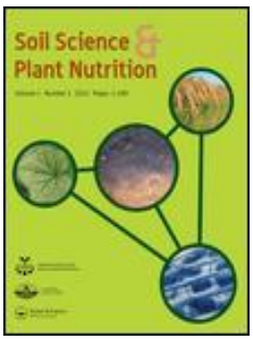

Soil Science and Plant Nutrition

ISSN: 0038-0768 (Print) 1747-0765 (Online) Journal homepage: http://www.tandfonline.com/loi/tssp20

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To cite this article: JianJun Kang, LiJun Yue, SuoMing Wang, WenZhi Zhao & AiKe Bao (2016): Na compound fertilizer stimulates growth and alleviates water deficit in the succulent xerophyte Nitraria tangutorum (Bobr) after breaking seed dormancy, Soil Science and Plant Nutrition, DOI: 10.1080/00380768.2016.1232600

To link to this article: http://dx.doi.org/10.1080/00380768.2016.1232600

Published online: 18 Oct 2016.

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SOIL SCIENCE AND PLANT NUTRITION, 2016
http://dx.doi.org/10.1080/00380768.2016.1232600

ORIGINAL ARTICLE

Na compound fertilizer stimulates growth and alleviates water deficit in the succulent xerophyte *Nitraria tangutorum* (Bobr) after breaking seed dormancy

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ABSTRACT

Nitraria tangutorum (Bobr), a typical succulent xerophyte with high level of seed dormancy, is one of the few shrubs found to date that can develop and form fixed dunes in desert regions. Our studies have demonstrated that the strong drought tolerance of the succulent xerophytes was strongly linked to high sodium (Na^+) accumulation in the photosynthesizing branches (PB) as well as leaves. The study is to explore a method that can rapidly promote the seed germination of *N. tangutorum*, and then investigate the positive effects of Na compound fertilizer (NaCF) on the growth and drought tolerance of *N. tangutorum* and ecological environment by short-term pot experiment in a greenhouse and long-term field and pot experiment in a desert environment. The results indicate that the germination rate of seeds obtained a maximum by 69% when seeds were treated with 150 mg L^{-1} gibberellic acid (GA_3) for 48 h followed by soaking in concentrated sulfuric acid (98% H_2SO_4) for 55 min, and then germinated (25/5°C) in darkness for 8 d. After breaking seed dormancy, the NaCF significantly stimulated growth of *N. tangutorum* and, concomitantly, improved its ability to cope with water deficit (30% of field water capacity) by increasing Na^+ more than Potassium (K^+) accumulation for osmotic adjustment in greenhouse and desert conditions. The contribution (take the pot experiment in the desert, for example) of Na^+ to the osmotic potential (compared with control) varied from 13.9% in plants subjected to diammonium phosphate [$(NH_4)_2HPO_4$] to, surprisingly, 63.9% in plants grown in the presence of NaCF under water deficit. The distribution characteristics of the total Na^+ (1620 mg) in the NaCF indicate that 691.2 mg (42.7%) is absorbed by plants, 848.8 mg (52.4%) remained in the pot and 80 mg (4.9%) leached, which accounted for 2.2% of the nursery soil, respectively. The positive effect of NaCF on the drought resistance of *N. tangutorum* and the ecological environment were also confirmed in the field experiments. These findings suggest that the rapid seed germination technology of *N. tangutorum* combined with the popularization and application of NaCF can shorten the seed germination period and make the seedling establishment much easier, which may be an effective strategy to restore and reconstruct degraded vegetation in many desert regions.

ARTICLE HISTORY
Received 14 February 2016
Accepted 31 August 2016

KEY WORDS
application; break seed dormancy; drought resistance; Na compound fertilizer; *Nitraria tangutorum*; popularization; succulent xerophyte

1. Introduction

Drought stress is the main abiotic stress resulting in serious threats to the ecological environment and agricultural productivity throughout the world (Martínez *et al*. 2003; Ben-Hassine *et al*. 2010). Though most crops are highly sensitive to drought stress, some succulent xerophytes—*Nitraria tangutorum* (Bobr) (Nitrariaceae), *Haloxylon ammodendron* (C. A. Mey.) and *Zygophyllum xanthoxylum* (Bunge) Maxim—grown in arid regions have, however, evolved multiple adaptive mechanisms to survive and grow well in these harsh conditions (Wang *et al*. 2004; Ma *et al*. 2012, 2014; Yue *et al*. 2012). Our previous studies showed that *H. ammodendron* and *Z. xanthoxylum* can absorb a greater quantity of Sodium (Na^+) than potassium (K^+) that was transported to photosynthesizing branches (PB) as well as leaves for osmotic adjustment (Wang *et al*. 2004). Further investigations revealed that 50 mM sodium chloride (NaCl) significantly stimulated growth and mitigated deleterious impacts of *Z. xanthoxylum* by improving relative water content and increasing leaf turgor pressure, and improving photosynthetic activity to adapt to water deficit (Dennis and Andrea 2012; Ma *et al*. 2012, 2014; Yue *et al*. 2012). On these bases, we developed an Na compound fertilizer (sodium, nitrogen and phosphorus) in the laboratory through a sand and pot experiment, which could significantly promote growth and improve drought tolerance of *Z. xanthoxylum* and *H. ammodendron* (Kang *et al*. 2013; Wang *et al*. 2014).

