

Effects of Environmental Factors on Seed Germination of *Salix linearistipularis*

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Molecular Soil Biology, 2018, Vol.9, No.1 doi: [10.5376/msb.2018.09.0001](https://doi.org/10.5376/msb.2018.09.0001)

Received: 27 Nov., 2018

Accepted: 04 Dec., 2018

Published: 29 Dec., 2018

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Preferred citation for this article:

Ma S.R., Xu W.D., Qi Q., Li Y., Shi M.Y., and Liu S.K., 2018, Effects of environmental factors on seed germination of *Salix linearistipularis*, Molecular Soil Biology, 9(1): 1-9 (doi: [10.5376/msb.2018.09.0001](https://doi.org/10.5376/msb.2018.09.0001))

Abstract The effects of soil moisture, temperature, storage condition, drought, and saline-alkali stress on seed germination of *Salix linearistipularis* were studied by simulating natural field conditions in the laboratory. (1) *S. linearistipularis* seeds demonstrate a fast water absorption rate, with a maximum absorbed amount of 200% within 30 min. (2) The seeds require accurate soil moisture and start to germinate when they contain sufficient water. The optimal soil moisture ranged from 100% to 200%, and the seeds did not germinate when the water content was lower than 25%. (3) The suitable temperature for seed germination ranged from 25 °C to 30 °C, but the process was inhibited when the temperature was lower than 15 °C or higher than 35 °C. (4) The seeds were short-lived with a germination rate of 3% in 45 d at room temperature. After storage at -80 °C for 1 year, the seed germination rate remained 50% and (5) was significantly inhibited by saline-alkali and drought stresses. Low concentrations of NaHCO₃ solution can promote the growth of the seedlings and young roots of *S. linearistipularis*.

Keywords *Salix linearistipularis*; Seed germination; Temperature; Water stress; Saline-alkali stress

Background

Salix linearistipularis is a small tree that belongs to *Salix* of *Salicaceae* and is mainly distributed in Heilongjiang, Jilin, Liaoning, and Hebei. This plant is cold resistant, flood tolerant, and saline-alkali tolerant and often grows by the river, in plains with low attitude and high humidity, and in saline-alkali land (Feng, 2013). *S. linearistipularis* is an ideal plant for windbreak, sand fixation, and landscaping because of its resistance against drought, barren soil, and saline alkali (Shang et al., 2010). This woody plant is rare and naturally distributed in Songnen Plain (Li, 2006); this species can grow in saline-alkali land with pH as high as 9.2. *S. linearistipularis* not only exhibits strong saline-alkali tolerance, but also improves soil pH (Ishida, 2009). Therefore this plant is suitable for saline-alkali afforestation. Despite that *S. linearistipularis* exhibits sexual and asexual reproduction, seed regeneration on saline-alkali soil of Songnen Plain is difficult. Only a small amount of seedlings can be found in wetlands within the distribution area, hence natural regeneration is primarily through asexual reproduction. Although *S. linearistipularis* presents high seed production rates and produces a large number of seeds every year (Feng, 2013), most of them exist as a single plant in Songnen Plain. The population size is very small and has not yet formed into large natural distribution. The seeds exhibit very low survival ratio, and the underlying cause must be further investigated.

Few studies focused on *S. linearistipularis*, and reports on genetic transformation (Han, 2013), sexual reproduction (Feng, 2013), tissue culture (Wu et al., 2009), windbreak, and sand fixation (Shang et al., 2010) are limited. No research has been conducted on seed germination and seedling growth characteristics. This study aims to study the effects of water content, drought stress, saline-alkali concentration, temperature, and storage condition on seed germination by simulating natural field conditions. We also investigated environmental factors that affect the natural regeneration of *S. linearistipularis* to reveal the main reason for its large yield but very few seedlings. This study provides theoretical basis and experimental data for further development and utilization of *S. linearistipularis* resources in saline-alkali land and for improvement of saline-alkali soil.

