

Variation in tolerance to high temperature and water stress in weedy *Bidens pilosa* seeds from Yunnan, SW China

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Abstract

Using *Bidens pilosa* seeds from three provenances in Yunnan, this study comprised an investigation of changes in seed viability and germination under high temperature and water stress. It was found that *B. pilosa* seeds had a wide temperature range for germination, as all seeds germinated in the incubation temperature range of 10–30°C, under no more than a 6-h daily thermoperiod at 40°C and moisture regime no less than –0.6 MPa, regardless of the seed provenance. The seeds showed no substantial viability loss after no longer than 8 d of continuous heating at 40°C or 30 min heating at no higher than 50°C. However, obvious intra-specific variation was found among seeds from these provenances when further stress was applied. Seeds from Xishuangbanna (N21°41') always exhibited the most high-temperature tolerance, whereas those from Diqin (N27°11') had the least and those from Chuxiong (N25°10') had intermediate tolerance, regardless of whether this was assayed based on the ceiling temperature for germination, the maximal daily thermoperiod, the longest continuous heating duration at 40°C, or the endurable maximum temperature for 30 min of heating, indicating the adaption of invasive plants to invaded habitats. This study supported our hypothesis that *B. pilosa* seeds from different provenances have intra-specific divergence in germination traits and high-temperature tolerance to adapt to local conditions. It was concluded that high-temperature tolerance is an important seed trait that affects plant invasion success in open habitats in Xishuangbanna.

Introduction

Successful invasion is thought to be a context-specific process (Hobbs and Humphries, 1995; Rashid et al., 2007), depending on the attributes of an invader, the community characteristics and environmental conditions (Maurer and Zedler, 2002), and anthropogenic events, such as globalisation, land-usage changes, and intentional or accidental introduction, which facilitate this process (Fumanal et al., 2008). Although understanding the mechanisms of plant invasions has been a challenging task, it is crucial for invasive species management and the prediction of future invaders. This will subsequently minimise their impact on agricultural production and biological diversity through preventive methods at the early stages (Ren and Zhang, 2009), because management options become more constrained along the sequence of invasion transitions (Kolar and Lodge, 2001). Approximately 14,000 non-native plant species have been established globally, and between 2500 and 4375 of these are considered invasive (Huebner, 2022). It seems that no generalised 'invasive traits' are applicable at all, but a general feature seems to be that plant-history traits associated with adaptation to invaded habitats are of vital importance for successful plant invasion. The relationship between invasiveness and species life-history traits relies both on the general species characteristics and the post-introduction evolution of the traits in areas in which species are introduced (Beckmann et al., 2011), i.e., phenotypic plasticity and evolutionary adaptation.

The germination stage is critical in the plant life-history and is also a key process during the expansion of species ranges into new environments (Vilà and Weiner, 2004). It was suggested that exotic species exhibit more rapid and prolific germination across a variety of environmental cues and in response to increased resource availability compared with native species; for example, they exhibit both germination

plasticity and robustness (Wainwright and Cleland, 2013). In addition, invasive seeds were found to possess higher tolerance to high-temperature stress, as they often need to survive huge temperature fluctuations in invaded habitats after dispersal (Wen, 2015; Wen et al., 2015). Thus, it is anticipated that studies on germination and seed tolerance to high temperatures can provide a reference to understand such invasion mechanisms.

As a common invasive plant, *Bidens pilosa* L. is distributed worldwide in subtropical and tropical regions with its centre of diversification in Mexico (Ballard, 1986; Fenner, 1980a; Huang and Kao, 2014). It is an erect, herbaceous, annual, autogamous, and exozoochorous plant, with large genetic variability within populations (de Brito et al., 2016). The first report of its appearance in China came from Hong Kong in 1857 (Hong et al., 2004), and now it has spread to almost all subtropical and tropical areas of China and even to temperate regions in China, such as Shandong, Hebei, and Liaoning Province (Ma, 2013). Located in Southwest China, Yunnan is a mountainous province situated at a low latitude with various climates, from boundary tropics to cold temperate, where *B. pilosa* is widely distributed. This prompted us to speculate on whether *B. pilosa*, with such a wide distribution in Yunnan, exhibits different germination traits and whether the seeds from cold areas have the same high-temperature tolerance as those from hot areas. Considering that adaptive evolutionary changes possibly occurred, we hypothesised that seeds from different climate zones would have distinct germination traits and high-temperature tolerance with adaptation to local environments. The present study was designed to investigate the effects of high temperature and water stress on seed viability and germination to test this hypothesis.

Materials And Methods

Seed sources and climate conditions of seed provenances

Bidens pilosa seeds were collected from three sites, referred as three provenances in Yunnan, Southwest China, including the following: (1) Xishuangbanna, from Menglun Town in Mengla, Xishuangbanna; (2) Chuxiong, from Guangtong Town in Lufeng, Chuxiong; and (3) Diqing, from Hutiaoxia Town in Xianggelila, Diqing. The seeds were harvested in October and December of 2019 when they were mature, with geographic information listed (Table 1). As heteromorphic achenes have been frequently reported in *Bidens* species, which demonstrate obvious differences in seed shape, dispersal capacity, dormancy, and germination requirements (Brändel, 2004; Brown and Mitchell, 1984; Forsyth and Brown, 1982; Rocha, 1996; Whitaker et al., 2010), we intentionally collected central achenes only and avoided the usage of short achenes in this study (achenes were referred to as seeds thereafter for convenience in this study). After being taken to the laboratory, thousand-seed weights, moisture contents, and initial viability were assessed (Table 1), with the remaining seeds stored in paper bags at 15°C until initiation of the experiments, for up to no more than half a year.

Table 1
Seed traits and climate conditions of provenances

Provenance	Seed collecting sites: town, county and prefecture, with longitude, latitude and altitude	Climate: climate type, annual mean temperature (°C), annual mean precipitation (mm)	Seed traits: 1000-seed weight (g), Moisture content (%) and Initial germination percentage (%)
Xishuangbanna	Menglun, Mengla, Xishuangbanna, E101°25', N21°41', 570 m	Tropical monsoon, 21.4°C, 1493 mm	1.129 g, 10%, 100%
Chuxiong	Guangtong, Lufeng, Chuxiong, E101°45', N25°10', 1570	Subtropical monsoon, 16.2°C, 948.5 mm	1.949 g, 7.84%, 100%
Diqin	Hutiaoxia, Xianggelila, Diqin, 100°3', 27°11', 1850 m	River valley with a subtropical climate, 13.2°C, 730 mm	1.963 g, 10.98%, 100%

Effect of incubation temperature on seed germination

In this experiment, seeds were first sown in Petri dishes containing 1% plain agar, and then, these Petri dishes were assigned randomly to incubators set at constant temperatures of 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C, as well as an alternating temperature of 30/20°C, with light provided by fluorescent lights.

