



The Effect of Temperature on Seed Germination and Seedling Growth of Two Invasive Plants in *Rorippa*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors QW and MS designed the study. Authors JM and YX performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LM and BQ managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: By studying the response of seed germination and seedling growth of invasive plants, *Rorippa amphibia* and *Rorippa sylvestris*, to temperature, the influence of temperature on the invasive ability of two species of *Rorippa* were further analyzed, which provided a theoretical basis for revealing the diffusion and invasion mechanism of two invasive plants in *Rorippa*.

Study Design: Seed germination and seedling growth test of two invasive plants in *Rorippa* at different temperature was studied by means of laboratory culture. The germination percentage, germination index, germination potential of the seeds and the total leaf number, root length, lateral root number, biomass and root shoot ratio of seedlings were determined.

Place and Duration of Study: Seeds were collected from the Shenyang Agricultural University of Liaoning Province in July 2018. Experiments were done in the College of Bioscience and Biotechnology, and conducted in April 2019 for a month.

Methodology: The petri dish method was used at the experiment of seed germination. The seedling growth experiment was carried out by pot sowing.

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Results: At the seedling stage, *R. amphibia* has the strongest tolerance at 30°C, while the *R. sylvestris* does at 35°C. The low temperature is more beneficial to the accumulations of the seedlings, *R. amphibia* is the most tolerant at 25°C, and *R. sylvestris* is at 30°C at the seedling stage.

Conclusion: The response of the seeds and seedlings of the two species to temperature was basically the same. Higher temperature promoted seed germination and inhibited seedling growth, while lower temperature inhibited seed germination. The response of seeds and seedlings of *R. sylvestris* to high temperature makes it more invasive in the process of global warming.

Keywords: *Rorippa*; temperature; seed germination; seedling growth.

1. INTRODUCTION

The spread of invasive species has badly threatened the global biodiversity, the ecosystem structure and function, social production and human health [1]. In the context of global warming, the temperature in northern China has risen sharply. Over the past 46 years, the average temperature in Northeast China had a clear tendency to warm up, and the rate of increase in the minimum temperature in Liaoning Province was about twice that of the maximum temperature [2]. As the foundation of weed propagation and spread harm [3], The seed germination is the key link from potential population to the real population, and plays an extremely important role in the reproduction, settlement, diffusion of individuals and resistance against the adverse environment [4,5]. Seedling is an important stage in the process of plant growth and development [6]. The adaptability of seedling to the external environment will directly affect whether the plant could successfully complete the whole growth term. In the terms of invasive species, this will affect the establishment of the whole population. The temperature is one of the most important factors that affects plant invasion, seed germination and seedling growth [7,8], which provides an opportunity for the successful invasion of alien species.

R. amphibia and *R. sylvestris* are perennial plant of *Rorippa* in the family Brassicaceae. *R. amphibia* originated in Europe and was firstly found in Dalian, China in 2006. *R. sylvestris* originated in Europe and Southwest Asia. It was firstly distributed in Yining of Xinjiang Province, and then diffused to such regions as the Qinghai, Tibet and Gansu, etc [9]. In recent years, it has spread to Dalian, Shenyang, Tieling, and has been listed as the new record species in Liaoning [10,11,12]. Both have similar ecological habits which could reproduce through seeds and root buds. Generally, the new invasion point is mainly

seed propagation. When the large population was formed as the invasion point, the buds of root tillers are used to spread [13]. The root system is developed, while the ability to invade soil, asexual reproduction and environmental adaptability are strong [14]. At present, both *Rorippa* have formed many single excellent communities in the green belt in Liaoning regions, which have shown explosive growth trend and have the huge harm feature.

By studying the response of seed germination and seedling growth of invasive plants, *R. amphibia* and *R. sylvestris*, to temperature, The influence of temperature rise on the invasive ability of two species of *Rorippa* were further analyzed, which provided a theoretical basis for revealing the diffusion and invasion mechanism of two invasive plants in *Rorippa*.

2. MATERIALS AND METHODS

2.1 Materials

R. amphibia seeds were collected through the artificial turf on the South side of the Shenyang Agricultural University in July 2018. The weight of 1000 seeds are 0.0704 g. The seeds of *R. sylvestris* were collected on the North side of Shenyang Agricultural University and the weight of 1000 seeds are 0.0524 g. Mature and plump seeds were selected and stored in a cool and dry place.