Nitraria tangutorum, a typical succulent xerophyte native to the desert areas of northwest China, is one of the few shrubs found to date that can develop and form the fixed dunes in desert regions (Wang and Kang 2005; Kang *et al*. 2015). The species is considered an important recommended plant and is widely used for sand fixation and for soil and water conservation in desert regions. Meanwhile, its high palatability and nutrient value make *N. tangutorum* attractive as a forage crop in local regions, the 'ginseng of the desert' *Cynomorium Songaria* Rupr parasitized in *N.*

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tangutorum has high medicinal value and its fruits are known as desert 'cherry' (Hao *et al*. 2012; Kang *et al*. 2013). However, due to the strong dormant characteristic of *N. tangutorum* seeds, research, establishment and utilization of this excellent plant resource have severely been limited (Zhao 1991; Zhang *et al*. 2004). Our study found that *N. tangutorum* seeds were tightly wrapped by a layer of stone and thick seed coat which significantly affected the water permeability of the seeds, and impeded the germination inhibitor discharge from the embryo and radicle, thus eventually leading to seed dormancy (Adkins *et al*. 2002). To date, only a few studies have been reported on promoting seed germination of *N. tangutorum*. First, seeds were stratified for about 3 months followed by soaking with warm water (40–50°C) for 48–72 h, and then germinated in darkness under fluctuating temperature (Zhao 1991; Ji *et al*. 2004). Second, seeds were immersed in water for 10 d and then germinated in darkness under fluctuating temperature (Zhao 1989, 1991). Third, seeds were soaked with water for 24–48 h, then mixed with wet sand (3:1), and then wrapped with a mouth in the plastic to germinate in the leeward place where there is light (Yue and Wang 2009). The optimal method was the first type and the germination rate reached 69% when seeds germinated for 14 d (Zhao 1991). However, this method is labor intensive and needs a long period (at least 60 d) for the seed germination of *N. tangutorum*. Result has showed that the sulfuric acid (98% H_2SO_4) and mechanical scarification, fluctuating temperature and gibberellic acid (GA_3) treatments were the effective measures for breaking seed dormancy caused by seed coat (Baskin and Baskin 1998; Finch-Savage and Leubner-Metzger 2006).

Very little is known about the effect of the abovementioned methods on breaking seed dormancy of *N. tangutorum* caused by the seed coat. Furthermore, after breaking seed dormancy, the feasibility for NaCF on *N. tangutorum* to be popularized and applied in desert areas is not clear. In this work, we try to explore a method that can rapidly break the seed germination of *N. tangutorum*, and then investigate the positive effect of NaCF on the growth and drought resistance of *N. tangutorum* and ecological environment by short-term pot experiment in a greenhouse and long-term field and pot experiment in a desert environment, with the purpose of laying a solid foundation for the large-scale popularization and application of NaCF on *N. tangutorum* in desert areas.

2. Material and methods

Mature fruits of *N. tangutorum* were collected in August 2014 from wild plants in the Gansu Minqin National Studies Station for Desert Steppe Ecosystems (MSDSE), which belongs to the Chinese Ecosystem Research Network (38°34′28″N, 102°59′05″ E; elevation: 1300–1700 m). The area is surrounded by the Badain Jaran Desert in the west and north, and by the Tengger Desert in the east, a typical desert and oasis area in China. The mean annual precipitation and temperature are 110 mm and 7.6□, respectively, and the mean annual evaporation is 2644 mm (Zhao *et al*. 2011). Fruits were gently crushed to release seeds and washed to remove impurities. Seeds were stored in a hop-pocket in a refrigerator (−7°C) after drying at room temperature.

2.1. Seed size, weight, moisture content and purity degree

Four groups of 1000 seeds were weighed, and four groups of 20 seeds were measured after collection, to measure the average weight of 1000 seeds and seed size. The seed moisture content tests were conducted according to the rules of the International Seed Testing Association (1985). The value of seed purity degree was calculated using the following formula: seed purity degree (%) = (sample weight − impurity and empty seeds weight)/sample weight ×100%.