Seed tolerance to extreme high temperature

This experiment was designed to investigate the effects of extreme high ground temperature on seed viability. A water bath was used to create a temperature gradient from 30°C to 95°C at increments of 5°C. Seeds, in two states, specifically air-dried and imbibed, spread in a layer in a big triangular flask, were first heated for 30 min at a set temperature and then sown on 1% plain agar for viability assessments at 25°C.

Effects of continuous high-temperature treatment on seed viability

Seeds sown in Petri dishes containing 1% plain agar were first incubated at 40°C for 1 day, 2 days, and up to 40 days. After a designed period of heating treatment, the Petri dishes containing seeds were transferred to 25°C for viability assessments.

Effects of periodic high temperature on seed germination

In this experiment, Petri dishes containing seeds sown in 1% plain agar were physically transferred between incubators set at 40°C and 25°C every day, allowing the seeds to be incubated for a designed period at 40°C, which increased over 3, 6, and up to 21 h as different treatments, with 0 and 24 h as the controls.

Effects of water availability on seed germination

Testing solutions with water potentials of 0, - 0.1, - 0.2, and up to - 1.5 MPa were created using polyethylene glycol (PEG) 8000 according to Michel (1983). Seeds were sown on Petri dishes lined with filter paper, moistened with 2.5 ml testing solutions, and placed in an incubator set at 25°C for germination. To minimise changes in water potentials during the experiment, Petri dishes subjected to the same treatment were sealed in a resealable double-clear plastic bag. Seed germination was scored once every 3–4 days, with filter paper and testing solutions refreshed simultaneously. Four weeks later, the ungerminated seeds were released from water stress and incubated at 0 MPa to test if they were still viable.

Effects of NaCl stress on seed germination

This was performed in the same manner as the water availability experiment previously described, except that testing solutions with water potentials of 0, - 0.1, - 0.2, and up to - 1.5 MPa were created using NaCl according to Lang (1967).

Seed viability and germination assessment

In this study, six replicates of 50 seeds were used for every treatment. Unless otherwise stated, seed viability was assessed using germination tests in the following manner: incubation of seeds sown on 1% plain agar at 25°C, with a 12 h photoperiod of $25 \mu \text{mol m}^2 \text{s}^{-1}$ irradiance provided by a white fluorescent light; germination was scored once per week for at least 4 weeks. Protrusion of the radicle up to 5 mm was used as the criterion for germination or survival for stress-treated seeds, and the formation of morphologically normal seedling was considered seedling formation. Non-germinated seeds were pinched with forceps. Those that were still white and firm were assumed to be viable and the remainder were considered non-viable.

Data analysis

As germination data usually do not follow a normal distribution, seedling percentages and germination or survival percentages in germination or stress experiments, respectively, were taken separately as dependent variables for data analysis after arcsine-square-root transformation in this study. A two- or three-way analysis of variance was employed, with all environmental factors treated as fixed effects. Interactions between factors were also evaluated; however, only treatments resulting in germination were included. All analyses were carried out using SPSS 16.0 for Windows. Data were presented as means and standard errors of six replicates of 50 seeds.

Results

Though seeds collected from the three provenances varied in the 1000-seed weight and moisture content, they all had perfect initial viability, with 100% seedling formation in an initial germination test in this study (Table 1).

Effect of incubation temperature on seed germination

The seeds had a wide temperature range for germination, from 10°C to 40°C, and both the incubation temperature and seed provenance had a significant effect on germination, as assessed by both germination and seedling percentages ($P < 0.001$ for all, Table 2). When incubated at $\leq 30^\circ\text{C}$ and $30/20^\circ\text{C}$, all seeds germinated and formed morphologically normal seedlings, regardless of the seed provenance; however, differences occurred when seeds were incubated at 35°C and 40°C . Evidently, seeds from Xishuangbanna exhibited a higher level of adaptation to high temperatures, and these germinated 90% at 35°C and 40% at 40°C . Meanwhile, those from Chuxiong and Diqin exhibited a marked decrease in germination at these temperatures. The seedling percentage differed from the germination percentage only for seeds incubated at 40°C (Fig. 1).

Table 2

Analysis of variance for seedling and germination or survival percentage of *Bidens polosa* seeds

Experiment	Effect	df	F-value	
			Seedling	Germination/Survival
Incubation temperature	Provenance	2	77.5 ^{***}	40 ^{***}
	Temperature ¹	7	2503.4 ^{***}	1287.6 ^{***}
	P×T	14	34.3 ^{***}	17.6 ^{***}
Extreme high-temperature	Provenance	2	189.2 ^{***}	175 ^{***}
	Hydration status	1	2614.1 ^{***}	2614.4 ^{***}
	Temperature ²	12	3870 ^{***}	3645.3 ^{***}
	P×H	2	302.9 ^{***}	291.1 ^{***}
	P×T	24	32.7 ^{***}	31.3 ^{***}
	H×T	12	256.1 ^{***}	231.2 ^{***}
	P×H×T	24	49.9 ^{***}	48 ^{***}
Continuous heating	Provenance	2	74.1 ^{***}	54.9 ^{***}
	Duration	14	479.4 ^{***}	387.9 ^{***}
	P×D	28	10.4 ^{***}	8.5 ^{***}
Periodic heating	Provenance	2	1166.9 ^{***}	1240.5 ^{***}
	Duration	8	767.9 ^{***}	786.9 ^{***}
	P×D	16	162.1 ^{***}	160.6 ^{***}
Water availability	Provenance	2	249 ^{***}	285.5 ^{***}
	Water potential ³	8	1731.5 ^{***}	1998 ^{***}

“***” $p < 0.001$;

¹Data for the alternative temperature of 30/20°C was included in this analysis;

²Data for the temperature of 95°C was not included in this analysis because no seed germination;

³Data for water potential of -1.5 MPa was not included in this analysis because no seed germination

