Effects of Temperature on Seed Germination of Invasive Plant *Rorippa amphbia* （L.）Besser[1]

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Article Information

ABSTRACT

Aims: Global warming and biological invasion are major environmental issues faced in the world. In the study, *Rorippa amphibia*, a perennial invasive clone plant in northern China, was used as a material to study the germination characteristics of the seeds at different temperatures.

Study Design: Germination test of *R.amphibia* seeds at different temperature was studied by means of laboratory culture. The germination percentage, germination index, germination potential, bud height and root length of the seeds were determined.

Place and Duration of Study: Samples were collected from the west side of the swimming pool of Shenyang Agricultural University of Liaoning Province in August 2017. Experiments were done in the College of Biological Science and Technology, between October 2017 and June 2018.

Methodology: The petri dish method was used in the experiment. Fifty seeds were randomly selected and soaked in distilled water for 12h. The seeds were placed in a petri dish covered with double filter paper, cultured at 10℃, 15℃, 20℃, 25℃, 30℃, 35℃ and 40℃ in light incubators for 8h darkness and 12h light (4000lux), with 3 repeats per processing. Seed germination was based on embryo root breakthrough seed coat ⩾ 1 mm. During the experiment, the numbers of seed germination were recorded every day, and the filter paper was kept moist until there was no new seed germination for 2 consecutive days, which was regarded as the end of germination. The numbers of seed germination should be counted regularly every day, and the beginning and duration of germination should be recorded. The germination rate, daily germination rate, germinating potential, germinating index and vigor index of *R. amphibia* seeds at different temperature were calculated by measuring bud height and radicle length on the 10th day after germination.

Results: The temperature range of seed germination of *R.amphibia* was wide, which could germinate at 15-40℃. Lower temperature delayed the peak period of seed germination at some extent and the germination rates of *R. amphibia* peak at 30-35℃, which were 44.67% and 50% respectively. At 35℃, germination potential and germination index were 25.33% and 29.46, reaching the maximum value.

Conclusion: The reason for the wide temperature range of seed germination and the low germination rate might be the candidate method for clonal plant population establishment in temperate zone. The higher germination rate of high temperature condition suggested that clone invasive plants in temperate regions were more invasive during global warming.

Key words: Rorippa amphibia; invasive plant; temperature; seed germination.

1 Introduction

Global warming and biological invasion are the significant environmental issues faced in the world[1]. Due to the increasing concentration of CO_2 and other greenhouse gases in the atmosphere caused by human activities, the

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earth's surface temperature has risen by an average of about 0.74℃ in the past 100 years. It is predicted that the global average temperature will rise by 1.6 - 6.4℃ in 2100[2]. Global warming will lead to changes in plant distribution patterns. Successful plant invasion is one of the results of global climate change[3]. The proliferation of invasive species has seriously threatened the security of global biodiversity, the structure and function of ecosystem, the production of agriculture, forestry, animal husbandry and fishery, and even human health[4,5]. Studies have shown that temperature can affect the growth, development and reproduction[6,7], interspecific competition[8] and migration and invasion[9,10] of plants. There are also studies showing that warming will further promote the spread of invasive plants to higher latitudes[11].

R. amphbia(L.) Besser is genus of *Rorippa* in the cruciferous family, which is a kind of herbaceous perennid, native to Europe, which is a common alien invading grass in Liaoning area of China(North temperate zone)[12], and its life forms are various. In general, the new invasive sites are mainly seed propagation. When the invading sites have formed a large population, they are mainly spread by the bud of root tillers[13]. Seed germination is not only the basis of plant growth, but also an important link in the process of plant reproduction[14]. In addition to its own factors, temperature, light, water, air and soil can affect the seed germination of plants[15]. Wills et al makes a study of the reaction of seed germination of two exotic plants in England to the external environment, and believes that temperature is one of the important factors affecting plant invasion, which affects the final germination rate and germination rate of seeds[16-18]. *R. amphbia* belongs to the plant that sprouted and flowered earlier in early spring. Temperature directly affects its sprouting time. At present, there are no measures to prevent and control *R. amphbia* in China. Under the global warming environment, the temperature rise in the north is aggravating. Especially in recent 44 years, the average air temperature in Northeast China has an obvious warming tendency, and Liaoning is a weak warming area, however, the temperature increasing rate of the lowest temperature is about twice the maximum temperature[19], which will certainly lead to *R. amphibia* germinating prematurely and occupying niche earlier, and foreshadows a possibly increasing in its hazards.