2.2. Mechanical scarification of seed

Seeds were abraded with ground sand until a tiny part of the embryo appeared. Fifty abraded seeds were placed in Petri dishes (11 cm diameter) with two filter papers moistened with distilled water in an incubator at 25°C in darkness. Four replications were used for the germination experiment. Seeds not abraded with ground sand were used as a control. The germination percentage was used as the germination potential after 4 d of incubation. The final germination percentage was recorded after 10 d of incubation. Germination was checked every 24 h and seedlings removed (the same as below).

2.3. Sulfuric acid scarification of seed

Seeds were sterilized with 75% ethanol for 1 min, rinsed 8 times with distilled water and then soaked with 98% H_2SO_4 for 0, 45, 50, 55 and 60 min, respectively. Seeds were washed with distilled water until the potential of hydrogen (pH) of the seed surface was close to the distilled water pH value. Germination of seeds was tested in Petri dishes which were sealed with parafilm and cultured in an incubator at 25°C under darkness conditions. Four replications were used for each germination experiment, and seeds not soaked in 98% H_2SO_4 were used as a control. Other methods and progress of this section are the same as above.

2.4. Seed germination in fluctuating temperatures in darkness

Seeds were soaked in 98% H_2SO_4 for 55 min and washed with distilled water until the pH value of the seed surface was 6.7. Germination percentages of *N. tangutorum* seeds were determined at a constant temperature of 25°C (control) and fluctuating temperatures of 25/5, 25/10 and 25/15°C, in Petri dishes with filter paper in darkness. Other methods and progress of this section are same as above.

2.5. Seed germination at different concentrations of gibberellic acid (GA_3) solution

Seeds were soaked in 98% H_2SO_4 for 55 min and washed with distilled water until the pH value of the seed surface was 6.7.

Table 1. Summary statistics of soil properties in nursery site.

WS (μmol g^{-1})		CU (μmol g^{-1})		AN (μmol g^{-1})	AP (μmol g^{-1})	AK (μmol g^{-1})	BD (g cm^{-3})
9.1 (0.6)	2.2 (0.4)	14.6 (1.7)	6.3 (0.6)	0.33 (0.03)	0.12 (0.02)	4.7 (0.2)	1.26 (0.5)

WS: water soluble Na^+, K^+ concentrations (μmol g^{-1}); CU: changeable and unchangeable Na^+, K^+ concentrations (μmol g^{-1}); AN: available nitrogen (μmol g^{-1}); AP: available phosphorus (μmol g^{-1}); AK: available potassium (μmol g^{-1}); BD: soil bulk density (g cm^{-3}).

Germination of seeds was tested in 50, 100 150 and 200 mg L^{-1} GA_3, then seeds were sealed with parafilm and cultured in incubators for 48 h in darkness at fluctuating temperature of 25/5°C. Seeds were germinated in distilled water as the control. Other methods and progress of this section are same as above.

2.6. Plant growth conditions and drought treatments in pot experiments in greenhouse condition

Seeds of *N. tangutorum* were germinated as described above. The tested soil samples were collected from the MSDSE where the halophyte species *N. tangutorum* was naturally distributed. The summary statistics of soil properties are described in Table 1. NaCF was used for this test which was composed of the following components: sodium nitrate ($NaNO_3$:0.14 g kg^{-1} dry soil), sodium phosphate monobasic dihydrate ($NaH_2PO_4.2H_2O$: 0.121 g kg^{-1} dry soil) and NaCl (0.27 g kg^{-1} dry soil).

The pot experiments were conducted with the purpose of preliminarily investigating the positive effects of NaCF on the growth and drought resistance of *N. tangutorum* (October to December 2014). The soil described above was dried out, crushed and screened with a 2-mm sieve. Seeds of *N. tangutorum* were germinated and then seedlings were transplanted into plastic pots (26 cm height × 20 cm diameter) filled with 4 kg sandy soil and irrigated with water. Soil water content (SWC) was maintained at 70% of field water capacity (FWC) by weighing. After 4 weeks, plants were thinned out and five uniform plants were kept, and then plants were divided into three groups: control (C), drought (D) and drought with additional NaCF treatment (D+Na). The amount of fertilizer in each pot culture treatment was calculated as $T_a = 4 \times T_f$ (Kang *et al.* 2013, 2014), where T_a is the total amount of fertilizer in each pot, and T_f is the amount of fertilizer in 1 kg dried soil. The SWC in the C group was maintained at 70% of FWC by irrigating with water during the experimental period. The SWC in the D and D+Na groups was maintained at 70% of FWC for 15 d, then water was withheld 5 d to induce drought stress gradually. When the SWC has reduced to 30% of FWC, this value was maintained by irrigating with the corresponding water. Each treatment consisted of 10 pots containing five plants each. To minimize the effects of environmental gradients in the greenhouse, pots were randomly reassigned to new positions every 2 d. After 3 weeks, the plants were harvested; eight pots of each treatment were dug out and separated into roots, stems and leaves for physiological and morphological analysis.