	P×W	16	104.8 ^{***}	116.1 ^{***}
NaCl stress	Provenance	2	30.3 ^{***}	56.5 ^{***}
	Water potential ³	8	2204.3 ^{***}	1334.2 ^{***}
	P×W	16	10.7 ^{***}	20.1 ^{***}
“***” $p < 0.001$;				
¹ Data for the alternative temperature of 30/20°C was included in this analysis;				
² Data for the temperature of 95°C was not included in this analysis because no seed germination;				
³ Data for water potential of -1.5 MPa was not included in this analysis because no seed germination				

Seed tolerance to extreme high temperature

It was found that the seed provenance, heating temperature, and water status, specifically air-dried or imbibed, all significantly affected seed viability ($P < 0.001$ for all, Table 2), with little difference between germination and seedling formation. Heating for 30 min at a temperature of no more than 45°C had no detrimental effect, whereas seed viability exhibited different response to further increase in heating temperature, depending on provenances. Among seeds from the three provenances, those from Xishuangbanna had the most tolerance to high temperature, and with an air-dried status, they survived up to 70°C without a loss of viability, though this temperature killed half of all seeds or more from the other provenances. Meanwhile, for seeds from the same provenance, those that were air-dried exhibited higher tolerance than those that were imbibed, thus yielding a large gap of viability between air-dried and imbibed seeds; this was especially prominent for seeds from Xishuangbanna (Fig. 2).

Effects of continuous high-temperature treatment on seed viability

Both the seed provenance and continuous heating duration significantly affected seed viability, according to survival and seedling percentages ($P < 0.001$ for all, Table 2). Eight days of heating negatively affected neither the seedling percentage nor the survival percentage, whereas 10 days of heating had an effect, and after that, each increase in the heating duration produced additional damage. Moreover, after approximately 10 days of heating, some seeds germinated but failed to form seedlings, making the seedling percentage lower than the survival percentage. However, the seeds from the three provenances differed from each another. Among them, seeds from Xishuangbanna had the strongest tolerance to continuous heating. After the longest heating duration (40 days), 60% of seeds from this provenance germinated and two thirds of them formed seedlings; these values were halved or less for seeds from the other provenances (Fig. 3).

Effects of periodic high temperature on seed germination

The daily thermoperiod and seed provenance had significant effects on seed germination, as assessed by both germination and seedling percentages ($P < 0.001$ for all, Table 2). Seeds from Xishuangbanna demonstrated the strongest tolerance to daily heat treatment, exhibiting a decrease in seedling and germination percentages only when the daily heating duration increased to 24 h, whereas seeds from Chuxiong and Diqin exhibited marked reductions in germination when the daily heating duration reached 18 and 9 h, respectively (Fig. 4).

Effects of water availability on seed germination

Water stress and seed provenance were important factors that determined seed germination, as demonstrated by both germination and seedling percentages ($P < 0.001$ for all, Table 2). Seeds from the three provenances differed from each another. For seeds from Chuxiong and Diqin, water stress up to -0.6 MPa resulted in no difference, but a further decrease in water potential gradually reduced the germination and seedling percentage. However, this turning point was -0.8 MPa for Xishuangbanna. At -1.0 MPa, a seedling percentage greater than 40% was observed for seeds from Xishuangbanna, whereas this value was nearly zero for seeds from the other provenances (Fig. 5). Most of the seeds which did not germinate when incubated at water potentials of -0.8 to -1.5 MPa germinated soon after released from stress (data not shown).

Effects of NaCl stress on seed germination

Similarly, NaCl stress and seed provenance substantially influenced seed germination, based on both the germination and seedling percentage ($P < 0.001$ for all, Table 2). Moreover, among seeds from the three provenances, those from Xishuangbanna had the strongest tolerance. All seeds germinated and formed normal seedlings with water potentials up to -0.6 MPa, but few germinated at -1.2 MPa and more negative water potential. In the intermediate range of -0.8 to -1.0 MPa, seeds from Xishuangbanna had the highest germination, and those from Diqin had the lowest (Fig. 6). And similarly, most of the ungerminated seeds incubated at water potentials of -0.8 to -1.5 MPa germinated soon after released from stress (data not shown).

Discussion

Using *B. pilosa* seeds from three locations, this paper reports a study of the effects of high temperature and water stress on seed viability and germination. It was found that *B. pilosa* seeds had a wide temperature range for germination, as all seeds germinated when incubated at 10 – 30°C , although germination was repressed at 35°C and inhibited at 40°C , with the extent varying with the provenance of the seeds. Regardless of whether it was generated with PEG or NaCl, the seedling percentage was 100% under water potentials up to -0.6 MPa, with this tolerance extending to -0.8 MPa for seeds from Xishuangbanna. Depending on the seed provenance, the seeds could tolerate a 6–21 h daily

thermoperiod at 40°C without effects on germination and 8–10 days of continuous heating at 40°C and 30 min of heating at 50–70°C without a loss of viability. Marked variation in tolerance to high temperature for *B. pilosa* seeds was revealed based on these three provenances, with seeds from Xishuangbanna always demonstrating the highest viability after heat treatment and the highest tolerance during germination under stress.

Germination is a key step for the invasion of most plants. The ability of a species to germinate rapidly under a wide set of environmental conditions has been regarded as an important trait for invasive species (Vilà and Weiner, 2004), which was supported by the results of the present study. Compared to the results of our previous studies on invasive plant seeds in Xishuangbanna, such as *Piper aduncum* (Wen et al., 2015), *Tithonia diversifolia* (Wen, 2015), *Amaranthus spinosus* (Ye and Wen, 2016), and three invasive Asteraceae weeds (*Crassocephalum crepidioides*, *Conyza canadensis* and *Ageratum conyzoides*, Yuan and Wen, 2017), *B. pilosa* seeds from the Xishuangbanna provenance had an even broader temperature range for maximum germination, demonstrating complete germination when incubated at temperatures ranging from 10 to 30°C, almost complete germination at 35°C, and partial germination at 40°C. Only *C. crepidioides* and *C. canadensis* were found to have a similar minimum temperature for germination, whereas *A. spinosus* has a similar maximum temperature.