2.2 Methods

2.2.1 Experiment of seed germination

The petri dish method was used at the experiment. 50 seeds were randomly selected and soaked for 12h in distilled water. The seeds were placed in a petri dish covered with double filter paper, cultured at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C in the light incubators for 8h darkness and 12h light (4000lux), Each process was repeated three times. The standard of seed germination was that the radicle breaking

out the seed coat was ≥ 1 mm. During the experiment, the number of the seed germination was recorded every day, and the filter paper was kept moist until no new seed germination for 2 consecutive days, which was regarded as the end of germination. The number of seed germination was counted regularly every day, and the time of initiative germination and the duration of germination were recorded. The germination rate, germination trend, germination index and vigor index of *Rorippa* species seeds at different temperatures were calculated.

2.2.2 Seedling growth experiment

The full seeds of *R. amphibia* and *R. sylvestris* were evenly scattered on the bud bed of moist soil germinated in a light incubator at 30°C with the 16h light (the intensity 4000 lux) and 8h darkness. The seeds were cultured at room temperature after seed germinated. After two months, 50 seedlings of *R. amphibia* and *R. sylvestris* were transplanted into 8* 8 cm small flowerpots, cultured at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C in the light incubators for 8h darkness and 12h light (4000lux), Repeat 10 times for each temperature at gradient. After 30 days, such growth indexes as the number of leaves, the length of roots, the number of lateral roots and the number of secondary lateral roots were recorded.

2.3 Determination Index

Germination rate $G = n / N \times 100\%$

In the formula, G is Germination rate, n is the number of normal seedling after the seedling experiment, N is the number of seeds to be tested.

Germination vigor $G_r = m / N \times 100\%$

In the formula, G_r is germination potential, m is number of germinated seeds at the germination peak, N is the number of seeds to be tested.

Germination indexes $G_i = \sum(G_j / D_t)$

In the formula, G_i is germination index, G_t is germination number on Day t , D_t is days of germination.

Germination vigor index $G_v = G_i \times$ seedling root length

Plant height, the distance from the base to the top of the plant.

Total number of leaves, all functional leaves of the whole seedling.

Root length, the length is from the root-stem junction to the root tip.

Number of lateral roots per plant, the number of taproots that grow to a certain length and laterally generate many branches internally at a certain location.

Total dry weight, the dry weight of the whole seedling.

Root to shoot biomass ratio= the dry weight of root to shoot biomass.

Value of subordinate function $R(X_i) = (X_i - X_{min}) / (X_{max} - X_{min})$.

In the formula, X is the measured value of a certain index of the test plant. X_{max} and X_{min} are the maximum and minimum values of the index in all the tested materials. The relative germination rate, the relative germination potential, the index of the relative germination and the length of the relative root were obtained. The average value of subordinate function of each index can be accumulated to obtain the total function value. The calculation of the total function value of each temperature at seedling stage is the same as above.

2.4 Data Processing

The data were analyzed by SPSS 22.0 software, the significant difference between species and temperature was tested by single factor variance analysis (one-way ANOVA), LSD significance test method was used to compare the differences between different treatments, and Excel was used to map the difference between different treatments.

The temperature tolerance of 2 foreign invasive *Rorippa* species at different temperatures during seed germination and seedling growth was evaluated with the modulus and mathematical subordinate function. the greater the total function value is, the greater the temperature tolerance will be [5,15].

3. RESULTS

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

3.1.1 Effects of temperatures on seed germination of two *Rorippa* seeds

The germination rate, germination potential, germination index and vigor index of *R. amphibia*

and *R. sylvestris* were significantly affected by temperature ($P<0.05$). The germination rate, germination potential and germination index of *R. amphibia* and *R. sylvestris* seeds had the same trend in change, namely, they increased at first and then decreased as the temperature rise, and reached the peak at 35°C. The germination rate, germination potential and germination index of *R. amphibia* were max at 30-40°C. *R. sylvestris* did not germinate at 15°C, while the germination rate, germination potential and germination index were higher at 25-35°C. The vigor index of *R. amphibia* also increased at first and then decreased. At 30-35°C, the vigor was higher than that at 25°C and 35°C. The low peak showed at 30 °C. The results showed that higher temperature promotes seed germination while the too-much higher or lower temperature inhibited the radicle growth (Table 1).