1 Materials and Methods

1.1 Sampling location and seed collection

Samples were collected from Anda city, which is located in the hinterland of southwest Heilongjiang Province on longitude 124°53'~125°55' and latitude 46°01'~47°01'. This area is in the temperate continental semi-arid monsoon climate zone and experiences cold and dry winter and hot and rainy summer, with low annual rainfall, large evaporation, and drought condition (Guan et al., 2009).

At the end of May 2013, *S. linearistipularis* seeds were collected from the Anda Experimental Base of the Northeast Forestry University Alkali Soil Natural Experimental Science Center and then transferred into the laboratory. Well-stacked and pest-free seeds were selected and stored at 25 °C, 4 °C, -20 °C, and -80 °C (Feng, 2013).

Seed germination begins with the emergence of radicle; the sprouting of seed is indicated by the length of radicle, which reaches 1/2 of the seed length (Liang, 1995).

1.2 Storage condition for seed germination experiment

Seeds were stored at 25 °C, 4 °C, -20 °C, and -80 °C. Experiment was conducted using culture dish-paper germination method every 2 d from seed collection and every 5 d after 30 d. After 90 d, germination test was performed once per month until the seeds stopped germinating. Data on germination rate were collected (Andrew et al., 2013; Duc et al., 2015).

1.3 Seed imbibition experiment

Four groups were established with 200 seeds in each group. Dry weight was obtained. Seeds were soaked in warm water, collected every 5 min, and placed on a dry filter paper. After water on the seed surface was absorbed by the paper, the seeds were weighed until a constant weight (Zheng et al., 2013).

1.4 Temperature in seed germination experiment

A total of 50 *S. linearistipularis* seeds were placed in a culture dish with a diameter of 10 cm and covered with a double layer of filter paper. The dish was then placed in an incubator at 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C, and each treatment was performed for four times. Seed germination was observed and recorded daily, and water was added (Andre et al., 2014).

1.5 Soil moisture in seed germination experiment

Approximately 20 g of sterilized sand and 50 seeds were placed into a culture dish with a diameter of 10 cm. The culture dish was sprayed with 5, 10, 20, 30, 40, and 60 mL of distilled water, which corresponded to soil water content of 25%, 50%, 150%, 200%, and 300%, respectively. The culture dish was cultivated in a ZPQ-400 climate-smart incubator with an illumination time of 12 h/d, level 5 light intensity, and RH of 19%~23 % at 25 °C. Each treatment was repeated for four times, and observation and recording were conducted daily (Marcos et al., 2013).

1.6 Drought stress in seed germination experiment

Polyethylene glycol (PEG) 6000 solution with concentrations of 0 (distilled water only), 0.05, 0.1, 0.15, and 0.2 g/mL were prepared, with the corresponding water potential gradient of solution of 0, -0.10, -0.20, -0.40, and -0.60, respectively (Zhou et al., 2012). A total of 50 seeds were placed in a culture dish with double filter paper. The dish was placed in a climate-smart incubator at 25 °C. The experiment was repeated for four times, and observation and recording were conducted daily.

1.7 NaCl and NaHCO₃ stress in seed germination experiment

NaCl concentration was set at 0, 50, 100, 150, 200, and 250 mmol/L, and NaHCO₃ concentration was divided into six gradients, namely, 0, 10, 20, 30, 40, and 50 mmol/L. For treatment with each gradient, 50 seeds were placed in a culture dish with double layer of filter paper. The dish was then placed in a climate-smart incubator at 25 °C. Each treatment was repeated for four times, and observation and recording were conducted daily.

Non-germinating seeds were placed in a container with distilled water and subjected to germination experiment, and germinative number was then recorded (Zeng et al., 2006; Guan et al., 2009; Yan et al., 2013).

1.8 Data analysis

SPSS 13.0 statistical analysis software was used to determine significant differences in seed germination rate. $p < 0.05$ indicates significant difference, and $p < 0.01$ implies extreme significant difference. Tukey test was applied for multiple comparison. The difference level of treatment items was determined to construct a linear regression equation. Excel 2003 software was used for chart preparation (Luiza et al., 2012).