2.7. Plant cultivation conditions in the field and pot culture experiments in desert conditions

The field and pot experiments were carried out with the intent to popularize and apply NaCF by cultivating strong drought-resistant *N. tangutorum* seedlings in desert areas (MSDSE; Apri1 to September 2015). Fertilizers of NaCF and diammonium phosphate (DP) were used for this test; the compositions of NaCF (N, P -Na) are the same as the pot experiments in greenhouse conditions, and the DP has the same content of N and P with NaCF which is composed of Diammonium phosphate ($(NH_4)_2HPO_4$) (0.11 g kg^{-1} dry soil). The plant cultivation in the pot experiment is similar to that in the greenhouse. Germinated seeds of *N. tangutorum* were sown in each disposable plastic pot (28 cm diameter × 36 cm height) filled with 10 kg sandy soil and irrigated with water, and then all pots were covered with a white plastic film (for 15 d) in order to decrease the water loss of soil. The SWC was maintained at 70% of FWC by weighing for 6 weeks, then plants were thinned out (three uniform plants) and divided into three groups: drought with control (D+C), drought with DP treatment (D+DP) and drought with NaCF treatment (D+Na). The SWC in the three groups was maintained at 70% of FWC for 15 d by irrigating with water; then water was withheld 5 d to induce drought stress gradually. When the SWC had reduced to 30% of FWC, this value was maintained by irrigating with the corresponding water. Each treatment consisted of 30 pots containing three plants each. After the plants were harvested, 10 pots of each treatment were dug out and separated into aboveground parts and roots for physiological and morphological analysis.

The plant cultivation in the field experiment is as follows: The soil fertilization treatments were the same as those in the pot experiment. Each plot (4 m length × 4 m width) was surrounded by an isolated zone (0.5 m), and then we calculated the total amount of fertilizer of each plot using $T = A \times S_1 \times B_1 \times T_1$ (Kang *et al.* 2013), where T is the total amount of fertilizer of each plot, A is the area (12 m^2) of each plot, S_1 is the soil depth (30 cm depth), B_1 is the soil bulk density (1.26 g cm^{-3}) and T_1 is the amount of fertilizer in 1 kg dried soil. When the fertilizer was applied evenly to each plot (30 cm in depth), germinated seeds of *N. tangutorum* were sown with wide drilling (3 cm in depth; 30 cm of row space distance) and irrigated with water (70% of FWC). When the plants grew to about 15 cm (8 weeks), the SWC in the D+C, D+DP and D+Na groups was maintained at 70% of FWC for 10 d by irrigating with water; then water was withheld to induce drought stress gradually. When the SWC had decreased to 30% of FWC, this value was maintained by irrigating with the

corresponding solution (water). Each treatment consisted of three plots containing 300–400 plants each. After the plants were harvested, 20 uniform plants of each treatment were dug out and separated into aboveground parts and roots for physiological and morphological analysis.

3. Measurements of samples

3.1. Measurements of growth-related parameters

Plant height (PH) and main root length (MRL) were measured using a ruler (0.3 m), and the fresh weight (FW) and dry weight (DW) (70°C for 4 d to constant weight) were measured using an electronic scale with 0.001 g precision. The relative growth rate (RGR) of plants was determined by the formula RGR = $(\ln W_u - \ln W_o)/\triangle t$, where W_u and W_o represent ultimate and original dry biomass, respectively, and $\triangle t$ is the time consumed (days).

3.2. Determination indexes related to photosynthesis of plants

Net photosynthesis rate (Pn) and stomatal conductance (Gs) were measured with an automatic photosynthetic measuring apparatus (LI-6400, LI-COR Biosciences, Lincoln, NE, USA). The water use efficiency (WUE) was calculated by the formula WUE = Pn/Gs (Yue *et al*. 2012; Liu *et al*. 2005). The measurement of Chlorophyll a (Chl a) content was estimated according to the method described by Porra *et al*. (1989), and determined by ultraviolet spectrophotometry (UV-2102C, Unico Instrument Co., Ltd, Shanghai, China).