3.1.2 Evaluation on the adaptability of seed germination of two *Rorippa* on temperature

The relative germination rate, the relative germination potential, relative germination index and relative root length of two invasive species of *Rorippa* at different temperatures were comprehensively evaluated with the modulus subordinate function. The results showed that the subsequence of temperature tolerance of *R.amphibia* was 30□>25□>20□>35□>40□>15□. The subsequence of temperature tolerance of *R. sylvestris* was 35□>25□>30□>40□>20□>15□. The tolerance of seed germination of two *Rorippa* species to the high temperature was higher than that at the lower or high temperature, which was not conducive to the seed germination (Table 2).

3.2 Effects of Temperatures on the Growth and Adaptability of Two *Rorippa* Seedlings

3.2.1 Effect of different temperature on seedling growth of two *Rorippa*

The seedling height, total leaf number, root length and number of lateral roots for two *Rorippa* species were significantly affected by different temperatures. Plant height, total leaf number and root length increased first and then decreased as temperature rise. The number

of lateral roots of *R.amphibia* decreased gradually as the temperature rise while the *R. sylvestris* gradually increased. Total dry weight and root shoot ratio decreased as the temperature increased ($P<0.05$).

The plant height of *R. amphibia* was the highest at 20 °C, *R. sylvestris* were the highest at 25 °C. Low value at 15°C for both spp. the total number of leaves was the highest at 15°C - 30°C for *R.amphibia*. Where as 30°C for *R. sylvestris*. Low value at 35°C for both spp. Root length of two *Rorippa* species were the highest at 20 °C, Low value at 35°C > 30°C > 15°C > 25°C. The number of lateral roots were the highest at 35 °C for *R.amphibia*, and at 15°C > 20°C > 30°C > 35°C > 25°C for *R. amphibia*. Low value at 25°C for both spp. The lower temperature was more favorable to the growth of *R.amphibia* seedlings (Fig. 1 A, B, C, D).

Total dry weight and root shoot ratio decreased as the temperature increased. The biomass of two *Rorippa* species were the highest at 20°C and root shoot ratio was the highest at 15°C. while the *R. amphibia* increased slightly at 35°C. *R. sylvestris* increased slightly at 30°C. The biomass and root shoot ratio of *R. amphibia* were the smallest at 35°C, the biomass and root shoot ratio of *R. sylvestris* were the smallest at 15°C and 35°C, respectively. Low temperature was conducive to biomass accumulation of two *Rorippa* and its biomass distribution to roots (Fig. 1 E, F).

3.2.2 Evaluation of adaptability of two *Rorippa* on different temperatures at seedling stage

The above results showed that different temperatures had a great effect on the seedling growth. The tolerance of two *Rorippa* species to different temperature was comprehensively evaluated by the math subordinate function in four indexes at seedling stage. The results showed that the subsequence of tolerance of *R. amphibia* to different temperatures at seedling stage was 25□>20□>15□>30□>35□. The tolerance sequence of *R. sylvestris* at seedling stage to different temperatures was 30□>35□>15□>20□>25□ (Table 3). The seedlings of *R.amphibia* were well tolerated at low temperature, while the *R. sylvestris* was well tolerated at the higher temperature.

Table 1. Effects of temperatures on germination of two kinds *Rorippa* plants Seeds

| | Temperature(°C) | Germination rate(%) | Germination vigor (%) | Germination indexes | Germination vigor index |
|----------------------|-----------------|---------------------|-----------------------|---------------------|-------------------------|
| <i>R. amphibia</i> | 15 | 0.67 ±0.67d | 0.67 ±0.67 d | 0.05±0.05d | 0.00±0.00c |
| | 20 | 4.67 ±0.67d | 3.33 ±0.67cd | 0.36±0.06d | 0.05±0.01c |
| | 25 | 16.00±1.15c | 10.00±1.15bc | 1.44±0.07d | 2.85±0.14b |
| | 30 | 44.67±2.91a | 18.00±2.00ab | 6.54±0.65b | 5.29±0.52a |
| | 35 | 50.00±4.16a | 26.00±4.16a | 9.82±1.08a | 4.79±0.53a |
| | 40 | 35.33±2.40b | 21.33±4.37a | 4.77±0.61c | 0.73±0.09c |
| <i>R. sylvestris</i> | 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 |
| | 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 significant differences in species at different temperatures ($P<0.05$)