In addition to germination traits, seeds tolerance to high temperature is regarded as a key factor contributing to invasive capacity (Wen, 2015; Wen et al., 2015), as the seeds must survive before germination. Plant invasion mostly occurs in open habitats, where the maximum ground temperature reached by open soil can be very high on sunny days, such as > 60°C in Xishuangbanna with an extreme value of 71.4°C recorded (Wen et al., 2015). Meanwhile, after dispersal, seeds usually need to remain on the ground for a period until conditions become suitable for germination, and thus, high-temperature stress is unavoidable, making high-temperature tolerance necessary for successful invasion. This was supported by our previously mentioned studies and again by the present study, as air-dried seeds of all of these invasive species tolerated 30 min of heating at 70°C without substantial viability loss, except for *P. aduncum*, which tolerated temperatures up to 65°C and exhibited a small viability loss at 70°C as an invader occurring at the forest edge in Xishuangbanna (Wen et al., 2015). This inter-specific convergence of high-temperature tolerance for seeds indicates its importance for plant invasion success in Xishuangbanna.

Comparative studies between invasive and native species and between invasive species and non-invasive congeners, as well as comparative studies of invasive species between native and invasive origins/populations, comprise an important approach to identify plant traits conferring invasion capability and include those of Bochenek et al. (2016), Cervera and Parra-Tabla (2009), Erfmeier and Bruehlheide (2005), Mandák (2003), van Clef and Stiles (2001) and Wainwright and Cleland (2013). Many studies have found adaptive evolutionary changes in seed dormancy and germination traits, which contribute to plant invasion capability, as demonstrated by Beckmann et al. (2011), Hierro et al. (2009), Kudoh et al. (2007), Leiblein-Wild et al. (2014), Udo et al. (2017) and Xia et al. (2011). By comparison among seeds from different provenances, this study found intra-specific variation in *B. pilosa*

germination traits, mainly for germination at 35 and 40°C and with a 9–24 h daily thermoperiod, indicating the effects of seed provenance on germination traits. This intra-specific variation also provides an explanation for the reported discrepancy/inconsistency in the germination temperature range of *B. pilosa* seeds in the literature. For example, Reedy and Singh (1992) reported that its optimum temperature range for germination was 25/20 to 35/30°C (day/night, 12/12 h) and that temperatures below 15/10°C were unfavourable for germination. Yan et al. (2013) recorded germination percentages of 70% at 10°C and 45% at 40°C, whereas these were less than 20% at 35°C in another study (Hong et al., 2004). However, previous studies paid little attention to the variation in seed tolerance to high temperatures even though invasive plants usually have a wide geographical distribution. This study revealed marked intra-specific variation in *B. pilosa* seeds among the three provenances, with their tolerance to high temperature always correlated with the climate conditions in the provenance of seed origin; for example, seeds from Xishuangbanna demonstrated the highest adaptation to high temperatures, whereas those from Diqin had the least and those from Chuxiong had intermediate tolerance, assessed by both enduring maximum temperature for 30-min heating and longest continuous heating duration at 40°C. This variation with that in the germination trait together revealed that *B. pilosa* seeds have the ability to evolve fast and adapt to local conditions. We suggest that both inter-specific convergence among weedy seeds in Xishuangbanna and intra-specific *B. pilosa* divergence with respect to seed high-temperature tolerance and germination traits reflect the adaptation of invasive plants to local conditions.

As a common invasive plant in tropical and subtropical regions (Huang and Kao, 2014), *B. pilosa* has been extensively studied, and it exhibits many seed traits contributing to its invasiveness. For example, this weed is comprised of individuals exhibiting two types of flowering-fruiting phenologies, which differ in seed production, seed mass, germination speed, and total germination (Gurvich et al., 2004). It is also a prolific seed producer, as its entire reproductive cycle can be completed in 57–70 days and can occur 5–6 times per year in some areas, depending on local climates; thus, a single plant can produce 3000–6000 seeds per year. Their burred seeds can be spread by attaching to animals' fur, birds' feathers, and people's clothing. Moreover, this plant produces dimorphic seeds, which differ in dispersal capability, with central achenes often dispersed earlier than peripheral achenes (Rocha, 1996), and dormancy status, with long achenes germinating readily under a wide range of conditions and short achenes showing fairly precise germination requirements (Forsyth and Brown, 1982), as well as a light requirement that can be induced by leaf canopy shade (Fenner, 1984a,b). Furthermore, this study revealed the divergence in germination traits and high-temperature tolerance of *B. pilosa* seeds. It appears that the variation in germination traits and seed high-temperature tolerance as an evidence of adaptation to local environments, in addition to the previously investigated features, could be responsible for the invasiveness of *Bidens pilosa* transferred by seeds. We concluded that high-temperature tolerance is an important seed trait that contributes to plant invasion success in open habitats in Xishuangbanna.

Declarations

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Availability of data and material All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Research did not involve human subjects or animal handling.

Consent for publication All authors consent to publication. This manuscript has not been submitted to any other journals.

References

1. Ballard R (1986) *Bidens pilosa* complex (Asteraceae) in North and Central America. *Am J Bot* 73: 1452–1465
2. Beckmann M, Bruelheide H, Erfmeier A (2011) Germination responses of three grassland species differ between native and invasive origins. *Ecol Res* 26: 763–771
3. Bochenek A, Synowiec A, Kondra B, Szymczak M, Lahuta LB, Gołaszewsk J (2016) Do the seeds of *Solidago gigantea* Aiton have physiological determinants of invasiveness? *Acta Physiol Plant* 38: 159.
4. Brändel M (2004) Dormancy and germination of heteromorphic achenes of *Bidens frondosa*. *Flora* 199: 228–233
5. Brown NAC, Mitchell JJ (1984) Germination of the polymorphic fruits of *Bidens bipinnata*. *S Afr J Bot* 3: 55–58
6. Cervera JC, Parra-Tabla V (2009) Seed germination and seedling survival traits of invasive and noninvasive congeneric *Ruellia* species (Acanthaceae) in Yucatan, Mexico. *Plant Ecol* 205: 285–293
7. de Brito IPFS, Marchesi BB, Pucci C, Carbonari CA, Velini ED (2016) Variation in the sensitivities of hairy beggarticks (*Bidens pilosa*) plants and their progenies to Glufosinate Ammonium. *Weed Sci* 64: 570–578

