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Effects of Different Temperature Treatments on Seed Germination and Seedling Growth of *Rorippa sylvestris*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Temperature is one of the important conditions for plant growth, and an important factor affecting the establishment and spread of invasive plants. In this study, the northern perennial invasive plant (*Rorippa sylvestris*) was taken as an example. By comparing the seed germination rate, germination potential, germination index and vigor index, the total number of leaves, root length, root length, vigor index, leaves of seedings, seedling biomass and root- shoot ratio under different temperature treatments (15, 20, 25, 30, 35, 40℃), the subordinate function was used to compare the temperature tolerance of *R. sylvestris in seed germination. sylvestris* in seed germination and seedling growth. To study the response and tolerance of seed germination and seedling growth of invasive *R. sylvestris to different temperatures, the subordinate function was used to compare the temperature tolerance of R. sylvestris* in seed germination and seedling growth. The results showed

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that high temperature promoted seed germination of *R. sylvestris* and limited radicle growth, and it had the most tolerance at different temperatures. The results showed that high temperature promoted seed germination of R. sylvestris and limited radicle growth, and it had the strongest tolerance at 35℃. Lower temperature was more beneficial to material accumulation of seedlings, and the tolerance of *R. sylvestris was more* favorable. The results provide theoretical basis for revealing the diffusion and invasion mechanism of *R. sylvestris.*

Keywords: Rorippa sylvestris; temperature; tolerance; membership function method.

1. INTRODUCTION

Global warming and biological invasions are important factors affecting biodiversity and ecosystem functioning. The spread of invasive species has seriously threatened global biodiversity, ecosystem structure and function, social production and human health [1]. Under the general environment of global warming, the temperature rise in northern China has intensified. Over the past 50 years, there has been an obvious warming trend in the average temperature of the northeast region, and the warming rate of the minimum temperature in Liaoning Province is about twice that of the maximum temperature [2]. Seed germination, as the basis for the expansion and spread of weeds [3], is a key link in the transformation of potential populations into actual populations, and plays an extremely important role in the reproduction, settlement, dispersal of individual populations as well as in the resistance to adverse environments [4,5], seedling stage is an important period in the process of plant development [6], the adaptability of the seedling to the outside world directly influences the ability of the plant to complete the entire reproductive period successfully, and, in the case of invasive species, it is related to the establishment of the entire population. In the case of invasive species, this will be related to the establishment of the whole population. Temperature is one of the most important factors affecting plant invasion, seed germination and seedling growth [7,8], providing opportunities for successful invasion of exotic species.

Rorippa sylvestris are Brassicaceae *Rorippa* perennial herbaceous plants, early spring sprouting earlier, more sensitive to temperature. It is native to Europe and southwestern Asia, China was initially distributed in Xinjiang Yining, and then gradually spread to Qinghai, Tibet, Gansu and other places have distribution [9], in recent years jumped to Dalian, Liaoning, Shenyang, Tieling, etc., is listed as a new record species in Liaoning [10,11,12]. *Rorippa sylvestris* can reproduce through seeds and root tiller buds. Generally, seed reproduction is the mainstay of new invasions, and when a large group has formed at the invasion site, it mainly relies on the sprouting of root tillers to spread; the root system is well-developed, and it has a strong ability to invade the soil, asexual reproduction ability, and the ability to adapt to the environment [12]. At present, *Rorippa sylvestris* has formed several single-optimized communities in the green belts of Liaoning area, and has shown explosive growth, which is more harmful.

Given that *Rorippa sylvestris* is able to reproduce rapidly and establish a single-optimal community after invading a new ecosystem, this experiment was conducted to investigate the response and tolerance of *Rorippa sylvestris* seed germination and seedling growth to different temperatures by using the seeds and seedlings at the early stage of invasion, with a view to providing theoretical basis for the study of *Rorippa sylvestris*'s invasion mechanism.