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

| Species | Temperature(°C) | subordinate function values | | | | Total function value | Ranking |
|----------------------|-----------------|-----------------------------|----------------------------|------------------------------|----------------------|----------------------|---------|
| | | Relative germination rate | Relative germination vigor | Relative germination indexes | Relative Root length | | |
| <i>R. amphibia</i> | 15 | 0.33 | 0.33 | 0.33 | 0.20 | 0.30 | 6 |
| | 20 | 0.33 | 0.67 | 0.38 | 0.38 | 0.44 | 3 |
| | 25 | 0.50 | 0.50 | 0.60 | 0.34 | 0.49 | 2 |
| | 30 | 0.47 | 0.67 | 0.35 | 0.49 | 0.50 | 1 |
| | 35 | 0.43 | 0.43 | 0.35 | 0.45 | 0.42 | 4 |
| | 40 | 0.42 | 0.38 | 0.42 | 0.38 | 0.4 | 5 |
| <i>R. sylvestris</i> | 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 two kinds of *Rorippa* plant sseedlings to different temperatures during the growth period

| Species | Temperature(°C) | subordinate function values | | | | Total function value | Ranking |
|----------------------|-----------------|-----------------------------|---------------------------------|----------------------|--|----------------------|---------|
| | | Relative Plant height | Relative Total number of leaves | Relative Root length | Relative number of lateral roots per plant | | |
| <i>R.amphibia</i> | 15 | 0.35 | 0.50 | 0.59 | 0.60 | 0.51 | 3 |
| | 20 | 0.63 | 0.68 | 0.46 | 0.41 | 0.55 | 2 |
| | 25 | 0.52 | 0.79 | 0.58 | 0.56 | 0.61 | 1 |
| | 30 | 0.39 | 0.53 | 0.40 | 0.48 | 0.45 | 4 |
| | 35 | 0.52 | 0.20 | 0.33 | 0.53 | 0.40 | 5 |
| <i>R. sylvestris</i> | 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 |