8. Erfmeier A, Bruelheide H (2005) Invasive and native *Rhododendron ponticum* populations: is there evidence for genotypic differences in germination and growth? *Ecography* 28: 417–428
9. Fenner M (1980a) The inhibition of germination of *Bidens pilosa* seeds by leaf-canopy shade in some natural vegetation types. *New Phytol* 84: 95–101
10. Fenner M (1980b) The induction of a light requirement in *Bidens pilosa* seeds by leaf canopy shade. *New Phytol* 84:103–106
11. Forsyth C, Brown NAC (1982) Germination of the Dimorphic Fruits of *Bidens pilosa* L. *New Phytol* 90: 151–164
12. Fumanal B, Girod C, Fried G, Bretagnolle F, Chauvel B (2008) Can the large ecological amplitude of *Ambrosia artemisiifolia* explain its invasive success in France? *Weed Res* 48: 349–359.
13. Gurvich DE, Enrico L, Funes G, Zak MR (2004) Seed mass, seed production, germination and seedling traits in two phenological types of *Bidens pilosa* (Asteraceae). *Aust J Bot* 52: 647–652
14. Hobbs RJ, Humphries SE (1995) An integrated approach to the ecology and management of plant invasions. *Conserv Biol* 9: 761–770.
15. Hong L, Shen H, Yang QH, Cao HL, Ye WH (2004) Studies on seed germination and storage of the invasive alien species *Bidens pilosa* L. *Journal of Wuhan Botanical Research* 22: 433–437 (in Chinese with an English abstract)
16. Huang YL, Kao WY (2014) Different breeding systems of three varieties of *Bidens pilosa* in Taiwan. *Weed Res* 54: 162–168.
17. Huebner CD (2022) Effects of global climate change on regeneration of invasive plant species from seeds. In: Baskin CC and Baskin J (eds) *Plant Regeneration from Seeds. A Global Warming Perspective*. Academic Press, pp 243–257.
18. Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends Ecol Evol* 16: 199–204
19. Kudoh H, Nakayama M, Lihová J, Marhold K (2007) Does invasion involve alternation of germination requirements? A comparative study between native and introduced strains of an annual Brassicaceae, *Cardamine hirsuta*. *Ecol Res* 22:869–875.
20. Lang ARG (1967) Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40°C. *Aust J Chem* 20: 2017–2013
21. Leiblein-Wild MC, Kaviani R, Tackenberg O (2014) Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. *Oecologia* 174:739–750
22. Ma JS (2013) *The Checklist of the Chinese Invasive Plants*. Beijing, Higher Education Press (*in Chinese*)
23. Mandák B (2003) Germination requirements of invasive and noninvasive *Atriplex* species: a comparative study. *Flora* 198: 45–54
24. Maurer DA, Zedler JB (2002) Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. *Oecologia* 131: 279–288.

25. Michel BE (1983) Evaluation of water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiol* 72: 66–70
26. Rashid I, Reshi Z, Allaie RR, Wafai BA (2007) Germination ecology of invasive alien *Anthemis cotula* helps it synchronise its successful recruitment with favourable habitat conditions. *Ann Appl Biol* 150: 361–369
27. Reddy KN, Singh M (1992) Germination and Emergence of Hairy Beggarticks (*Bidens pilosa*). *Weed Science* 40: 195–199
28. Ren MX, Zhang QG (2009) The relative generality of plant invasion mechanisms and predicting future invasive plants. *Weed Res* 49: 449–460
29. Rocha OJ (1996) The effects of achene heteromorphism on the dispersal capacity of *Bidens pilosa* L. *Int J Plant Sci* 157: 316–322
30. Udo N, Tarayre M, Atlan A (2017) Evolution of germination strategy in the invasive species *Ulex europaeus*. *J Plant Ecol* 10: 375–385
31. Van Clef M, Stiles EW (2001) Seed longevity in three pairs of native and non-native congeners: assessing invasive potential. *Northeast Nat* 8: 301–310
32. Vilà M, Weiner J (2004) Are invasive plant species better competitors than native plant species? – evidence from pair-wise experiments. *OIKOS* 105: 229–238
33. Wainwright CE, Cleland EE (2013) Exotic species display greater germination plasticity and higher germination rates than native species across multiple cues. *Biol Invasions* 15: 2253–2264
34. Wainwright CE, Cleland EE (2013) Exotic species display greater germination plasticity and higher germination rates than native species across multiple cues. *Biol Invasions* 15: 2253–2264
35. Wen B, Xue P, Zhang N, Yan Q, Ji MY (2015) Seed germination of invasive species *Piper aduncum* as affected by high temperature and water stress. *Weed Res* 55: 155–162
36. Wen B (2015) Effects of high temperature and water stress on seed germination of the invasive species Mexican Sunflower. *PLoS ONE* 10: e0141567
37. Whitaker C, Beckett RP, Minibayeva FV, Kranner I (2010) Alleviation of dormancy by reactive oxygen species in *Bidens pilosa* L. seeds. *South African Journal of Botany* 76: 601–605
38. Xia HB, Xia H, Ellstrand NC, Yang C, Lu BR (2011) Rapid evolutionary divergence and ecotypic diversification of germination behavior in weedy rice populations. *New Phytol* 191: 1119–1127
39. Yan WB, Quan GM, Zhang JE, Kang XW, Guo J (2013) Effects of environmental factors on seed germination of *Bidens pilosa* and *Bidens bipinnata*. *Ecology and Environmental Sciences* 22: 1129–1135 (in Chinese with an English abstract)
40. Ye J, Wen B (2017) Seed germination in relation to the invasiveness in spiny amaranth and edible amaranth in Xishuangbanna, SW China. *PLoS ONE* 12: e0175948.
41. Yuan X, Wen B (2018) Seed germination response to high temperature and water stress in three invasive Asteraceae weeds from Xishuangbanna, SW China. *PLoS ONE* 13(1): e0191710.