By studying the response of the seed germination of the invasive plant, *R. amphibia*, to temperature rise, The influence of temperature rise on the invasive ability of *R. amphibia* was further analyzed, which provided a theoretical basis for monitoring the spread of *R. amphibia* and integrated control.

2 Materials and Methods

2.1 Materials

The *R. amphibi*a seeds were collected from the west side of the swimming pool of Shenyang Agricultural University of Liaoning Province in August 2017, with a 1000-grain weight of 0.0769 grams, then selected mature, plump seeds and store them in a well-ventilated room for backup.

2.2 Methods

The petri dish method was used in the experiment. Fifty seeds were randomly selected and soaked in distilled water for 12h. The seeds were placed in a petri dish covered with double filter paper, cultured at 10℃, 15℃, 20℃, 25℃, 30℃, 35℃ and 40℃ in the light incubators for 8h darkness and 12h light (4000lux), with 3 repeats per processing. Seed germination was based on embryo root breakthrough seed coat ⩾ 1 mm. During the experiment, the numbers of seed germination were 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 numbers of seed germinations were counted regularly every day, and the beginning and duration of germination were recorded. The germination rate, daily germination rate, germinating potential, germinating index and vigor index of *R. amphibia* seeds at different temperature were calculated by measuring bud height and radicle length on the 10th day after germination.

2.3 Determination index

The specific determination index was as follows.

The germination rate was GR=(N0/N)×100%(In this formula, N0 was the total number of seeds germinated at germination stage, and N was the number of seeds tested).

Daily germination rate (%) = (normal number of germinated grains on each day / total number of seeds tested) × 100%.

The germination vigor was GE=n/N×100%（In the formula, n was the highest seed germination rate per day and N was the number of seeds tested.）

The germination index was GI=Σ(Gt/Dt) (In the formula, Gt was the daily germinating number, and Dt was the germinating day).

Activity index GV=GI ×length of seedling root(cm)[20-22].

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.

3 Results and Discussion

3.1 Daily relative germination rate of *R. amphibia* seeds at different temperature

Daily relative germination rate of *R. amphibia* seeds increased at first and then was stabilized. On the second day, seeds in petri dish began to sprout, which clearly falled into three stages: slow growth period, rapid growth period and stable period. At 10℃, the seeds did not germinate. At 15℃, the seeds began to germinate on the 7th day and ended on the 8th day. At 20℃, *R. amphibia* began to germinate on the 6th day and ended on the 8th day. At 25℃, *R. amphibia* began to germinate on the 5th day until the 9th day. At 30℃, *R. amphibia* began to germinate on the 2nd day, then became stable, and began to germinate by repetiton on the 6th day, while the rate of daily germination was lower than that of the first time, until the end of germination on the 8th day. At 35℃, *R. amphibia* began to germinate on the 2nd day and entered a slow growth period. On the 6th day, it began to germinate by repetiton and continued to germinate until the 8th day. At 40℃, *R. amphibia* began to germinate on the 3rd day, then became stable, and began to germinate by repetiton on the 6th day, and continued to germinate until the 9th day. The results showed that the germination date of seeds was earlier and had the characteristics of secondary germination with high temperature, and the seed germination was slow when the temperature was low(Fig.1).

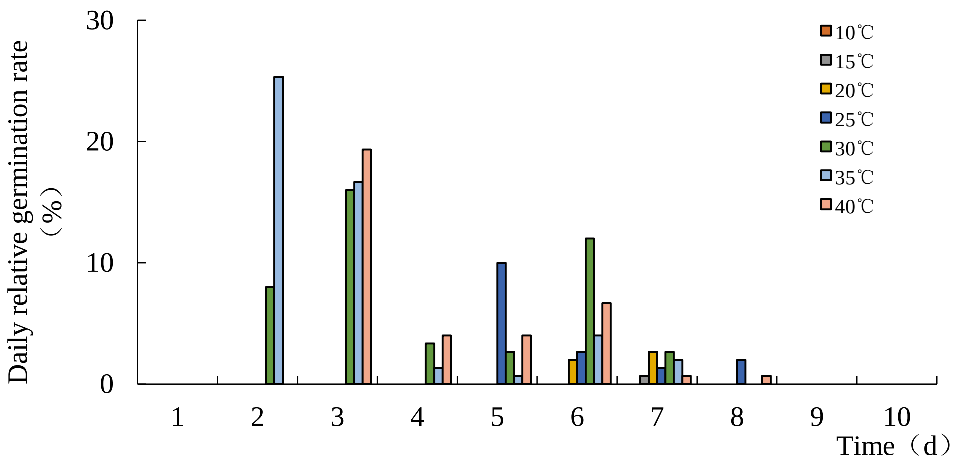

Fig. 1 Daily relative germination rate of *R. amphibia* seeds at different temperature