2 Results

2.1 Imbibitional characteristics of *S. linearistipularis* seeds

The absorption process of ordinary seeds can be divided into three stages. In the first stage (lasts for 0~5 h), seeds absorb a considerable amount of water and their water absorption rate is very high. In the second stage (lasts for 5~10 h), seeds still absorb water but their water absorption rate is lower than that in the previous stage. In the third stage (lasts for 10~40 h), the water absorption reaches the saturation point and the seed enters into pre-germination status (Bregman et al., 1997). *S. linearistipularis* seeds exhibit a very high water absorption rate (Figure 1); in the first, second, and third stage (last for 1~10, 10~30, and 30~120 min, respectively), water absorption reaches 133% at the 10th min, obtains the maximum of 200.50% at the 30th minute, and becomes saturated, respectively.

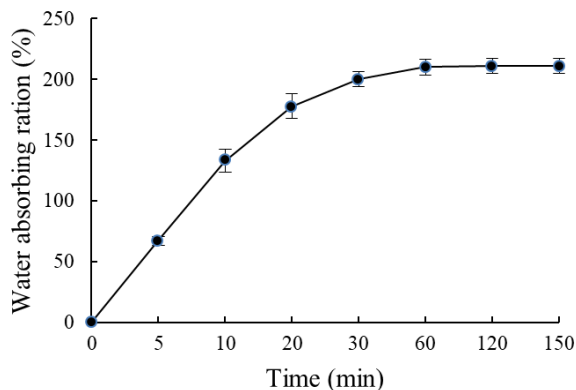


Figure 1 Water absorption ration of *S. Linearistipularis* (0~150 min)

On the average, plant seeds begin to germinate 40 h after absorbing water (Bregman et al., 1997). *S. linearistipularis* seeds exhibit a very fast germination rate. Under appropriate environmental conditions, the seeds enter into the germination status 8~12 h after absorbing water, the radicle breakthrough after 12~24 h, and germination is terminated after 48 h. The germination rate reaches 91.00% \pm 3.51%. Figure 2 shows the whole process of seed germination.

2.2 Effect of storage condition on seed germination

The seed germination rate reaches 3% after storage at 25 °C for 45 d (Figure 3), which is consistent with the experimental results reported in a relevant study (Feng, 2013). Low storage temperatures can extend seed longevity. After storage at -80 °C, the germination rate of seed reaches more than 50% after 1 year.

2.3 Effect of temperature on seed germination

S. linearistipularis seeds can germinate at 15 °C to 35 °C (Figure 4). On the fifth day at 15 °C, some seeds do not germinate and the final germination rate is only 74.00% \pm 2.84%, which is significantly lower than the seed germination rate at other temperatures ($p < 0.01$). After 5 d, some seeds continue to germinate but the germination rate is very low. At 20 °C to 30 °C, the final seed germination rate does not significantly differ ($p > 0.05$). Figure 4 shows that low (15 °C) or high temperatures (35 °C) significantly delay the seed germination rate. Therefore, the most suitable temperature for *S. linearistipularis* seed germination ranges from 25 °C to 30 °C.

