased by 14.0%, 32.7%, 42.9%, 9.3%, and 61.5%, respectively. Although the MDA content at 1000 m was not significantly different from those at 800 m and 1600 m, it was significantly related at other altitudes.

Plasma membrane permeability

As can be seen from Fig. 1, the plasma membrane permeability of the *Lonicera caerulea* initially decreased and then increased with increasing altitude. In both the low and high altitude areas, the plasma membrane permeability was higher; while in the intermediate altitude areas, the plasma membrane permeability was lower. The maximum value of 3.20% occurred at 1800 m above sea level, and it is also higher at 1600 m, with a value of 1.80%. The minimum value of 0.30%

occurred at 1000 m above sea level. The plasma membrane permeability at 1800 m was significantly different from those at other altitudes.

Superoxide anion content

As shown in Fig. 1, the superoxide anion content of the *Lonicera caerulea* initially decreased and then increased with increasing altitude, the maximum superoxide anion content of 55.20 g/g occurred at 1800 m, an increase of 73.9% compared with that at 1600 m. The minimum value of 26.26 g/g occurred at 1200 m above sea level. The superoxide anion content at 1800 m was significantly different from those at 800 m, 1000 m, 1200 m, 1400 m, and 1600 m; and the superoxide anion content at 1200 m was significantly different from that at 800 m, 1000 m, 1600 m, and 1800 m.

Correlations between environmental factors and various indicator traits of the *Lonicera caerulea* leaves at different elevations

Table 2 shows the correlation analysis of the osmotic regulation substances and environmental factors for the *Lonicera caerulea* leaves at different elevations. Among the possible correlations between leaf traits and all environmental factors (soil and climate), organic matter, mean annual precipitation, moisture index, days with snow cover and total P content were positively correlated. By contrast, bulk soil density, available K, total K content, salt leaching pH, mean annual temperature, >5°C accumulated temperature, average temperature in January, average temperature in July, and drying index were negatively correlated. Finally, among the possible correlations between superoxide anions and all environmental factors (soil and climate), the average annual temperature, >5°C accumulated temperature, and average temperature in July were positively correlated.

Table 2 Correlation analysis of each leaf trait indicator and environmental factors

Osmotic regulating substance	Soluble protein	Soluble sugar	Malondialdehyde	Superoxide anion	Plasma membrane permeability
Bulk soil density	-0.67	-0.92*	-0.61	-0.65	-0.50
Hydrolysis N	0.81	0.91*	0.84	0.71	0.49
Total N	0.55	0.83	0.67	0.63	0.32
Available K	-0.93*	-0.35	0.14	-0.05	-0.54
Total K	-1.00**	-0.97**	-0.78	-0.87*	-0.85*
Available P	-0.21	-0.48	-0.38	-0.67	-0.98
Total P	-0.32	0.23	-0.02	0.71*	0.62
Organic matter	0.91*	0.97**	0.63	0.81*	0.72*
Salt leaching pH	-0.96**	-0.65	-0.36	-0.49	-0.93
Average annual temperature	-0.88*	-0.96*	-0.91*	1.00**	0.56
Mean annual precipitation	0.86	0.96**	0.91*	0.99**	0.57*
>5°C accumulated temperature	-0.92*	-0.94*	-0.90*	0.94**	0.50
Precipitation from June to September	0.86	0.96**	0.91*	0.45	0.55*
Average temperature in January	-0.88*	-0.96*	-0.91*	0.63	0.67
Average temperature in July	-0.88*	-0.96*	-0.91*	0.87*	0.74
Annual frost-free period	-0.90*	-0.95*	-0.91*	0.21	-0.68*
Days with snow cover	0.88*	0.96*	0.91*	0.89*	0.55*
Drying index	-0.91*	-0.94*	-0.91*	-0.59*	-0.81*
Moisture index	0.88*	0.96*	0.91*	0.67*	0.76*

** denotes highly significant ($P < 0.01$), *denotes significant ($P < 0.05$).

Regression analysis of environmental factors to predict leaf indicator traits of *Lonicera caerulea* at different elevations

To determine the leading factors affecting the contents of the osmotic regulation substances and the plasma membrane permeability, multiple linear stepwise regression analysis was performed on the data using the stepwise screening method. These results are presented in Table 3. Evidently, the dominant environmental factor affecting the soluble protein content was the total K content (X), with a fitted regression equation is $Y = 2.37 - 0.46X$. The dominant environmental factors influencing the soluble sugar content were the total K content (X_1), the total P content (X_2), and the organic matter (X_3). The degree of influence of these three environmental factors follows the order total K content > total P content > organic matter, and the fitted regression equation is $Y = 44.67 - 12.75X_1 + 170.06X_2 - 0.42X_3$. The dominant environmental factor affecting the MDA content was the hydrolyzability N (X), and the fitted regression equation is $Y = 5.49 + 0.0075X$. The dominant environmental factors affecting the superoxide anion content were the total K content (X_1) and the total P content (X_2); and the fitted regression equation is $Y = 128.80 + 0.76X_1 - 1.15X_2$. The dominant environmental factor affecting the permeability of the plasma membrane was the total K content (X), and the fitted regression equation is $Y = 9.142 - 0.54X$.