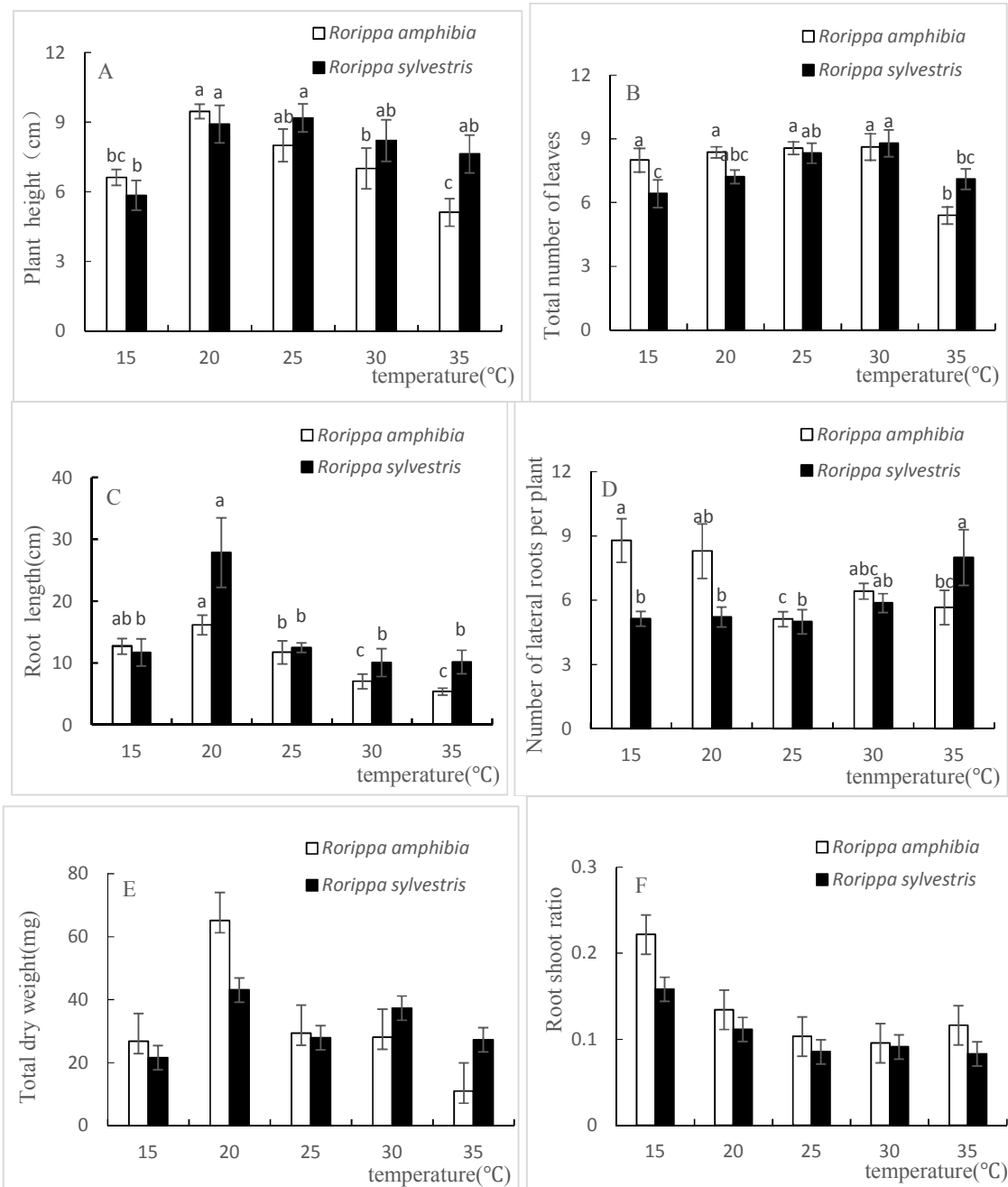


Fig. 1. Effects of temperature on plant height (A), total leaf number (B), root length (C), number of lateral roots (D), biomass (E) and root-shoot ratio (F) of two *Rorippa* plants seedlings
 Note: Different letters indicate significant differences in species at different temperatures ($P < 0.05$)

4. DISCUSSION

Plant growth was not only controlled by genetic material, but also by many environmental factors, such as light, temperature, water, soil nutrients and so on [16]. Seed germination was one of the key links in the life cycle of plants. The response

of seeds to germination conditions reflected the ecological strategy of its adaptation to the environment [17], which was also the key for invasive plants to enter the new environment. As an important environmental variable regulating seed germination, temperature was one of the key ecological factors for affecting seed

germination. Too high or too low temperature will affect the plant's normal growth and development. Wang Yuqin [18] found that the germination rate of *Oxytropis ochrocephala* seeds was lower at the low temperature. Zhang Tianxiang [19] found that both low and high temperatures were not conducive to the germination of *Moringa oleifera* seeds. In this experiment, it was found that too high or too low temperature (15°C, 40°C) will constrain the radicle growth and affected the vigor index of seed germination of two *Rorippa* species. The germination rate, germination potential and germination index were inhibited at lower temperature while the germination was promoted at higher temperature.

Germination rate and germination potential reflect the speed and uniformity of seed germination, germination rate was strong, indicating that the plant germination was rapid and uniform. Germination rate was high, germination potential was low, indicating that the seedling was uneven and weak in seedlings[20]. The germination rate and vigor of two *Rorippa* species were the highest at 35°C, and the high temperature help the seed germination and growth. However, the effects of temperature on seed germination were various, and it was one-sided to use a single index to evaluate the effects. At present, it was generally accepted that the comprehensive evaluation of plant tolerance was performed with the subordinate function [5,21,22]. The tolerance of two *Rorippa* species to different temperature could be reflected more completely and accurately by the subordinate function. In this experiment, the relative values of features were used to evaluate comprehensively the temperature tolerance of seed germination of two *Rorippa* species at different temperatures. The results showed that the tolerance of seed germination of two species was poor when the temperature was too high or too low. However, the tolerance of *R. amphibia* was highest at 30°C while that of *R. sylvestris* had the highest at 35°C. As *R. sylvestris* does not germinate at 15°C, its root length is less than 1mm at 20°C and 40°C. The temperature of *R. amphibia* germination is wider than that of *R. sylvestris*. The germination of two *Rorippa* species' seeds was promoted by the higher temperature, which might be an adaptive strategy to the global warming.