3.2 Germination rate of *R. amphibia* seeds at different temperature

From fig. 2, seed germination rate of *R. amphibia* increased first and then decreased with the increase of temperature. At 10℃, no seeds of *R. amphibia* were germinated. At 15℃, the germination rate was 0.67%, 4.67%

at 20℃, 16% at 25℃, 44.67% at 30℃, about 50% at 35℃, but just 35.33% at 40℃. The germination rates between 30℃ to 35℃ reached the maximum, and the germination rate at 30℃ (44.67%) was slightly lower than that at 35℃ (50%). While there was no significant difference between 30℃ and 35℃, and they were significantly higher than the other groups. The germination rate (35.33%) of *R. amphibia* seeds decreased at 40℃, but was significantly higher than those of 10-25℃. The results showed that *R. amphibia* seeds could germinate at 15-40℃, and both low temperature and high temperature had effects on seed germination, and low temperature had a greater effect on *R. amphibia* than high temperature.

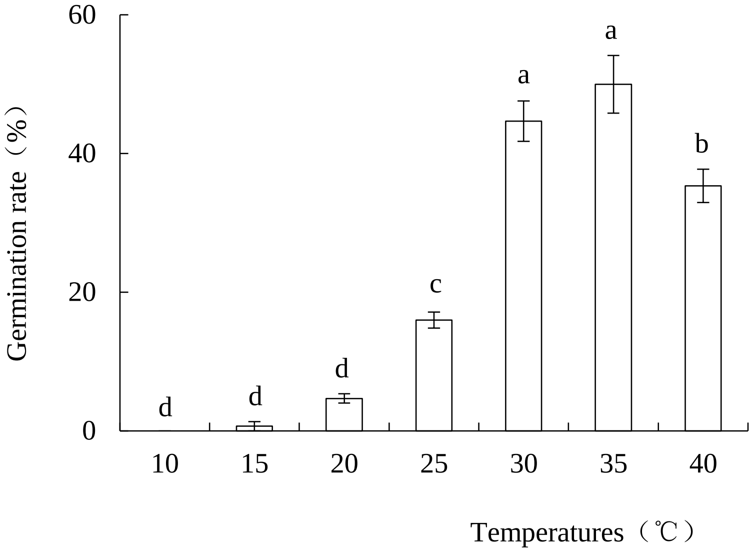

Fig. 2 Germination rate of *R. amphibia* seeds at different temperature

3.3 Germination vigor and germination index of *R. amphibia* seeds at different temperature

From fig. 3, seed germination vigor of *R. amphibia* showed a tendency of rising at first and then decreasing. The germination vigor of *R. amphibia* seeds reached the highest at 30℃,35℃, which was 24%, 25.33%. respectively. At 40℃, the germination vigor was 19.33%, And there was no significant difference among 30℃, 35℃ and 40℃. At 25℃, the germination vigor was 10%, in which there was no significant difference between 25℃ and 40℃, but significantly lower than 30℃ and 35℃. There was no significant difference between 10℃ and 20℃, and the germination vigor was 0, 0.67%, 4.67%, respectively.