2. MATERIALS AND METHODS

2.1 Materials

Rorippa sylvestris seeds were collected from the north side of the College of Plant Protection, Shenyang Agricultural University, and the weight of 1,000 grains was 0.0524 g. The seeds were kept in a dry environment after shade-drying.

2.2 Methods

2.2.1 Seed germination experiments

The experiment was conducted using the paper-dish method. Seeds of *Rorippa sylvestris* with normally ripe and full seeds were selected, soaked in distilled water for 12h, and placed in Petri dishes lined with double-layer filter paper (50 seeds per dish). They were placed in a light incubator at 15°C, 20°C, 25°C, 30°C, 35°C and 40°C, respectively, and the incubator was treated with 16h of light (intensity 4000lx) and 8h of darkness daily. Each treatment was replicated three times. Seed germination was measured by the radicle breaking through the seed coat ≥1 mm, and the number of seed germination was recorded every day during the test, and the filter paper was kept moist, the number of seed germination was counted every day, and the time of the first germination and the germination duration, and the germination was considered to be finished if no new seeds germinated for 2 consecutive days. The germination rate, germination potential, germination index and vigor index of *Rorippa sylvestris* seeds were calculated at different temperatures.

2.2.2 Seedling growth experiment

The seeds of *Rorippa sylvestris* were sown evenly on a bed of moist nutrient soil and placed in a light incubator at 30℃ with 16h of light (intensity 4000lx) and 8h of darkness every day to promote germination. After germination, the seeds were placed at room temperature, and after two months, 50 *Rorippa sylvestris* seedlings each of similar size and growth were selected and transplanted into small pots (8 cm of height and 8 cm of diameter), and subjected to different temperature treatments: the parameters of the light incubator were set to be 15, 20, 25, 30, and 35 ℃, The light-dark ratio to be 16/8h (light 4000lux), and 10 replications were done for each temperature gradient. After 30 days, the growth conditions were recorded, including: total number of leaves, root length, number of lateral roots, number of secondary lateral roots, and the root dry weight and crown dry weight of the seedlings were determined by using an electronic balance.

2.2.3 Statistics and calculations

Germination rate: $G=n/N\times100\%$.

Where: G is the germination rate, n is the number of normal seedling generated at the end of germination test; N is the number of seeds for test.

Germination vigor: $Gr = m/N\times100\%$,

Where: Gr is the germination potential,m is the number of germinated seeds at the peak of germination, and N is the number of test seeds.

Germination indexes: $Gi = \sum(Gt/D)t$

Where: Gi is the germination index,Gt is the number of germination on day t and Dt is the number of days to germination.

Vigor index (Germination vigor index) $Gv = Gi$ x seedling root length (cm) Plant height: the distance from the base to the top of the plant;

Total number of leaves (Total number of leaves): all functional leaves of the whole seedling; Root length (Root length): the length from the root-stem junction to the root tip;

Number of lateral roots per plant: the main root grows to a certain length, in a certain part of the lateral from the inside to produce a number of branch roots;

Seedling biomass (Total dry weight): dry weight of the whole seedling;

Biomass root to shoot biomass ratio (Root to shoot biomass ratio) = root dry weight/crown dry weight. The value of the affiliation function: $R(Xi)=(Xi-Xmin)/(Xmax-Xmin)$.

Where: X is the measured value of a certain index of the reference plant; Xmax and Xmin are the maximum and minimum values of the index in all the test materials, respectively. The relative germination rate, the relative germination potential, the relative germination index and the relative root length were obtained by the subordinate function value calculation of the germination indicators, and the average value of the subordinate function value of each indicator was summed up, which was the total function value. The total function value at each temperature in the seedling stage was calculated as above.

Overall evaluation: the larger the value of the total function, the more resistant to that temperature.

2.3 Data Analysis

Data were statistically analyzed using SPSS 22.0 software, plotted in Excel, one-way analysis of variance (ANOVA) was used to compare the significance of differences between different treatments of the same indicator, and multiple comparisons were performed using the DUNCAN method.