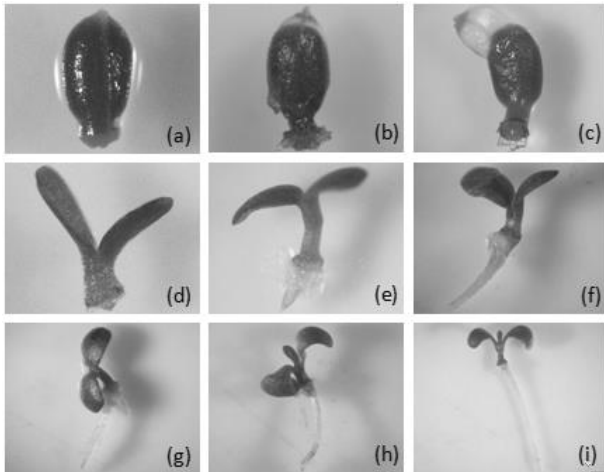


Figure 2 Morphological changes of *S. Linearistipularis* seed germination

Note: (a) Seed absorbed water, 1 h (bar = 500 μ m). (b) Seed coat cracking, 5 h (bar = 500 μ m). (c) Seed coat separated from the cotyledon, 8 h (bar = 500 μ m). (d) Cotyledon unfolded and radicle began to grow, 12 h (bar = 500 μ m). (e) Radicle elongation growth, 24 h (bar = 500 μ m). (f) Hypocotyl and radicle elongated rapidly, 48 h (bar = 1 mm). (g) Cotyledon continued to grow, and the first leaf begin to grow, 72 h (bar = 1 mm). (h) The first leaf is formed, 96 h (bar = 1 mm). (i) Cotyledon and the first leaf continued to grow, 120 h (bar = 1 mm)

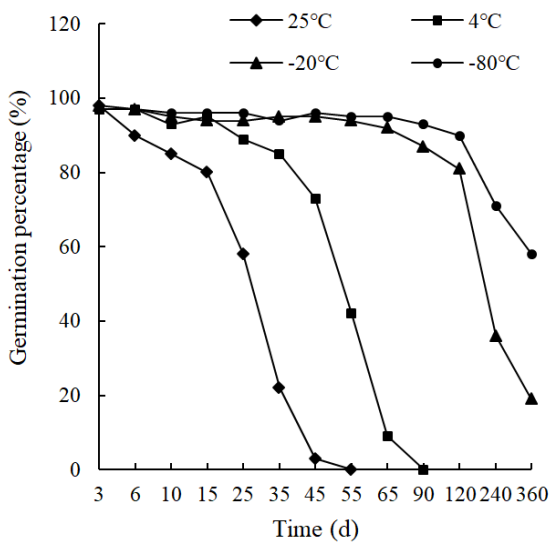


Figure 3 Effect of different storage temperatures on the seed germination percentage of *S. linearistipularis*

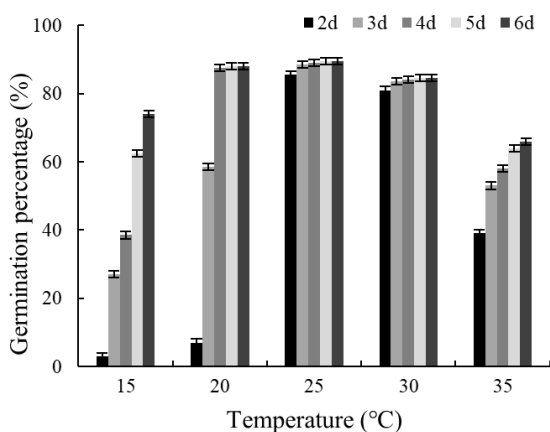


Figure 4 Effect of temperature (15 °C to 35 °C) on the germination percentage of *Salix linearistipularis*. Each point represents mean (1SD) of four replicates

Seedling length, germination vigor, germination index, and vitality index increase and then decrease (Table 1) with increasing temperature. At 30 °C, seedling length and vitality index reached their maximum values, which significantly differ from the other indices at other temperatures ($p < 0.01$). Germination vigor and index reach their maximum at 25 °C, and young seedlings do not grow at 15 °C. Therefore, the range of 25 °C to 30 °C is the optimum temperature for the growth of *S. linearistipularis* seedlings.