Figures

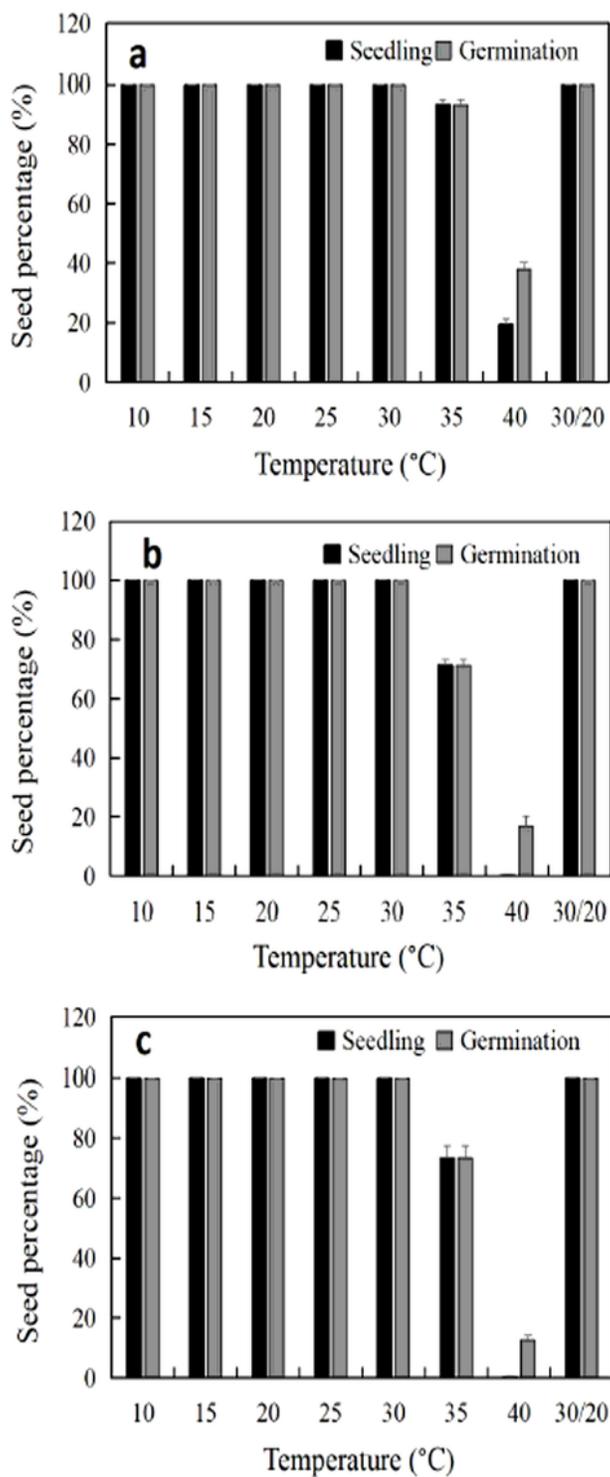


Figure 1

Germination of seeds incubated in light at 10-40 °C constant temperatures and alternating day/night temperature of 30/20 °C. Germination values are means±SE of 6 replicates of 50 seeds. a. from Xishuangbanna; b. from Chuxiong; c. from Diqi

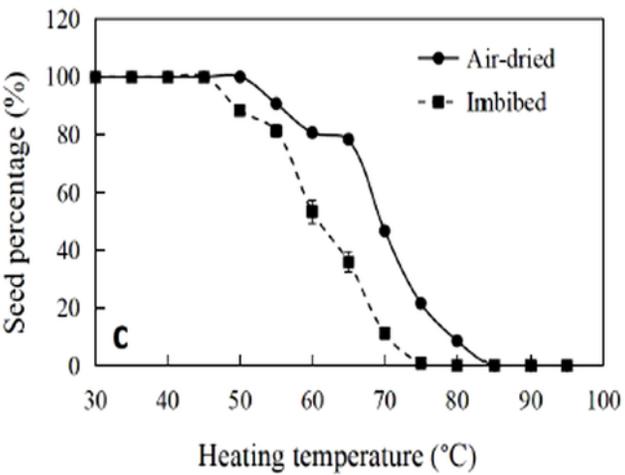
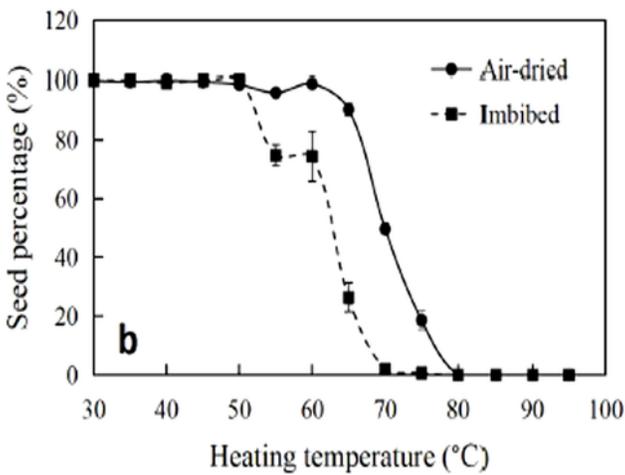
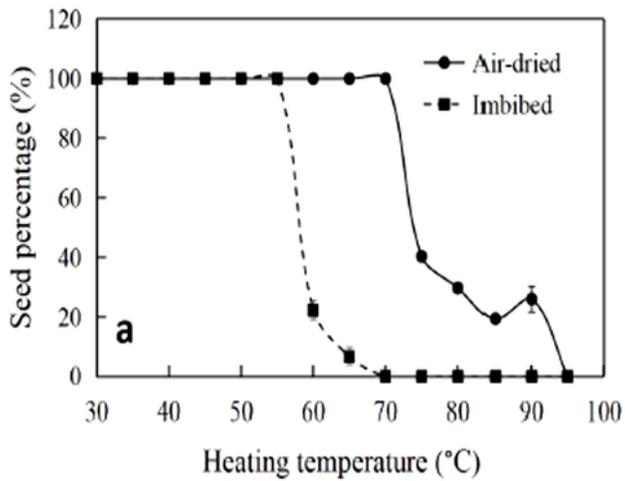


Figure 2

Effects of 30-min heat shocks at temperature from 30°C to 95°C on seed viability. Data for survival not shown because not essentially different from seedling percentage. Seeds, air-dried or imbibed, were heated at given temperature for 30 min, and then incubated at 25°C. Viability values are means \pm SE of 6 replicates of 50 seeds. a. from Xishuangbanna; b. from Chuxiong; c. from Diqi