Comprehensive evaluation of temperature tolerance of *Rorippa sylvestris* at different temperatures during seed germination and seedling growth stages, respectively, using the fuzzy mathematical affiliation function method [12].

3. RESULTS

3.1 Effects of Different Temperatures on Seed Germination of *Rorippa sylvestris* and Evaluation of Adaptability

3.1.1 Effect of different temperature treatments on the seed germination of *Rorippa sylvestris*

Temperature had a significant effect (P<0.05) on germination rate, germination potential, germination index and vigor index of *Rorippa sylvestris* seeds. These mentioned parameters showed an increasing and then decreasing trend with the increase of temperature, and peaked at 35℃. *Rorippa sylvestris* did not germinate at 15℃, and the parameters listed above reached the highest values at 25-35℃. The vigor index also had a tendency to increase and then decrease, with high vigor at 25℃ - 35℃ and a low peak at 30℃. It indicated that higher temperatures promoted seed germination and either too high or too low temperatures inhibited seed radicle growth (Table 1).

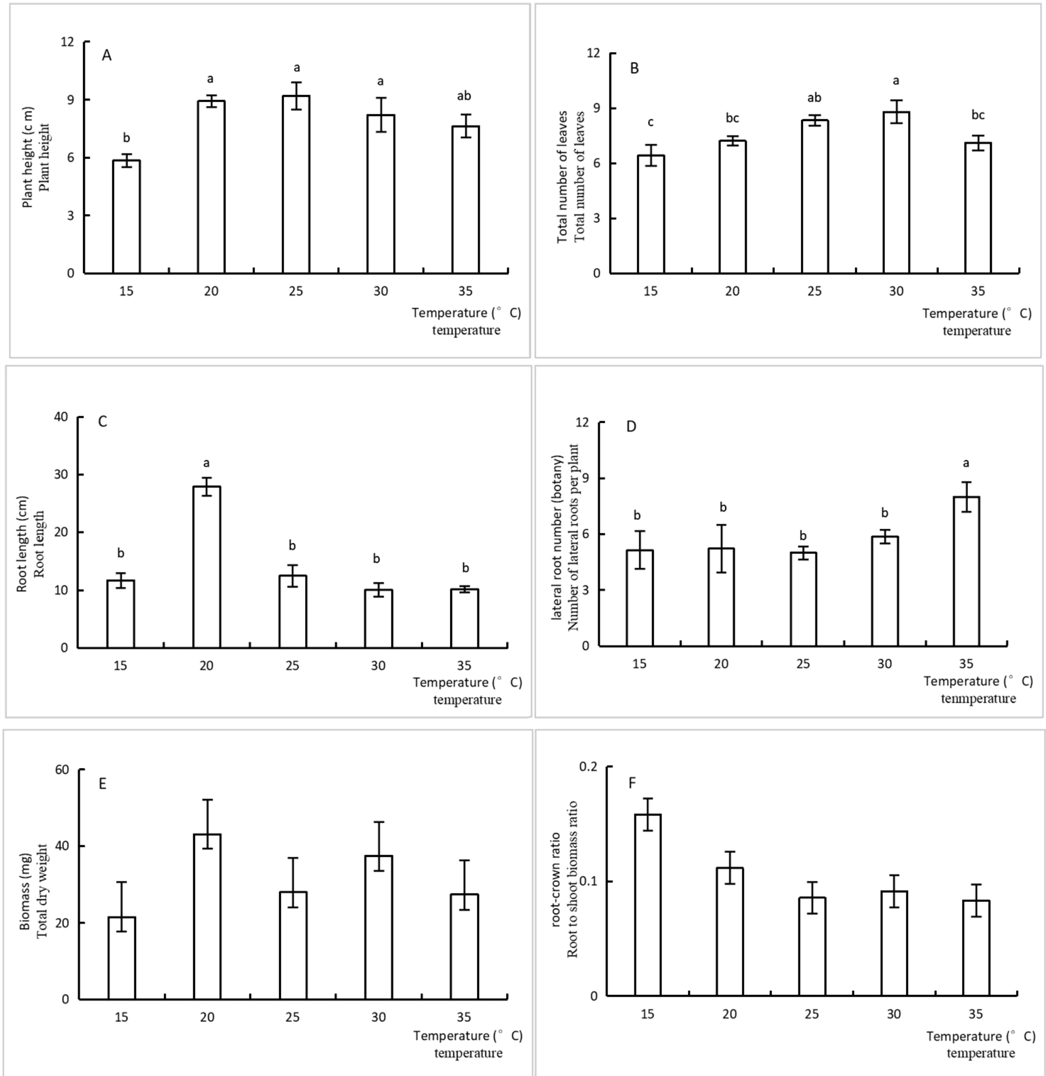

Fig. 1. Effects of different temperatures on plant height (A), total leaf number (B), root length (C), lateral root number (D), biomass (E), and root-crown ratio (F) of Rorippa sylvestris at seedling stage

Note: Different letters indicate that species differ significantly (P<0.05) at different temperatures

Note: Different letters indicate significant differences in species at different temperatures (P<0.05)

Table 1. Effects of different temperatures on germination of *R. sylvestris* Seeds

	Temperature(℃)	Germination rate(%)	Germination vigor(%)	Germination indexes	Germination vigor index
	15	0.00 ±0.00c	0.00 ±0.00d	0.00±0.00c	0.00±0.00c
	20	5.33 ±3.33bc	2.67 ± 0.67cd	0.39±0.25c	0.00±0.00c
R. sylvestris	25	18.00±7.57ab	6.00 ± 2.31c	1.58±0.67ab	3.28±1.38a
	30	18.67±4.67ab	12.00±2.31b	1.73±0.42ab	1.18±0.28bc
	35	30.00±3.06a	17.33±1.76a	2.66±0.30a	2.18±0.25ab
	40	6.00 ±1.15bc	4.00 ±1.15cd	0.88±0.15bc	0.00±0.00c