3.3. Measurements of Na^+ and K^+ concentrations in plants

Na^+ and K^+ concentrations were measured according to the method described by Kang *et al*. (2013) and Gulati and Jaiwal (1993). Briefly, Na^+ and K^+ were extracted from dried plant tissues (passing a 0.5-mm sieve) in 100 mM acetic acid at 90°C for 2 h. The ion analysis was performed using an atomic absorption spectrophotometer (2655–00, Cole-Parmer Instrument Co., Vernon Hills, IL, USA).

3.4. Measurements of soil-related parameters in pot culture experiments

Soils were sampled as 5–8 sub-samples from each pot culture treatment which were then mixed. For chemical analyses, soils were air-dried at laboratory temperature and sieved (2 mm). Soluble Na^+ and K^+ were extracted by soaking the soil in deionized water (water: soil, 5:1). Exchangeable and available non-exchangeable Na^+ and K^+ were extracted with 2 M cold Nitric acid (HNO_3) (Wang *et al*. 2004; Ma *et al*. 2012). Available Na^+ and K^+ included the sum of soluble, exchangeable and available non-exchangeable Na^+ and K^+. Concentrations of Na^+ and K^+ were determined with an atomic absorption spectrophotometer. Available N was measured by using the method of alkali-hydrolyzable diffusion; available P was measured by the Bray method; available K was measured by using the method of ammonium acetate extract; bulk density of soil was measured by the method of cutting ring (Kang *et al*. 2013, 2014).

4. Data analysis

All of the data were subjected to one-way analysis of variance (ANOVA) using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to detect significant differences between means at a significance level of $P < 0.05$.

5. Results

5.1. Seed size, weight, purity degree and moisture content of N. tangutorum seeds

The average diameter of seeds is 5.4 ± 0.2 mm, the average weight and purity degree of 1000 seeds were 38.8 ± 1.3 g and 97.2% respectively, and the average moisture content was 8.74%.

5.2. Mechanical scarification fails to break the dormancy of N. tangutorum seeds

All seeds of both abraded and control remained dormant at 25°C in darkness for 10 d (data not shown), suggesting the seed dormancy of *N. tangutorum* was not due to gaseous impermeability of endocarp.

5.3. Sulfuric acid scarification greatly reduces the dormancy of N. tangutorum seeds

The seed germination of *N. tangutorum* was greatly stimulated by 98% H_2SO_4 scarification (Fig. 1). The germination percentage in 98% H_2SO_4 treatments was significantly higher than that in control. The highest germination rate (26%) was observed when seeds were treated with 98% H_2SO_4 for 55 min, and the lowest germination percentage was observed in control (without treating with 98% H_2SO_4, and the seed germination rate is zero). Similarly, sulfuric acid scarification also induced a dramatic increase in germination potential of *N. tangutorum* seeds (Fig. 1). The germination potential in 98% H_2SO_4 treatments was significantly higher than that in control. The highest germination potential by 22% was also observed in the treatment with 98% H_2SO_4 for 55 min,

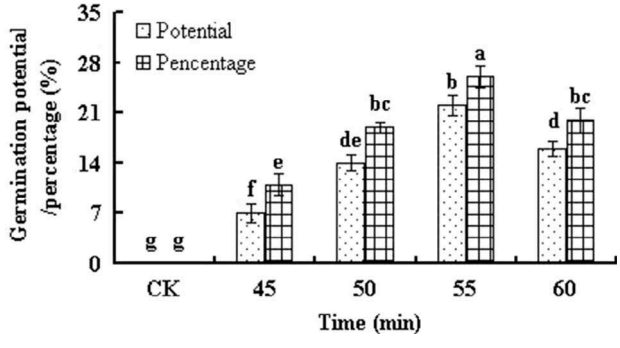

Figure 1. Effect of sulfuric acid scarification on the seed germination percentage/potential of *Nitraria tangutorum*. After treatment with sulfuric acid (98% H_2SO_4) for different time regimes (0, 45, 50, 55 and 60 min), seeds were germinated at 25°C in darkness for 10 d. Values are means ± SD (n = 50). Columns with different letters indicate significant difference at $P < 0.05$ (LSD test, the same as below).

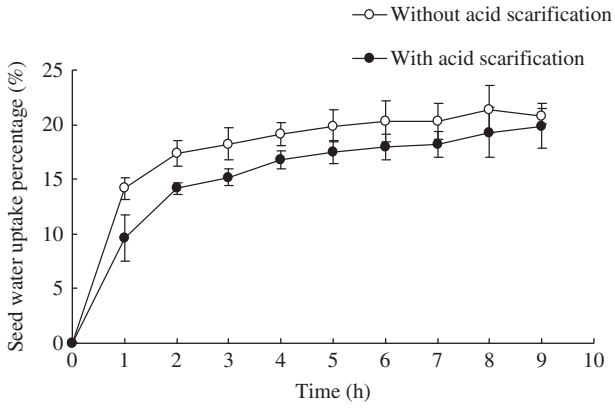

Figure 2. Effect of sulfuric acid scarification on seed water uptake percentage. Seeds soaked with sulfuric acid (98% H_2SO_4) for 55 min were placed in 11 cm Petri dishes with distilled water to imbibe water at room temperature (25°C) for 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 h, respectively. Values are means ± SD (n = 50).