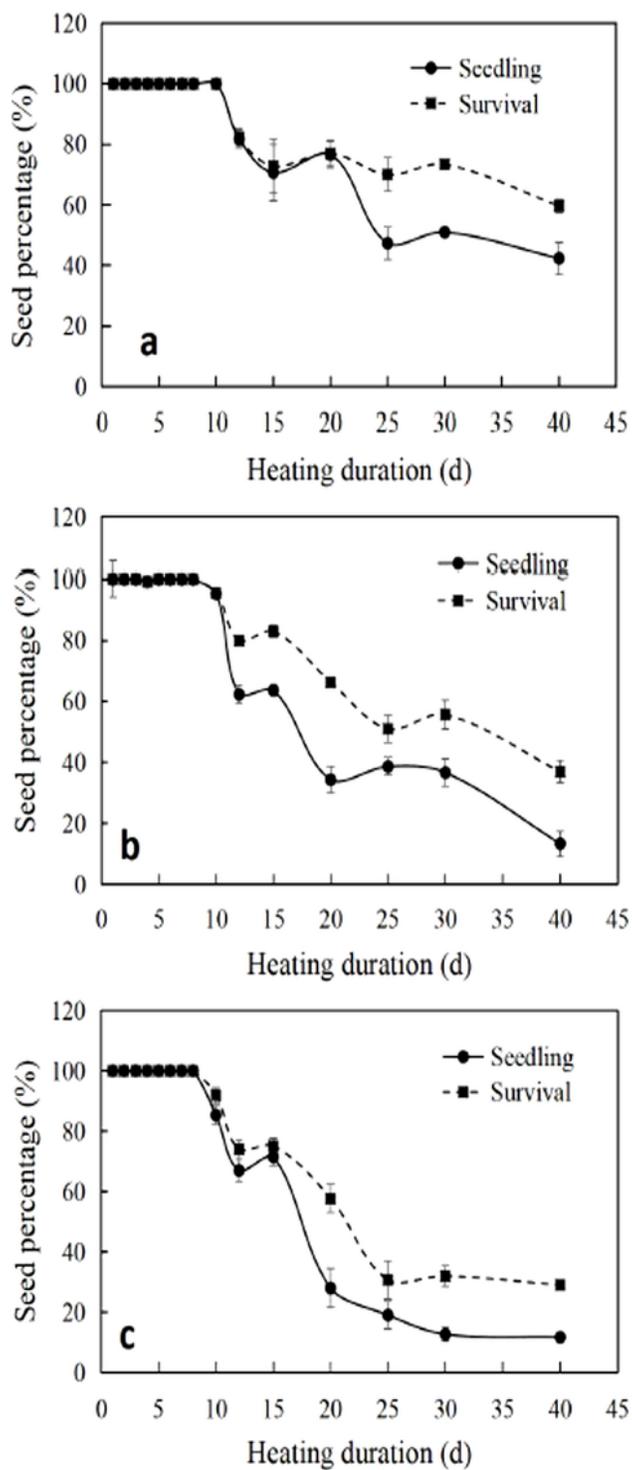


Figure 3

Effects of continuous heating at 40°C on seed viability. Seeds were heat-shocked for a given period of time at 40°C, and incubated at 25°C after released from stress. Seed viability values are means±SE of 6 replicates of 50 seeds. a. from Xishuangbanna; b. from Chuxiong; c. from Diqi

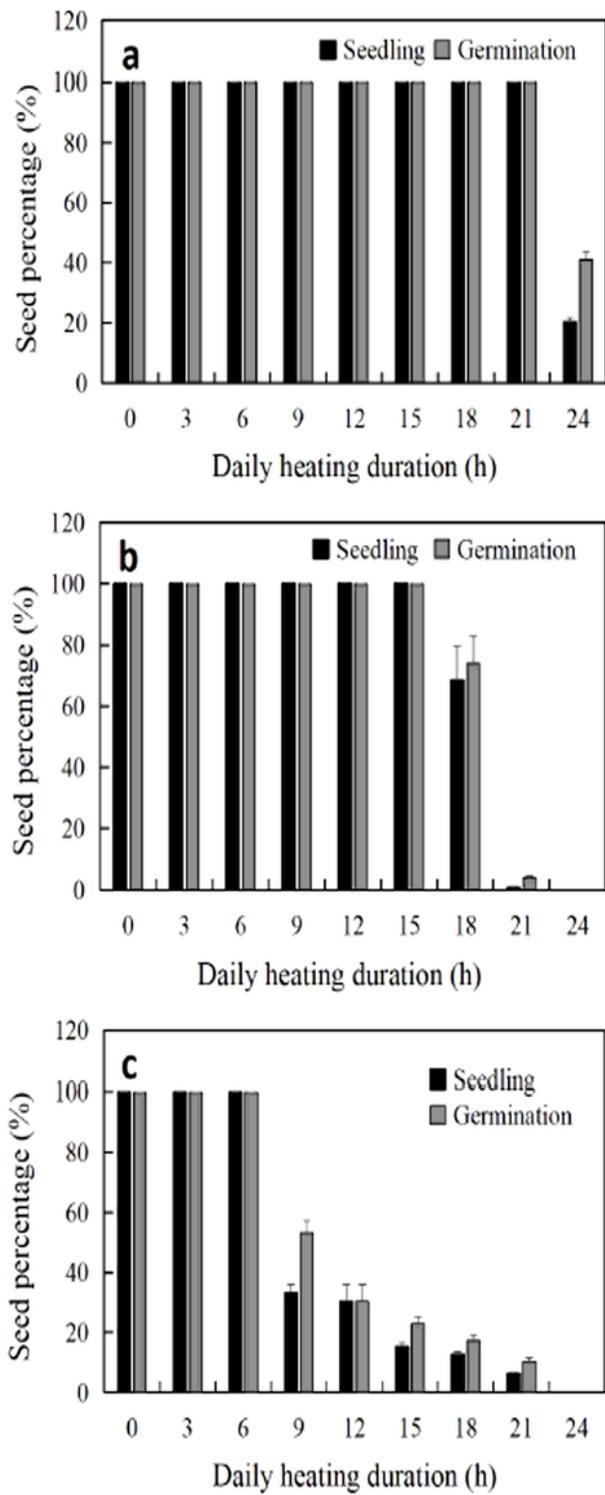


Figure 4

Germination of seeds under daily periodic high-temperature stress at 40°C. Heating of given thermoperiod at 40°C was imposed on seeds every day. Germination values are means±SE of 6 replicates of 50 seeds. a. from Xishuangbanna; b. from Chuxiong; c. from Diqi