Note: Different letters indicate that species differ significantly (P<0.05) at different temperatures
Note: Different letters indicate significant differences in species at different temperatures (P<0.05)

Table 2. The membership function value and comprehensive evaluation value of tolerance of *R. sylvestris* seeds during germination

		Subordinate function values					
Species	**Temperature**	**Relative germination rate**	**Relative germination vigor**	**Relative germination indexes(RGI)**	**Relative root length**	**Total function value**	**Ranking**
R. sylvestris	15	0.00	0.00	0.00	0.00	0.00	6
	20	0.33	0.33	0.36	0.00	0.26	5
	25	0.5	0.5	0.51	0.41	0.48	2
	30	0.54	0.5	0.53	0.29	0.47	3
	35	0.6	0.56	0.63	0.26	0.50	1
	40	0.5	0.5	0.64	0.00	0.41	4

Table 3. The membership function value and comprehensive evaluation value of the resistance of *R. sylvestris* seedlings to different temperatures during the growth period

		Subordinate function values					
Species	**Temperature (°C)**	**Relative plant height**	**Relative total number of leaves**	**Relative root length**	**Relative number of lateral roots per plant**	**Total function value (math.)**	**Ranking**
R. sylvestris	15	0.39	0.49	0.36	0.57	0.45	3
	20	0.48	0.41	0.38	0.31	0.40	4
	25	0.35	0.33	0.43	0.40	0.38	5
	30	0.46	0.47	0.40	0.63	0.49	1
	35	0.51	0.53	0.32	0.50	0.47	2

3.1.2 Evaluation of *Rorippa sylvestris* seed germination adaptation to temperature

The fuzzy mathematical affiliation function method was used to comprehensively evaluate the affiliation function values of relative germination rate, relative germination potential, relative germination index, and relative root length of *Rorippa sylvestris* seeds at different temperatures. The results showed that the order of the strength of *Rorippa sylvestris*'s tolerance to different temperatures was 35°C>25°C>30°C>40°C>20°C>15°C. The *Rorippa sylvestris*'s tolerance to different temperatures was higher at higher temperatures than at lower temperatures (Table 2), which was not favorable for seed germination as well as too high or too low temperature (Table 2).