2.4 Effect of soil water content on seed germination and seedling growth

Water content is the main environmental factor that affects the germination of *S. linearistipularis* seeds (Table 2). These seeds do not germinate when the soil water content is 25% but exhibit a higher germination rate at 50%. The nascent seedling growth is also not ideal. At soil water content $\geq 50\%$, the seed germination rate increases and then decreases but the difference is not significant ($p > 0.05$). At water content of 150%, the seed germination rate reaches the maximum. Seedling length, germination vigor, and germination index are not significantly different ($p > 0.05$) when the soil water content is $\geq 100\%$, but significantly different at water contents of 25% and 50% ($p < 0.01$). Vitality index reaches the maximum when the soil water content is 300%. Nevertheless, excessive water content causes excessive growth of the roots, as observed from the root length. Hence, this condition is not conducive to seedling growth.

2.5 Effect of drought stress (PEG 6000) on seed germination and seedling growth

Germination rate, seedling length, germination vigor, germination index, and vitality index increase and then decrease with increasing PEG (6000) concentration and reaches the maximum when the PEG = 0.05 g/mL (Table 3). At PEG = 0.15 g/mL, all index significantly differs from that of the control group ($p < 0.01$). At PEG = 0.2 g/mL, seeds begin to germinate on the third day and the final germination rate is 20.00% \pm 0.65%, which is significantly lower than that of the control group (86.00% \pm 2.31%) ($p < 0.01$). The other indices significantly differ from the control group ($p < 0.01$), and seedlings also exhibit poor growth.

Table 1 Effect of temperature on the growth of *S. linearistipularis* seedlings

Temperature (°C)	Seedling length (mm)	Germination potential (%)	Germination index	Vigor index
15	3.43 \pm 0.25 ^e	27.00 \pm 0.98 ^c	36.63 \pm 1.21 ^c	32.90 \pm 1.09 ^d
20	5.89 \pm 0.36 ^{cd}	58.50 \pm 2.86 ^b	67.13 \pm 2.32 ^b	70.48 \pm 2.35 ^c
25	9.57 \pm 0.76 ^b	88.50 \pm 3.88 ^a	99.85 \pm 3.68 ^a	185.71 \pm 7.25 ^b
30	10.61 \pm 0.67 ^a	83.50 \pm 3.24 ^a	94.38 \pm 3.98 ^a	277.48 \pm 10.37 ^a
35	6.72 \pm 0.37 ^c	53.00 \pm 2.94 ^b	57.05 \pm 2.12 ^b	76.45 \pm 1.23 ^c

Note: Data with different lowercase letters in the same row are significantly different at 0.05 level, the same below

Table 2 Effect of water content on the growth of *S. linearistipularis* seedlings

Water Germination Content (%)	Percentage (%)	SRL (m/g)	Seedling length (mm)	Germination potential (%)	Germination index	Vigor index
25	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^e	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^d
50	82.00 \pm 3.02 ^{ab}	11.32 \pm 0.35 ^c	8.22 \pm 0.58 ^b	73.50 \pm 2.86 ^b	57.30 \pm 1.92 ^{ab}	45.84 \pm 1.12 ^c
100	87.00 \pm 2.98 ^a	9.27 \pm 0.31 ^{cd}	10.22 \pm 0.76 ^a	81.50 \pm 2.88 ^a	62.50 \pm 2.13 ^a	75.00 \pm 2.13 ^b
150	88.60 \pm 3.13 ^a	8.85 \pm 0.37 ^{cd}	9.86 \pm 0.64 ^{ab}	84.67 \pm 2.24 ^a	64.50 \pm 2.24 ^a	93.52 \pm 2.92 ^{ab}
200	88.00 \pm 2.88 ^a	28.68 \pm 0.71 ^b	9.70 \pm 0.79 ^{ab}	85.50 \pm 1.94 ^a	64.60 \pm 1.98 ^a	115.92 \pm 3.40 ^a
300	86.60 \pm 2.65 ^a	39.52 \pm 1.02 ^a	9.59 \pm 0.71 ^{ab}	83.50 \pm 2.35 ^a	63.30 \pm 2.02 ^a	120.27 \pm 3.98 ^a