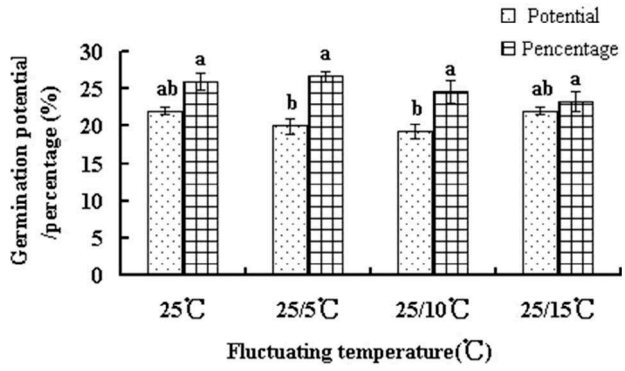

Figure 3. Effect of various temperature regimes on the seed germination percentage/potential of *Nitraria tangutorum*. After treatment with sulfuric acid (98% H_2SO_4) for 55 min, seeds were germinated in darkness at fluctuating temperature regimes (25/5, 25/10 and 25/15°C) and constant temperature regime of 25°C for 10 d. Values are means ± SD (n = 50). Columns with different letters indicate significant difference at $P < 0.05$.

and the lowest germination potential was observed in the control. The highest germination percentage and potential both were observed in seeds treated with 98% H_2SO_4 for 55 min, suggesting the optimal time was 55 min for 98% H_2SO_4 scarification.

5.4. Sulfuric acid scarification did not enhance the seed water uptake percentage

When seeds were soaked in water for 1, 2, 3 and 4 h, the water uptake percentage of *N. tangutorum* seeds in control was significantly higher than that in 98% H_2SO_4 scarification treatment, whereas no significant differences were observed between 98% H_2SO_4 treatments and control when seeds imbibed water for 5, 6, 7, 8 and 9 h (Fig. 2). It is suggested that the water uptake of seeds gradually tended to a plateau step followed by the rapid water uptake step of the first 2 h.

5.5. Fluctuating temperature did not increase the germination of N. tangutorum seeds but made the germination more uniform

The fluctuating temperature has no significant effect on seed germination percentage and potential of *N. tangutorum* scarified by 98% H_2SO_4 for 55 min (Fig. 3). However, the seed germination in fluctuating temperatures was more uniform than that in the constant temperature regime. The most uniform germination occurred when seeds germinated at 25/5°C.

5.6. Exogenous GA_3 increased the germination percentage and potential of N. tangutorum seeds treated with 98% H_2SO_4 for 55 min

Seeds soaked in 98% H_2SO_4 for 55 min were found to be very sensitive to exogenous application of GA_3 when seeds were incubated at 25/5°C in darkness (Fig. 4). Combining application of 98% H_2SO_4 scarification with GA_3 achieved a higher germination percentage than seeds only scarified by 98% H_2SO_4. The highest germination rate (69%) was observed when seeds were treated with 150 mg L^{-1} GA_3 for 48 h, and the lowest germination rate (27%) was observed in the control

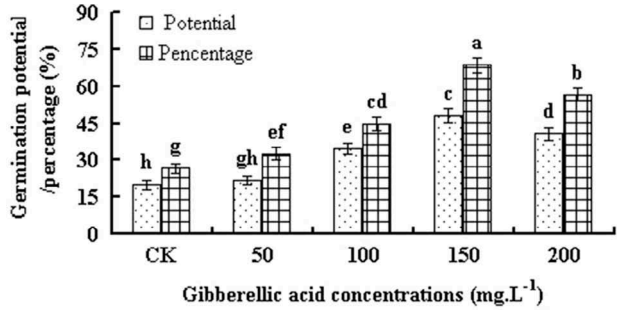

Figure 4. Effect of different gibberellic acid (GA_3) concentrations on the seed germination percentage/potential of *Nitraria tangutorum*. After treatment with sulfuric acid (98% H_2SO_4) for 55 min, seeds were placed at 25/5°C in darkness under different gibberellic acid (GA_3) concentrations (0, 50, 100, 150 and 200 mg L^{-1}) for 48 h, and then germinated at 25/5°C in darkness for 8 d. Values are means ± SD (n = 50). Columns with different letters indicate significant difference at $P < 0.05$.