Table 3 Multiple regression analysis between each leaf trait indicator and the environmental factors

Osmotic regulating substance	Environmental factors	Stepwise analysis results		
		R ²	F-value	Pr > F
Soluble protein	Total K	0.9918	361.11	0.0003
Soluble sugar	Total K	0.9438	50.44	0.0057
	Total P	0.0520	24.87	0.0379
	Organic matter	0.0042	585.79	0.0069
Malondialdehyde	Hydrolysis N	0.6943	9.09	0.0394
Superoxide anion	Total K	0.7542	170.84	0.0218
	Total P	0.2399	38.19	0.0129
Plasma membrane permeability	Total K	0.9507	313.01	0.0008

Discussion

The environmental factors in mountainous areas are fundamentally and strongly determined by altitude, such as rapid seasonal and daily changes in temperature, low atmospheric pressure, low CO₂ concentration, short period of vegetation growth, and enhanced solar ultraviolet radiation intensity, all of which are closely related to plant growth (Dinh et al.2019). Studies have shown that soluble protein can slow down excessive loss of water in plants and enable them to grow normally under adverse conditions, such as at low temperature and dry conditions, which is related to the cold-tolerance ability of plants (Veselá et al.2020; Li et al.2021; Silva et al.2020). In this study, the soluble protein contents of the *Lonicera caerulea* leaves initially increased and then decreased with increasing altitude. Some scholars have found that increases in UV-B radiation can lead to increased soluble protein contents (Lu et al.2019). At an elevation of 800 to 1400 m, *Lonicera caerulea* can increase the accumulation of soluble proteins to adapt to temperature, light, and radiation damage. When the elevation is above 1400 m, low temperatures, strong light and a large amount of radiation inhibit the accumulation of soluble proteins of *Lonicera caerulea* leaves, causing damage to *Lonicera caerulea* leaves. In addition, high intensity light at high elevation will lead to excessive accumulation of reactive oxygen species, leading to lipid peroxidation, protein damage, and eventually cell death (Kuang et al.2019).

Soluble sugar is an important water-retaining agent in plants. Some scholars have concluded that it functions crucially in protecting and preventing protein solidification at low temperature (Chen et al.2017), while others believe that soluble sugar is related to the cold-resistance of plants (Qian et al.2020). In this study, the soluble sugar content also initially increased and then decreased with increasing altitude, reaching its maximum at 1600 m. The soluble sugar content can reflect the ability of plants to convert sugars. The lower the soluble sugar content, the stronger the ability of the plant to convert sugar into starch, and vice versa (Zhou et al.2020). The soluble sugar content increased gradually at elevations of 800 to 1600 m, which improved the water retention capacity of *Lonicera caerulea*. As such, *Lonicera caerulea* grows well at moderate elevations. The soluble sugar content in the high elevation area decreased due to environmental factors such as low temperature and strong UV-B radiation on Changbai Mountain. We found that soluble sugar and soluble protein contents were significantly positively correlated, as expected (Cardoso et al.2020). The variation in the soluble sugar content was similar to that of the soluble protein content at the same altitude (800–1800 m). The decrease in the soluble sugar content at higher altitude may have arisen from the synergistic effect between soluble protein and soluble sugar.

With greater elevation, the temperature decreases, and UV-B radiation is augmented, among other changed abiotic factors. The plasma membrane is the primary site where plants are harmed by low temperature, strong UV-B radiation, and other adverse conditions; and this damage to the plasma membrane is mainly caused by ROS that are generated under adverse conditions. The presence of ROS triggers membrane lipid peroxidation, whose products include MDA that is highly toxic. It not only severely damages the cell membranes, but it also causes oxidative damage to the biological molecules in cells, such as proteins and nucleic acids. Therefore, the MDA content is indicative of the magnitude of damage incurred by the cytoplasm membrane (Riasat et al.2019). In our study, except for the low MDA contents at 1000 m and 1600 m, at all of the other elevations the MDA content increased slightly with altitude, indicating that *Lonicera caerulea* has a strong ability to mitigate stress. The study's results are similar to the changes in the MDA content of fruit trees at different elevations in the Dapanshan Nature Reserve in China found in a previous study (Kang et al.2008). Stimulated by adversity, *Lonicera caerulea* can stimulate the accumulation of osmotic regulating substances and enhance the activity of antioxidant enzymes, thus lessening the potential for damage and injury. In terms of plasma membrane stability, the MDA content was the lowest in the intermediate altitude area, suggesting the latter offers a more suitable growth environment for *Lonicera caerulea*.