Table 3 Effect of PEG (6000) on the growth of *S. linearistipularis* seedlings

PEG Concentration (g/ml)	Germination percentage (%)	Seedling length (mm)	Germination potential (%)	Germination index	Vigor index
0	86.00 \pm 2.31 ^a	9.88 \pm 0.32 ^{ab}	78.50 \pm 1.43 ^a	60.75 \pm 2.02 ^a	96.59 \pm 3.83 ^a
0.05	90.00 \pm 2.15 ^a	10.01 \pm 0.36 ^a	79.00 \pm 2.19 ^a	61.00 \pm 1.98 ^a	98.21 \pm 3.89 ^a
0.1	82.00 \pm 2.09 ^a	9.29 \pm 0.19 ^b	74.00 \pm 2.21 ^a	57.50 \pm 1.95 ^b	81.07 \pm 3.65 ^b
0.15	60.00 \pm 2.98 ^b	7.70 \pm 0.12 ^c	44.00 \pm 1.23 ^b	31.00 \pm 0.92 ^c	31.31 \pm 1.41 ^c
0.2	20.00 \pm 0.65 ^c	6.70 \pm 0.09 ^d	0.00 \pm 0.00 ^c	5.00 \pm 0.50 ^d	4.10 \pm 0.50 ^d

2.6 Effect of NaCl stress on seed germination and seedling growth

Under the influence of NaCl stress, seed germination rate, vitality index, and seedling length increase and then decrease with increasing stress concentration. A low NaCl concentration inhibits seed germination (Table 4). At NaCl = 50 mmol/L, the germination rate is $76.50\% \pm 3.12\%$, which is significantly lower than that of the control group (0 mmol/L, $87.00\% \pm 2.39\%$) ($p < 0.01$). The other indices are also significantly lower than those of the control group ($p < 0.01$). Moreover, seeds are forced to germinate on the next day when NaCl ≤ 200 mmol and on the third day when NaCl = 250 mmol. Under this condition, the germination rate is only $11.50\% \pm 0.45\%$, the cotyledons spread, and the radicle does not grow. Linear regression analysis was adopted to analyze different NaCl concentrations and relative germination rates. The linear regression equation is $y = -0.311x + 99.958$. The salt-tolerance limit, semi-lethal salt concentration, and salt concentration of *S. linearistipularis* seeds are 241.02, 160.63, and 80.25 mmol/L, respectively. Non-germinated seeds were transferred into a container with clean water for seed germination experiment. NaCl-treated seeds become dark brown and inactivated, and they cannot germinate again. Few seeds that do not change color germinate after water immersion. With increasing NaCl concentration, the proportion of seed inactivation gradually increases.

2.7 Effect of NaHCO₃ stress on seed germination and seedling growth

After treatment with different concentrations of NaHCO₃ solution, seeds germinate on the second day; the results in the initial germination day is not correlated with stress concentration. Under NaHCO₃ stress, seed germination rate, germination vigor, and germination index decrease with increasing stress concentration. Seedling length and vitality index also increase and then decrease (Table 5). At NaHCO₃ = 10 mmol/L, the seed germination rate is $77.00\% \pm 3.11\%$, which is significantly lower than that of the control group (0 mmol/L, $87.00 \pm 2.39\%$) ($p < 0.01$). At this concentration, seedling length and vigor index reach their maximum values. At NaHCO₃ = 30 mmol, the seedling length is not significantly different from that of the control group ($0.01 < p < 0.05$). At NaHCO₃ = 50 mmol, seed cotyledons can expand but the radicle growth is poor. Linear regression analysis was adopted to analyze the different NaHCO₃ concentrations and relative germination rates. The linear regression equation is $y = -1.036x + 101.062$, $R^2 = 0.989$. The alkali-tolerance limit, semi-lethal alkali concentration, and alkali concentration of *S. linearistipularis* seeds are 73.41, 49.29, and 25.16 mmol/L, respectively. Non-germinated seeds are transferred into a container with clear water for seed germination recovery test. The experimental results are similar to those obtained in NaCl treatment.