3.2 Effects of Different Temperatures on the Growth of *Rorippa sylvestris* Seedlings and Evaluation of Acclimatization

3.2.1 Effect of different temperatures on the growth of *Rorippa sylvestris* seedlings

Different temperatures had significant effects on plant height, total leaf number, root length and lateral root number of *Rorippa sylvestris* seedlings. All the mentioned parameters increased and then decreased with increasing temperature. However the number of lateral roots gradually increased with increasing temperature ($P<0.05$). *Rorippa sylvestris* plant height, total leaf number, root length, and lateral root number reached their maximum at 25℃, 30℃, 20℃ and 35℃, and their minimum at 15℃, 15℃, 30℃ and 25℃, respectively. Temperatures were too high or too low for the growth of *Rorippa sylvestris* (Fig. 1. A, B, C, and D).

The biomass of *Rorippa sylvestris* seedlings first increased and then decreased with temperature, and the root-crown ratio decreased with temperature, and *Rorippa sylvestris* slightly increased at 30℃. Biomass was greatest at 20°C and root-crown ratio was greatest at 15°C; *Rorippa sylvestris* biomass and root-crown ratio were smallest at 15°C and 35°C, respectively. Lower temperatures favored *Rorippa sylvestris* biomass accumulation and biomass partitioning to roots at lower temperatures (Fig.1 E and F).

3.2.2 Evaluation of the adaptability of *Rorippa sylvestris* to different temperatures at seedling stage

The above results showed that different temperature treatments had a great influence on the growth of *Rorippa sylvestris* seedlings. The tolerance of *Rorippa sylvestris* seedlings to different temperatures was evaluated by the fuzzy mathematical affiliation function through a comprehensive evaluation of the values of different temperature affiliation functions of four indicators at the seedling stage. The results showed that *Rorippa sylvestris* seedlings were tolerant to different temperatures in the order of 30℃>35℃>15℃>20℃>25℃ (Table 3). *Rorippa sylvestris* was tolerant at higher temperatures.

4. DISCUSSION AND CONCLUSION

Plant growth is not only controlled by genetic material, but also by numerous environmental factors, such as light, temperature, water, soil nutrients and so on. Seed germination is one of the key links in the life history of plants, and the response of seeds to germination conditions reflects their ecological response to adapt to the environment [7], and is also the key for invasive plants to settle into new environments. Temperature, as an important environmental variable regulating seed germination, is one of the key ecological factors affecting seed germination [7,8]. Too high or too low will affect the normal growth and development of plants. Yang LP et al. [13] found that the seed germination rate of *Glycine soja* was lower under lowlow temperatures. Also, Li R et al. [14] found that both low and high temperatures were *Tanacetum tatsienense* seed germination.