With a rising altitude, the air density decreases, the atmosphere becomes thinner, the water vapor and CO₂ contents decrease, the temperature drops, and the air molecules that absorb, radiate, and conduct heat becomes less abundant. Moreover, the UV-B radiation in high-altitude areas is significantly higher than that in low-altitude areas. Accordingly, we found that the plasma membrane permeability and superoxide anion contents were augmented in high altitude areas and diminished in the intermediate altitude areas, indicating that the harsh conditions at high elevations were more harmful to the *Lonicera caerulea* plants. This adversity led to an imbalance in the oxygen metabolism of the plants, the accumulation of large quantities of superoxide anion radicals, with relatively high amounts of membrane lipid peroxidation, which agrees with finding of other studies (Li et al,2018; Shen et al.2017). The conductivity measured in our study ranged from 6.6–17.99%, which is much lower than the degree of irreversible damage (50%) of the plasma membrane (Mansour.2019). Hence, it is not sufficient to directly cause plant cell death. A previous study showed that *Lonicera caerulea* populations at different elevation gradients responded to environmental changes by altering their phenotypic traits (Li et al.2020). The above results indicate that *Lonicera caerulea* cells developed well in the intermediate altitude areas where they could maintain their optimal growth state. The results demonstrated the studied shrub species is capable of strong phenotypic plasticity across an altitudinal gradient.

Most *Lonicera caerulea* have a wide distribution range, which requires the plants to have excellent regulatory mechanisms to adapt to changes in different habitat conditions. *Lonicera caerulea* is globally distributed and economically and socially valuable. As such, more ecological studies should be conducted to explore the interaction between geographic and climatic factors and the underlying mechanisms of aquaculture at the physiological and biochemical levels, to provide a basis for aquaculture and wild moss population protection, and to realize the ecological and economic benefits of *Lonicera caerulea*.

Conclusions

In summary, on the northern slope of Changbai Mountain, along an elevation gradient, the osmotic regulating substances and plasma membrane permeability of *Lonicera caerulea* change predictably. The leaves' soluble sugar and soluble protein contents were relatively high in the mid-altitude areas, while their plasma membrane permeability and superoxide anion and MDA contents were relatively low. Therefore, the intermediate altitude area of Changbai Mountain in China is deemed more favorable for the stable growth of *Lonicera caerulea* and its populations. Because of its edible and medicinal value, this plant is widely distributed in the Changbai Mountain region, where its growth ability is robust. Accordingly, this study's findings suggest the planting area of *Lonicera caerulea* could be expanded into this altitudinal zone, to lay a more secure foundation for increasing this shrub's fruit yield and quality.

Declarations

Ethics approval and consent to participate

This article has not been submitted to any other journal, and the manuscript has not been previously published (in part or in whole). Submission of this manuscript has been approved by all authors.

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Authors' contributions

All authors contributed to sampling design. Qi Qige led field resampling, data analysis, and writing. All authors contributed to data analysis and commented on drafts of the paper. All authors read and approved the final draft of the paper.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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Figures

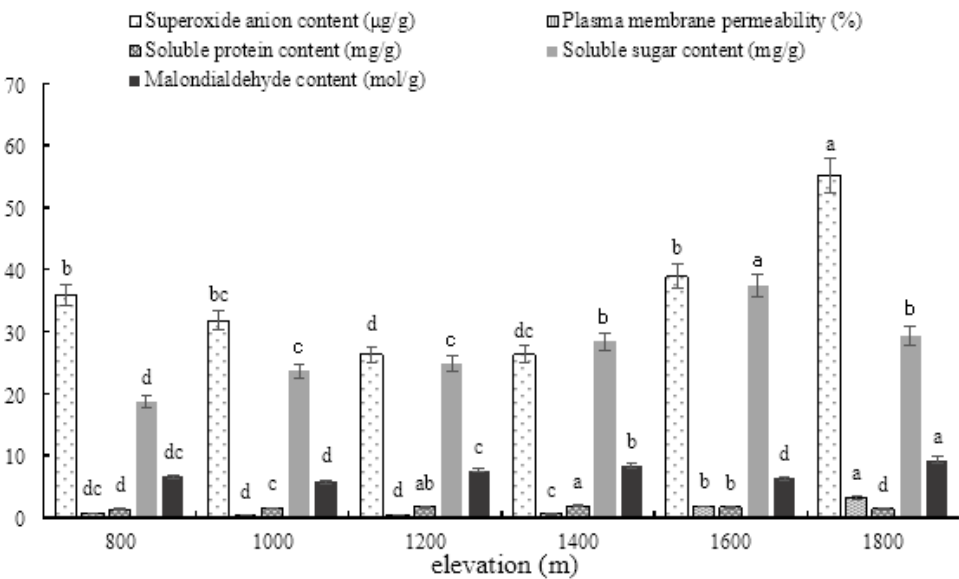


Figure 1

Changes in the contents of the three osmotic regulatory substances, the superoxide anion content, and the plasma membrane permeability of *Lonicera caerulea* leaves across the altitudinal gradient Note: The different lowercase letters indicate significant differences for a given leaf trait variable between the elevation levels ($P \leq 0.05$)