(without treating with GA_3). Similarly, GA_3 concentrations of 100, 150 and 200 mg L^{-1} also significantly increased the seed germination potential (Fig. 4). The highest germination potential (48%) was observed in seeds treated with 150 mg L^{-1} GA_3 for 48 h at 25/5°C in darkness, and the lowest germination potential (20%) was found in the control (without treating with GA_3). In addition, the seed germination potential under other GA_3 concentrations (100 and 200 mg L^{-1}) was also higher than that in the control.

5.7. The NaCF stimulates the growth of N. tangutorum seedlings

To understand the morphological and physiological role of NaCF on the growth and drought resistance of the *N. tangutorum*(after breaking seed dormancy), the PH, FW, DW and MRL were analyzed for plants grown in greenhouse and desert conditions (Fig. 5A, B; Table 3). After withholding water for 20 d, compared with C and D treatments, the NaCF treatment in pot experiment in greenhouse conditions significantly increased PH by 36% and 69%, and the leaves and roots fresh weights by 44 and 121%, and 22 and 57%, respectively (October to December 2014;

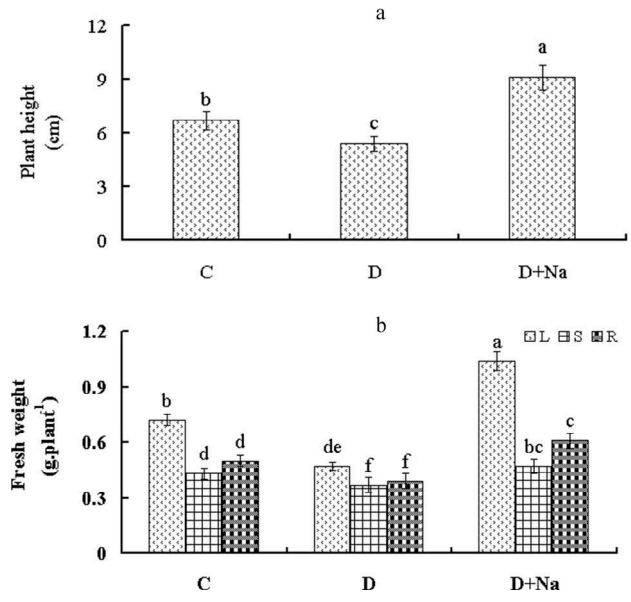

Figure 5. Plant height (A) and fresh weight (B) of the leaves, stems and roots of *Nitraria tangutorum* grown under irrigation as the control (C), drought stress (D) or drought with Na compound fertilizer (D+Na) for 20 d under pot experiments in greenhouse conditions (October to December 2014). Values are means ± SD (n = 8). Columns with different letters indicate significant difference at $P < 0.05$.

Fig. 5A, B). Moreover, compared with D+C and D+DP treatments, the D+Na treatment in the pot experiment in desert conditions significantly increased PH by 64.3 and 23.4%, FW by 61.2 and 27.4%, and MRL by 68.4 and 19.1% (April to September 2015; Table 3). The positive effect of NaCF on the growth of *N. tangutorum* in the field experiment was similar to that in the pot experiment in desert conditions (Table 3).

5.8. The NaCF alleviates the deleterious impact of water deficit on the photosynthesis and water status of N. tangutorum

To further investigate the effects of NaCF on the drought resistance of *N. tangutorum*, the RGR, Pn, Gs, WUE, leaf area and Chl a content were measured for plants grown in pots in the greenhouse. In comparison with the control (70% of FWC), RGR was significantly reduced when plants were subjected to water deficit (30% of FWC); however, a significant increase in RGR by 25.6% was observed in plants grown in the presence compared with the absence of NaCF in the presence of water deficit (Table 2). Likewise, photosynthesis was inhibited by water deficit, while the presence of NaCF significantly increased Pn, Gs and WUE by 69.2, 18.1 and 43.2%, respectively, compared with treatment without NaCF under water deficit (Table 2). In addition, the NaCF in the presence of water deficit also resulted in significant increases in leaf area and Chl a content of *N. tangutorum* (October to December 2014; Table 2).