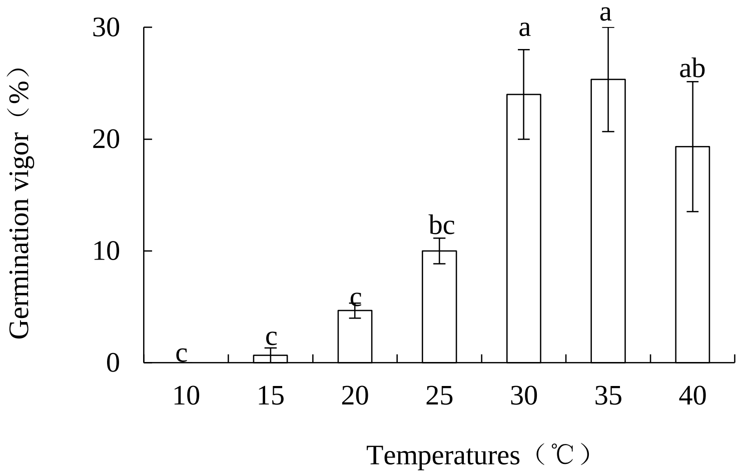

Fig. 3 Germination vigor of *R. amphibia* seeds at different temperat

From fig. 4, seed germination index of *R. amphibia* showed a tendency of rising at first and then decreasing. The germination index reached the highest at 35℃ (29.46) , which was significantly higher than other temperatures. The germination index at 30℃ was 19.62, germination index at 40℃ was 14.3, and germination index at 25℃ was 4.33. There was no significant difference between 10℃ and 20℃.

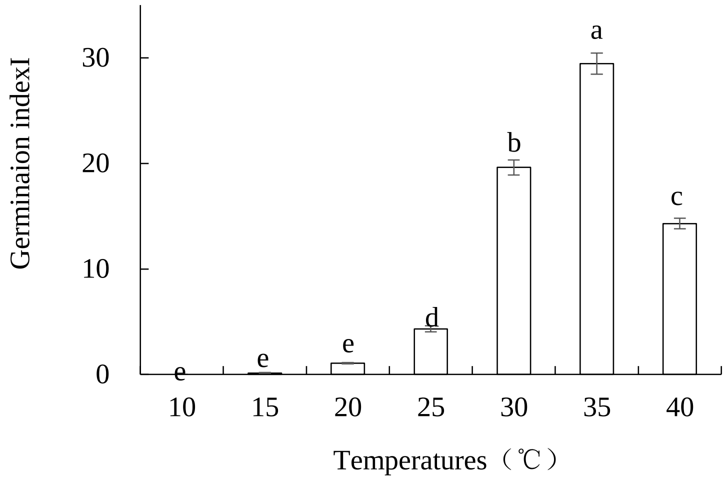

Fig. 4 Germination index of *R. amphibia* seeds at different temperature

3.4 Activity index of *R. amphibia* seeds at different temperature

Activity index of *R. amphibia* seeds was the highest at 30℃, which was 15.64, followed by 35℃ (14.38). Although the germination energy and germination index at 40℃ were significantly higher than those at 25℃, the germination vigor index was significantly lower than that at 25℃. The reason might be that the seed germinated at 40℃, but its radicle length was shorter, which was different from that of seed radicle growing at 25℃, so the vigor index was lower, which indicated that high temperature could promote seed germination. However, high temperature would inhibit the growth of seedlings(Table 1).

Table 1 Activity index of *R. amphibia* seeds at different temperature

Temperature（℃）	Length of root（cm）	Germination index	Activity index
10	0	0	0
15	0.07	0.14 ± 0.05^{e}	0.01 ± 0.01^{f}
20	0.1275	1.07 ± 0.06^{e}	0.14 ± 0.018^{e}
25	1.979	4.33 ± 0.30^{d}	8.57 ± 0.018^{c}
30	0.797	19.62 ± 0.71^{b}	15.64 ± 0.01^{a}
35	0.488	29.46 ± 1.01^{a}	14.38 ± 0.01^{b}
40	0.155	14.3 ± 0.50^{c}	2.22 ± 0.01^{d}

Note: Different letters represent significant differences at different temperature($P < 0.05$)

3.5 Discussion

The seeds have an active metabolic reaction during germination. Therefore, in a certain temperature range, the increase of temperature accelerates the process of germination. However, exorbitant temperature can cause some active substances to be inactive, for example, the denaturation of enzymes will affect the germination of seeds[23].

In this study, the results showed that the germination of *R. amphibia* seeds was greatly affected by temperature. When the temperature was between 15℃ and 25℃, the initial germination time was long, while the germination rate was low, and the germination rate increaseed with the increase of temperature. However, when the temperature was higher than 30℃, the seeds germinated rapidly and the germination rate was higher. With the increase of temperature, the germination rate increaseed first and then decreased. At low temperature (10℃), the seeds did not germinate. Under visible light, 30-35℃ was the most suitable for seed growth of *R. amphibia*, and if the temperature was too high (40℃) or too low (10℃), which would inhibit seed germination. In the range of germinable temperature (15-40℃), with the increase of temperature, the earlier the seed germination time was, the longer the seed germination time was, the more favorable factors could be obtained, which might be an adaptive strategy for *R. amphibia*.