Table 4 Effect of NaCl on the growth of *S. linearistipularis* seedlings

Concentration of NaCl (mmol/L)	Germination percentage (%)	Seedling length (mm)	Germination potential (%)	Germination index	Vigor index
0	87.00 ± 2.39^a	9.57 ± 0.76^a	82.50 ± 2.14^a	63.00 ± 4.00^a	129.78 ± 7.98^a
50	76.50 ± 3.12^b	5.42 ± 0.47^b	72.00 ± 2.86^b	55.12 ± 3.92^b	77.17 ± 5.32^b
100	56.00 ± 1.91^c	2.93 ± 0.29^c	52.00 ± 1.88^c	40.00 ± 3.23^c	44.80 ± 3.13^c
150	43.50 ± 1.19^d	2.12 ± 0.18^d	37.00 ± 1.10^d	29.37 ± 1.21^d	22.35 ± 2.89^d
200	38.88 ± 1.21^e	2.09 ± 0.12^e	27.00 ± 1.01^e	23.00 ± 1.02^e	15.80 ± 2.40^e
250	11.50 ± 0.45^f	2.07 ± 0.11^e	0.00 ± 0.00^f	1.50 ± 0.03^f	0.70 ± 0.02^f

Table 5 Effect of NaHCO₃ on the growth of *S. linearistipularis* seedlings

Concentration of NaHCO ₃ (mmol/L)	Germination percentage (%)	Seedling length (mm)	Germination potential (%)	Germination index	Vigor index
0	$87.00 \pm 2.39a$	$9.57 \pm 0.76b$	$82.50 \pm 2.14a$	$63.00 \pm 4.00a$	$129.78 \pm 7.98a$
10	$77.00 \pm 3.11b$	$10.72 \pm 0.67a$	$76.00 \pm 3.12b$	$57.75 \pm 3.88b$	$133.98 \pm 5.45a$
20	$72.00 \pm 2.99b$	$10.47 \pm 0.69a$	$70.00 \pm 1.89b$	$52.50 \pm 3.85c$	$95.03 \pm 4.65b$
30	$58.00 \pm 1.61c$	$8.12 \pm 0.31c$	$55.50 \pm 1.11c$	$42.00 \pm 3.01d$	$60.90 \pm 3.78c$
40	$54.50 \pm 1.79cd$	$4.23 \pm 0.16d$	$51.50 \pm 1.58cd$	$39.39 \pm 2.23e$	$46.85 \pm 2.01d$
50	$26.00 \pm 1.08d$	$2.01 \pm 0.12e$	$14.00 \pm 1.01d$	$27.00 \pm 2.02f$	$16.46 \pm 1.02e$

3 Discussion

3.1 Seed longevity of *S. Linearistipularis*

Longevity is the period when seed transforms from being fully mature to loss of vitality. The differences in seed longevity are caused by the genetic characteristics of seed and environmental conditions (Sun et al., 2007). Seed longevity is affected by many factors, such as seed water content, storage condition, and seed coat structure. Different seeds exhibit different longevities when stored in different environmental conditions (Dong et al., 2001). Short-lived seeds have high water content, and their skins demonstrate good air permeability under suitable conditions; these seeds also exhibit exuberant metabolic process, which consumes abundant materials stored in the seed and causes loss of viability in a short time (Liu et al., 2003). *S. linearistipularis* seeds are typically short-lived, and their germination rate is 22% and 3% on the 35th and 45th after falling, respectively. The longevity of the seeds can be extended when stored at low temperatures. After 1 year, the germination rate of a frozen seed at -80 °C is still 50%. Under low-temperature storage, seed respiration is very weak and the metabolism level is slow, consuming a very low amount of energy; changes in aging inside the cells are also reduced to a minimum degree, thus maintaining seed viability for a longer period and prolonging seed longevity (Hui et al., 2012).