In this experiment, it was found that too high or too low temperatures (15°C, 40°C) limited the growth of embryonic roots after *Rorippa sylvestris* seed germination and affected their vigor index. Lower temperatures inhibited the germination rate, germination potential, and germination index of seeds, however, higher temperatures promoted seed germination.Germination rate and germination potential reflect the germination speed and neatness of seeds. A high germination rate and strong germination potential predict fast and neat germination and strong seedlings. A high germination rate and low germination potential predict poor germination and weak seedlings [15]. *Rorippa sylvestris* germination rate and germination potential were highest at 35℃. High temperature promoted its seed germination and growth. However, the effect of temperature on seed germination is multifaceted, and the use of a single indicator to evaluate its effect is one-sided, and the use of the affiliation function method for the comprehensive evaluation of plant tolerance has been generally accepted [12], and the use of this affiliation function method can be more comprehensive and more accurate to reflect the tolerance of *Rorippa sylvestris* to different temperatures. In this experiment, the relative value of traits was used to analyze the temperature tolerance of *Rorippa sylvestris* seed germination at different temperatures, and the results showed that *Rorippa sylvestris* seed germination was less tolerant at too high or too low a temperature, but more tolerant at higher temperatures, and *Rorippa sylvestris* was the most tolerant at 35℃. Because *Rorippa sylvestris* did not germinate at 15℃, and root length was <1mm at 20℃ and 40℃. So, *this species* requires high germination temperature; *to initiate* seed germination, which may be its adaptive strategy to cope with global warming.

Temperature plays a very important role in the early growth of plant seedlings. Sha XM et al. [16] showed that low-temperature stress significantly inhibited the growth of seedlings, resulting in a decrease in the growth of plant height, stem thickness, and dry mass compared with the control. Zou H [17] showed that high-temperature stress decreased the accumulation of dry matter in the seedlings of *Rhododendron lαtoucheae*; and Wang JJ et al. [7] showed that different temperatures had different effects on root length and dry matter of *Festuca sinensis* seedlings. The results of this experiment showed that temperature had a significant effect on plant height, leaf number, root length and lateral root number of *Rorippa sylvestris* seedlings. Both plant height and root length had a tendency to increase and then decrease with the increase of temperature, and the effect of low temperature on the growth of plant height and root length of *Rorippa sylvestris* was more obvious, and its plant height was maximized at 25℃, and its root length was significantly higher at 20°C than at other temperatures. High temperature promotes the occurrence of leaves and lateral roots of *Rorippa sylvestris*, which has the highest number of leaves at 30℃ and the highest number of lateral roots at 35℃.

Rorippa sylvestris accumulated the most driest matter at 20℃, but tended to allocate more material to the roots at both low and high temperatures, probably because the growth of the above-ground part of the plant was limited at low temperatures, thus allocating more resources to the roots and showing a larger root-crown ratio, as the temperature increases.

It has been shown that warming can increase the nitrogen content in plant tissues and organs, and increase the supply of nutrients to the plant from the soil environment, thus causing a decrease in root partitioning and an increase in crown partitioning of photosynthesized products [17]. This may be the reason for the decrease in the root-crown ratio of *Rorippa sylvestris* seedlings at elevated temperatures.