Temperature played an important role in the growth of plant seedlings at the early stage. The results of study by Han Bing [23] showed that low

temperature could significantly inhibit the growth of seedlings and reduce the growth of plant height, stem diameter and dry weight. The result of study by Ma Baopeng [24] showed that the high temperature deduces the accumulation of dry matter in *Capsicum annuum* seedlings. The results of Wang Jianjun[6] showed that different temperatures had different effects on the root length and dry matter of *Festuca sinensis* seedlings. From the experiment result, it could be seen that temperature had a significant effect on the seedling height, leaf number, root length and lateral root number of two *Rorippa* species seedlings. As the temperature increases, the plant height and root length of two *Rorippa* species increased at first and then decreased. High temperature significantly inhibited the growth of plant height and root of *R. amphibia*. The plant height and root length were the highest at 20°C. The effect of low temperature on the plant height and root growth of *R. sylvestris* was more obvious. The maximum of plant height was obtained at 25°C, and root length at 20°C was significantly higher than that at other temperatures. The low temperature promoted the occurrence of lateral roots of *R. amphibia* and inhibited the production of leaves. The number of lateral roots was the highest at 15°C and the number of leaves was the highest at 30 °C. The high temperature promoted the occurrence of leaf and lateral root of *R. sylvestris*, and the number of leaf and lateral root were the highest at 30°C and 35°C, respectively. The dry matter of two *Rorippa* species accumulated was most at 20°C. However, at both low and high temperatures, it was more likely to distribute more material to the roots, in which the growth of the aboveground part of the plant was constrained at the low-temperature conditions, thus allocating more resources to the roots and showing a higher ratio of the roots and shoot. As the temperature increases, some studies showed that the increase of temperature could increase the content of nitrogen in plant tissues and organs, and enhances the ability of supplying nutrients to plants, so that the root allocation of photosynthetic products decreased as shoot allocation increased[25,26]. This may be the reason that the ratio of root to shoot of two *Rorippa* species seedlings decreased with the increase of temperature. At 35°C, the growth of seedlings of two species was not conducive. The results showed that higher temperature could inhibit the growth of seedlings. This was consistent with the conclusion that higher temperature could significantly inhibit the growth of *Festuca synesis*, *Leymus chinensis* and

Sedum sarmentosum at different temperatures conducted by Wang Jianjun, Xu Zhenzhu. and Zhang Lei [6,27,28], respectively. The plant height, total leaf number, root length and lateral root number of two *Rorippa* species were comprehensively evaluated with the subordinate function and it could be known that the tolerance of two *Rorippa* was higher at the lower temperature. The tolerance of *R.amphibia* was highest at 25 °C and *R. sylvestris* was highest at 30°C, respectively.

The response of seed and seedling of two *Rorippa* species to temperatures was basically synchronous. The higher temperature promoted seed germination, inhibits seedling growth while the lower temperature inhibits seed germination. The high temperature in summer under natural environment was not conducive to seedling growth, which might be an important reason why there were few seeds and seedlings of both clonal plants under natural environment. The seedlings of clonal plants often had a high mortality rate during the seedling stage, and the rate of success settlement was lower than that of the clone [29]. As far as invasive clonal plants were concerned, a seed germinates and passes through the seedling stage, and it could be successfully colonized by asexual propagation. Therefore, the study on the response of invasive clonal plant seeds and seedlings to the environment could partially showed the mechanism of their successful invasion.

5. CONCLUSION

The response of the seeds and seedlings of the two species to temperature was basically the same. Higher temperature promoted seed germination and inhibited seedling growth, while lower temperature inhibited seed germination. The response of seeds and seedlings of *R. sylvestris* to high temperature makes it more invasive in the process of global warming.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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