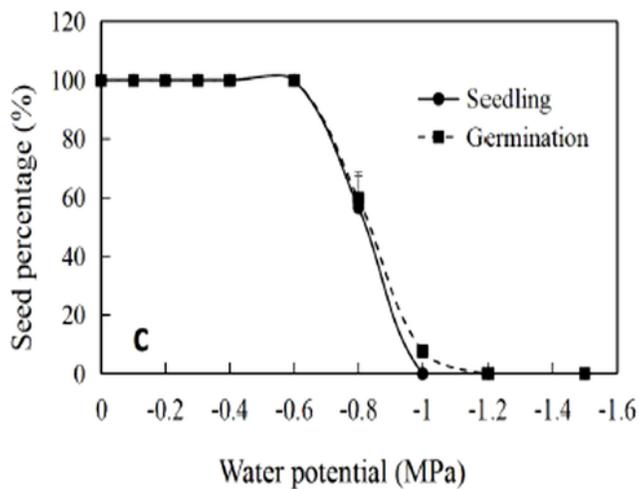
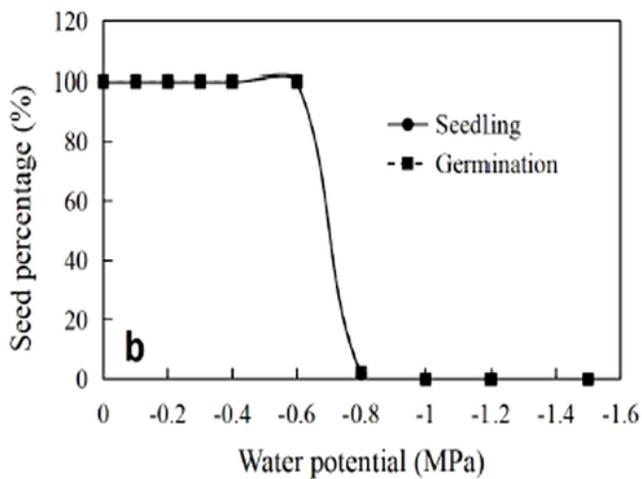
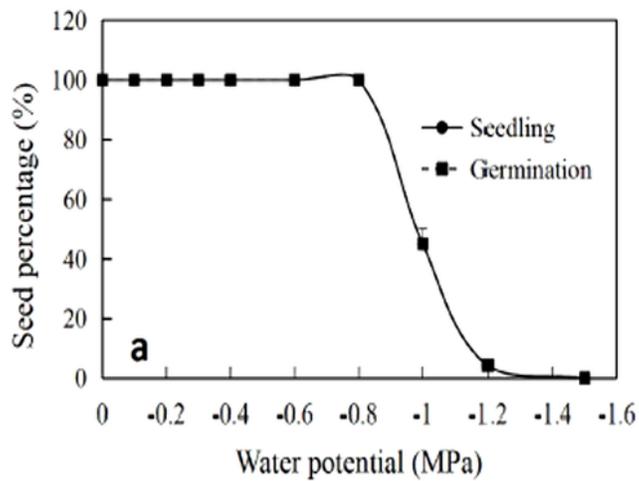


Figure 5

Effects of water availability on seed germination. Seed germination was scored for 4 weeks under water stress, and released from stress followed by two-more-week's incubation to check seed viability. Germination values are means \pm SE of 6 replicates of 50 seeds. a. from Xishuangbanna; b. from Chuxiong; c. from Diqi

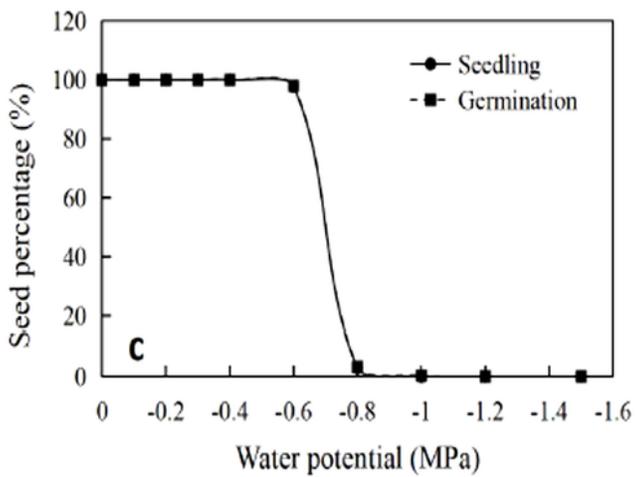
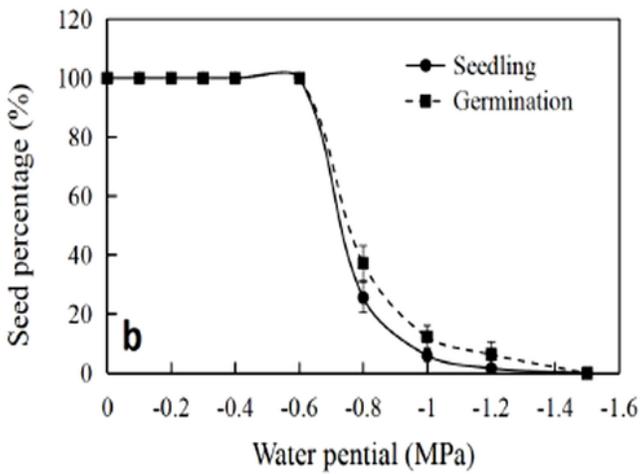
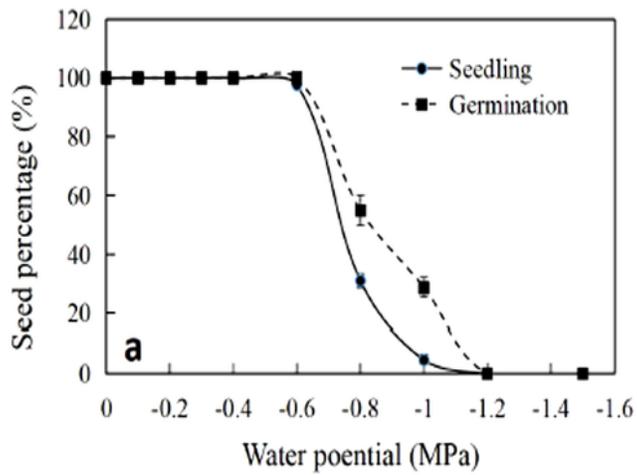


Figure 6

Effects of NaCl stress on seed germination. Seed germination was scored for 4 weeks under NaCl stress, and released from stress followed by two-more-week's incubation to check seed viability. Germination values are means \pm SE of 6 replicates of 50 seeds. a. from Xishuangbanna; b. from Chuxiong; c. from Diqi;