35℃ was detrimental to the development of *Rorippa sylvestris* seedlings, indicating that higher temperatures have an inhibitory effect on seedlings growth, which is consistent with the results of the research conducted by Wang JJ et al. [7], Liu FH et al. [18], and Peng LZ et al. [19] on the growth of *Festuca sinensis*, *Gleditsia sinensis, Peganum harmala* at different temperatures. Comprehensive evaluation of the affiliation function of *Rorippa sylvestris* seedlings in terms of plant height, total number of leaves, root length, and lateral roots number showed that this species was more tolerant to higher temperatures, with a maximum at 30℃. The response of *Rorippa sylvestris* seeds and seedlings to temperature was essentially synchronized, with both higher temperatures promoting seed germination and inhibiting seedling growth, and lower temperatures inhibiting seed germination. Under natural conditions high temperatures occur in the summer and are detrimental to seedling growth, which may be an important reason why this clone has very few seeded seedlings under natural conditions. Plants propagated by clonal growth tend to have a very high mortality rate at the seedling stage among the progeny produced by seed germination, and the rate of successful colonization is not as high as that of the cloned progeny. In the case of invasive clones, once seeds germinate and pass the seedling stage, they can be successfully colonized by asexual propagation. Therefore, the study of the response of seeds and seedlings of invasive clones to the environment can partly reveal the mechanism of their successful invasion.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rudgers J A, Orr S. Non-native grass alters growth of native tree species via leaf and soil microbes[J]. Journal of Ecology. 2009;97(2):247-255.
2. JIA J, GUO J. Characterization of climate change in Northeast China over the past 46 years[J]. Arid Zone Resources and Environment. 2011;25(10):109-115.
3. LI X F, ZHOU G Y, WANG J. Effects of temperature, light and moisture on seed germination and seedling growth of Fescue[J]. Grass Science. 2017;34(7): 1452-1458.
4. Baker P J. Seedling establishment and growth across forest types in an evergreen deciduous forest mosaic in western Thailand[J]. Natural History Bulletin of the Siam Society. 1997;45(1):17-41.
5. LI R, QI S, LIU Na,et al. Effects of lead, cadmium and copper on seed germination and seedling growth of four invasive plant species[J]. Journal of Northeast Normal University (Natural Science Edition). 2017; 49(04):101-108.
6. WANG J, WANG Z,MA An,et al. Effects of temperature and moisture on the growth of Chinese fescue seedlings[J]. Journal of Grass Industry. 2016;25(8):65-73.
7. LU X,ZHANG F,YANG Z et al. Effects of different temperature treatments on the physiology and biochemistry of seed germination of milky chicory[J]. Journal of Anhui Agricultural University. 2022; 49(06):913-919.
8. Tsung-Hsin H. *Rorippa sylvestris* (L.) Bess., a Newly Naturalized Mustard Species in Taiwan[J], Taiwania. 2005; 50(4):297-301.
9. ZHANG S, LI Z, WANG M, et al. New record plants of Liaoning[J]. Journal of Liaoning Normal University: Natural Science Edition. 2016;39(3):390-402.
10. MIAO Q, WANG Q, NIU Z, QU B, SHAO M. Occurrence and damage of regional new invasive plant *Rorippa sylvestris* in Shenyang area[J]. Plant Quarantine. 2019;(5)1-4.
11. XU Y F, LIU M, LV Y H,et al. Effects of Temperature on Seed Germination of Invasive Plant Rorippa amphibia (L.) Besser[J]. International. 2018;22(3):1-7.
12. ZHANG S, YUAN F, GUO J,et al. Comprehensive evaluation of salt tolerance in sweet sorghum seedlings using the affiliation function value method[J]. Journal of Plant Physiology. 2015;51(6):893-902
13. YANG L P, ZHANG C, ZHANG N Q. Effect of temperature on seed germination and seedling growth of wild soybean[J].Modern Agriculture. 2022;(10):15-18.
14. LI R, CHEN W L, SUN Z Y, et al. Effects of light, temperature and drought stress on seed germination of Chrysanthemum sinensis in western Sichuan[J].Chinese Traditional and Herbal Drugs. 2023 ;54(19):6443-6451.)
15. Wagdi GQ. LA, MarieHélène W, et al. The Concepts of Seed Germination Rate and Germinability: A Re-Evaluation for Cool-Season Grasses[J]. Agronomy. 2022; 12(6).
16. ZOU H. Effects of high temperature and drought synergistic stress on the growth and physiological characteristics of Rhododendron seedlings[D]. Central South Forestry University of Science and Technology ; 2021.
17. SHA X M, Li J, Patiguli A, et al. Effects of low temperature stress on seed germination of cotton[J].Seed. 2021; 40(02):80-85.
18. LIU F, WANG X, ZHAO Y et al. Effects of different temperatures on the growth and physiological characteristics of soap pod seedlings[J]. Journal of Mountain Agricultural Biology. 2022 ;41(06):22-29.
19. PENG M Z, JI X M, JIANG Li. Effects of temperature and salinity on seed germination and seedling growth of desert plant Camel canopy[J]. Northern Horticulture. 2023;(02):50-56.

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