The germination rate and germination vigor reflect the germination speed and uniformity of the seed. The germination rate is high and the germination vigor is strong, which indicates that the plant germinates quickly and neatly, and the seedlings are strong. High germination rate and low germination vigor indicate that the plants are not even in germination and there are many weak seedlings[24,25]. The germination rate of *R. amphibia* reached the highest at 30-35℃, which was higher than that of other temperature. The germination vigor at 30-35℃ was also significantly higher than that at other temperatures, and the germination index at 35℃ was significantly higher than that at other temperatures. However, the germination index at 30℃ was second only to that at 35℃, indicating that at 30-35℃, *R. amphibia* germinated neatly and had fewer weak seedlings. The seeds of *R. amphibia* germinated at 15-40℃, indicating that it had a wide range of adaptation to temperature. The germination vigor, germination index and vigor index of *R. amphibia* reach the maximum at 30-35℃. The germination vigor index at 40℃ was significantly lower than that at 25℃, which indicated that the increase of temperature promoted the germination and growth of *R. amphibia* seeds, while exorbitant temperature inhibited seedling growth.

Under harsh environmental conditions, there are many perennial plants which may have evolved two opposing germination strategies: one is that the seeds are still dormant or slow to germinate under suitable germination conditions, and the other is that they germinate rapidly once they meet favorable conditions[26]. Zhang Jingguang *et al*. classifies the types of seed germination into three types, namely, adventurous type, opportunity type and stable type[27]. The main feature of the adventurous type is that the most seeds germinate within a short period of time after germination. The main characteristic of the stable type is that the seed germination process is continuous and stable, and the cumulative germination rate of the seed increases slowly with the prolongation of the culture time. The opportunity-type seed germination is intermittent, and there is a long stop in the middle of the second germination. At the optimum germination temperature, the accumulative germination rate increases rapidly at the initial stage of germination, then tend to be stable, and at the end of the germination stage, the seed is germinated for a second time. The results of this study can be preliminarily determined that the type of seed germination of *R. amphibia* tends to be opportunity type.

The reason for the wide temperature range of seed germination and the low germination rate may be the candidate method for clonal plant population establishment in temperate zone. The higher germination rate of high temperature condition suggests that clone invasive plants in temperate regions are more invasive during global warming. So the summer is the key period to curb *R. amphibia* diffusion, cutting in the period can reduce seed dispersal. At the same time, it is suggested that the relevant departments strictly control the import of grass seeds, strengthen quarantine and prevent the entry of seeds. Of course, there are records that *R. amphibia* is palatable, non-toxic [28], can be vigorously promoted as a wild vegetable to eat, but also a good way to eliminate.

4 Conclusions

Global warming and biological invasion are major environmental issues faced in the world. In this study, *R. amphibia* a perennial invasive clone plant in northern China, was used as a material to study the germination characteristics of the seeds at different temperature. The temperature range of seed germination of *R. amphibia* was wide, which could germinate at 15-40℃. Lower temperature delayed the peak period of seed germination at some extent and the germination rates of *R. amphibia* peak at 30-35℃, which were 44.67% and 50%, respectively. At 35℃, germination potential and germination index were 25.33% and 29.46, reaching the maximum value. The reason for the wide temperature range of seed germination and the low germination rate might be the candidate method for clonal plant population establishment in temperate zone. The higher germination rate of high temperature condition suggested that clone invasive plants in temperate regions were more invasive during global warming.

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Authors' Contributions

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

Competing Interests

Authors have declared that no competing interests exist.