3.2 Effect of water content on seed germination

Seed germination and seedling survival depend on the soil water content and water absorption ability of the seedling roots (Luiza et al., 2012). The experimental results in soil water content indicate that when the soil water content is low, *S. linearistipularis* seeds exhibit delayed germination or even does not germinate. Low soil water content also inhibits the growth of roots; under this condition, the roots cannot properly absorb water and minerals and the internal physiological activity of the plant is decelerated. Seed germination and seedling growth are satisfactory when the soil water content reaches 100%~200%. By contrast, when the soil water content reaches 300%, soil lacks oxygen; this condition is not conducive to root's nutrient metabolism and may cause excessive root growth (Zeng et al., 2014). Under suitable water content conditions, seeds begin to germinate at 15 °C to 35 °C; the optimal temperature for germination ranges from 25 °C to 30 °C, and the germination rate is higher than 87%. These findings illustrate that water content is a major environmental factor that affects seed germination.

Imbibition is the first stage of seed germination. After imbibition occurs, the material and energy metabolism of seed converts from a quiescent state into an intense activity status (Liang, 1995). *S. linearistipularis* seeds exhibit high absorbance, and the seed coat demonstrates satisfactory permeability and germination rate, which reaches the saturation status after 30 min. The radicle also begins to break the seed coat after 24 h. When water is adequate in the environment, the high germination rate promotes the natural regeneration of the plant population (Sun et al., 2007). *S. linearistipularis* seeds mature in late May or early June and begin to fall thereafter. At this time, the average temperature of Songnen Plain ranges from 15 °C to 26 °C (Guan et al., 2009). Although the area reaches the optimum temperature for seed germination (25 °C), strong wind and minimal rain exist, with amount of rainfall ranging from 29.7 mm to 31.25 mm, resulting in very low water content on the soil surface (Guan et al., 2009). As a result, seeds cannot germinate because of lack of water. The seed longevity is also very short, and germination rate is only 40% after 25 d from the date of spreading. Before the rainy season in July, most seeds have lost vitality. The mismatch of seed longevity with local rainy season could be attributed to the large amount of *S. linearistipularis* seeds but very few seedlings in Songnen Plain.

3.3 Effect of saline-alkali stress on seed germination of *S. linearistipularis*

The seed germination rate under saline-alkali stress conditions can reflect the resistance of the seed (Guan et al., 2009). Various indices of seed germination gradually decrease with increasing NaCl concentration. However, under NaHCO₃ stress, low concentrations of NaHCO₃ solution promote not only seed germination, but also seedling growth. This finding indicates that seeds exhibit specific anti-alkali properties. To some extent, drought and salinity belong to osmotic stress; drought causes strong surface water evaporation and severe surface salt accumulation (Zeng et al., 2014). By simulating drought experiment with PEG (Zeng et al., 2006; Zhou et al., 2012), an appropriate concentration of PEG (6000) solution can promote germination. At PEG = 0.05 g/mL, the

seed germination rate is higher than that of the control group. Nevertheless, excessive osmotic stress can cause germination failure of *S. linearistipularis* seeds.

Saline-alkali and drought stresses significantly inhibit seed germination by lowering germination rate, delaying initial germination time, and extending germination period. These factors also affect the natural survival of *S. linearistipularis* seeds. However, low concentrations of NaHCO₃ solution can significantly promote seed germination, seedling growth, and radicle growth. These findings could be attributed to the evolutionary strategy of *S. linearistipularis* to adapt to saline-alkaline soils in Songnen Plain.

Authors' contributions

MSR and LSK conceived of the study and participated in the design and coordination of the study. XWD tuned and executed the study, collected data and performed the statistical analysis. QQ helped to draft the manuscript. LY and SMY helped in data collection and edit the drafted manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the Special Fund for Forest Scientific Research in the Public Welfare (201404220), Program for Changjiang Scholars and Innovative Research Team in University (IRT13053), and National High Technology Research and Development Program (863 Project, 2013AA102701-7).

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