References

1. Rahel FJ, Olden AJ. Assessing the effects of climate change on aquatic invasive species[J]. Conservation Biology, 2008;22: 521－533.
2. Schneider, B. Fits About Fit. Applied Psychology:An International Review, 2001;50: 141-152.
3. Huang JH, Han XG,Yang QER, Bai YF. Some Problems on the Biological and Ecological Basis of Alien Species Invasion [J]. Bio-diversity, 2003;03:240-247.
4. Pimentel D, Zuniga R, Morrison D. Update on the environmental and economic costs associated with alien-invasive species in the United States[J]. Ecological Economics. 2005; 52: 273-288.
5. Rudgers JA, Orr S. Non-native grass alters growth of native tree species via leaf and soil microbes[J]. Journal of Ecology. 2009; 97: 247-792.
6. Allen MF, Vargas R, Graham EA, Swenson W, Hamilton MP, Taggart M, Harmon TC, Rat'ko A, Rundel PW, Fulkerson B, Estrin DL. Soil sensor technology: life within a pixel[J]. Bioscience. 2007; 57: 859-867.
7. Li XY, Zhang YB, Pan KW, Sun CR, Wang KY, Wang JC, Qi DM. Effects of elevated temperature on reproductive phenology and growth of Allium xichuanense and Anemone rivularis in timber line ecotone[J]. Chinese Journal of Ecology. 2009; 28(1):12-18.
8. Wang K, Yang J, Chen JK. Comparison of morphological traits between alligator weed and two congeners under different water and nutrient[J]. Bio conditions diversity Science. 2010; 18 (6): 615–621.
9. Walther GR. Plants in a warmer world[J]. Perspect Plant Ecol Ecol Systemat,2003.6: 169-185.
10. Wills SG, Hulme PE. Does temperature limit the invasion of Impatiens glandulifera and Heracleum mantegazzianum in the UK? [J] Funct Ecol. 2002; 16: 530-539.
11. Huang W,Wang Y,Ding JQ. A review of adaptive evolution of defense strategies in an invasive plant species, Chinese tallow (Triadica sebifera). [J]. Chinese Journal of Plant Ecology, 2013;09:889-900.
12. Wang Y,Shi Y,Dai BQ. Turf-weed study in autumn in Shenyang Normal University[J]. Journal of Shenyang Normal University:Natural Science Edition ,2016;34(03):300-304.
13. Zhang SM, Li ZX, Wang Q, et al. A newly recorded species *Rorippa amphibia* (L.) Besser from China[J].

Journal of Tropical and Subtropical Botany. 2009; 17(2): 176-178.
14. Wang JJ, Yun JF. Characteristics and utilizing valueson the germplasm of Medicago falcata[J]. Journal of Inner Mongolia Agricultural University, 2008, 28(1): 215-216.
15. Leprince O, Walters-Vertucci C. A calorimetric study of the glass transition behaviors in axes of bean seeds with relevance to storage stability[J]. Plant Physiology, 1995, 109.
16. Aflakpui GKS, Gregory PJ, Froud-Williams RJ. Effect of temperature on seed germination rate of *Striga hermonthica* (Del.)Benth[J]. Crop Protection. 1998; 17(2): 129-133.
17. Probert RJ, Thompson PA. Effects of temperature and seed coat treatments on germination of sweet pea[J]. Scientia Horticulturae, 1976, 5(2): 139-151.
18. Tobe K, Zhang LP, Qiu GY, et al. Charaacteristics of seed germination in five non-halophytic Chinese desert shrub species[J]. Journal of Arid Environments. 2001; 47(2): 191-201.
19. Sun FH, Yang XQ, Lu S, Yang SY. The contrast analysis on the average and extremum temperature trend in northeast china[J]. Scientia Meteorologica Sinica. 2006; 02:157-163.
20. Yan QC. Principles and techniques of seed testing[M]. Hangzhou: Zhejiang University Press. 2001; 49.
21. Hu J. Seed biology[M]. Beijing: Higher Education Press. 2006; 89-91.
22. Zheng GH. Seed physiology[M]. Beijing: Science Press. 2004; 706.
23. Wang YF. Effect of temperature on the germination mechanism of plant seeds[J]. Protection Forest Science and Technology. 2015; (6): 76-78.
24. Li X, Yin X, Yang SH, et al. Effects of temperatures on seed germination characteristics of plant Stipa purpurea (Poaceae)[J]. Plant Diversity. 2014; 36(6): 698-706.
25. Qi SY, Dong JJ, Guo TT, et al. Effect of temperature on Seed Germination of invasive plant Galinsoga parviflora[J]. Journal of Shenyang University(Natural Science). 2014; 26(2): 87-90.
26. Green Berg CH, Smith LM, Levey DJ. Fruit fate, seed germination and growth of an invasive vine-An experimental test of "sit and wait" strategy[J]. Biological Invasions. 2001; 3(4): 363-372.
27. Zhang JG, Wang XP, Li XR, et al. Progress and Prospects of Research on the Countermeasures of Desert Plant Life History[J]. Journal of Desert Research. 2005; 25(3): 306-314.
28. Pang SY, Huang YQ. Common wild vegetables in Liaoning [M]. Shenyang Press. 2017;9:43-46.