5.9. NaCF improves the drought tolerance of N. tangutorum by accumulating a large number of Na^+ under water deficit

To further understand the physiological role of NaCF in the drought tolerance of *N. tangutorum*, the Na^+ and K^+ concentrations were analyzed for plants grown in greenhouse and desert conditions (Fig. 6E–G; Table 4). In comparison with the C and D, the K^+ concentrations of leaves in pot experiment in greenhouse were significantly decreased by 55 and 40% when plants were subjected to water deficit (30% of FWC); however, a significant increase in Na^+ concentrations of leaves and stems by 98 and 88%, and 28 and 22%, were observed in plants grown in the presence of the NaCF (October to December 2014; Fig. 6E–G). Similarly, compared with D+C and D+DP treatments, the D +NaCF treatment in the pot experiment in desert conditions significantly increased Na^+ concentrations by 63.9 and 43.9%, and significantly decreased K^+ concentrations by 34 and 19.1%, respectively, when plants were subjected to water deficit (30% of FWC; April to September 2015). The positive effect of NaCF on the drought resistance of *N. tangutorum* in field experiment was similar to that in the pot experiment in desert conditions (Table 4).

5.10. The NaCF has no obvious deleterious impact on the ecological environment

As shown in Table 5, NaCF had no obvious deleterious impact on the ecological environment. The distribution characteristics (pot experiment in greenhouse) of the total Na^+ (648 mg) in NaCF were that 258 mg (39.8%) was absorbed by plants, 330 mg (50.9%) remained in the pot and 60 mg (9.3%) leached (accounted for 4.1% of the nursery soil; October to December 2014). The distribution characteristics (pot experiment in desert condition) of the total Na^+ (1620 mg) in the NaCF were that 691.2 mg (42.7%) was absorbed by plants, 848.8 mg (52.4%) remained in the pot and 80 mg (4.9%) leached (accounted for 2.2% of the nursery soil; April to September 2015). The remaining Na^+ concentrations 330 mg (50.9%) in the pot experiment in the greenhouse, and 848.8 mg (52.4%) in the pot experiment in desert conditions, would be further absorbed by *N. tangutorum* in the following growth period.

Table 2. Relative growth rate (RGR), net photosynthesis rate (Pn), stomatal conductance (Gs), water use efficiency (WUE), leaf area and Chlorophyll a (Chl a) in leaves of *Nitraria tangutorum* seedlings.

Treatments	Relative growth rate (RGC) (g kg^{-1} day^{-1})	net photosynthesis rate (Pn) (μmol CO_2 m^{-2}s^{-1})	stomatal conductance (Gs) (mmol m^{-2}s^{-1})	water use efficiency (WUE) (μmol CO_2 m^{-2}s^{-1})	Leaf area (cm^2 plant^{-1})	Chlorophyll a (mg g^{-1} DW)
C	243.7 ± 6.8 a	19.3 ± 0.8a	377.6 ± 7.2a	51.1 ± 3.0a	22.7 ± 0.9a	18.4 ± 0.8a
D	166.5 ± 7.6 c	10.4 ± 0.6c	301.3 ± 8.3c	34.5 ± 2.4b	17.5 ± 0.7c	13.8 ± 0.7c
D+Na	209.1 ± 9.3 b	17.6 ± 0.9b	355.9 ± 10.6b	49.4 ± 2.7a	20.6 ± 0.7b	17.1 ± 0.8ab

Plants were treated with drought with control (C) (70% FWC), drought (D) (30% of FWC) and drought with Na compound fertilizer (D+Na) (30% of FWC) for 20 d under pot experiments in greenhouse conditions (October to December 2014). Values are means ± SD (n = 8). Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

Table 3. Plant height (PH), fresh weight (FW), dry weight (DW) and main root length (MRL) of *Nitraria tangutorum*.

		Field			Pot		
Year	Treatments	D+C	D+DP	D+Na	D+C	D+DP	D+Na
2015	Plant height (cm plant^{-1})	14.3 (1.0)c	19.2 (1.6)b	24.6 (2.0)a	15.4 (1.4)c	20.5 (1.2)b	25.3 (1.9)a
	Fresh weight (g plant^{-1})	4.6 (0.3)c	5.7 (0.6)c	7.1 (0.5)c	4.9 (0.4)c	6.2 (0.4)c	7.9 (0.6)c
	Dry weight (g plant^{-1})	0.83 (0.07)c	1.26 (0.09)c	1.73 (0.11)c	0.88 (0.09)c	1.44 (0.13)c	1.89 (0.14)c
	Main root length (cm plant^{-1})	19.1 (1.8)d	27.2 (2.5)b	33.8 (3.3)a	17.4 (1.6)de	24.6 (2.2)bc	29.3 (2.8)ab

Plants were treated with drought with control (D+C), drought with diammonium phosphate (D+DP) and drought with Na compound fertilizer (D+Na) and then were subjected to water deficit (30% of FWC) for 6 months (Apri1 to September 2015) under field and pot experiments in field conditions. Values indicate the means (SE); $n = 8$. Different letters in each line indicate significant difference at $P < 0.05$.

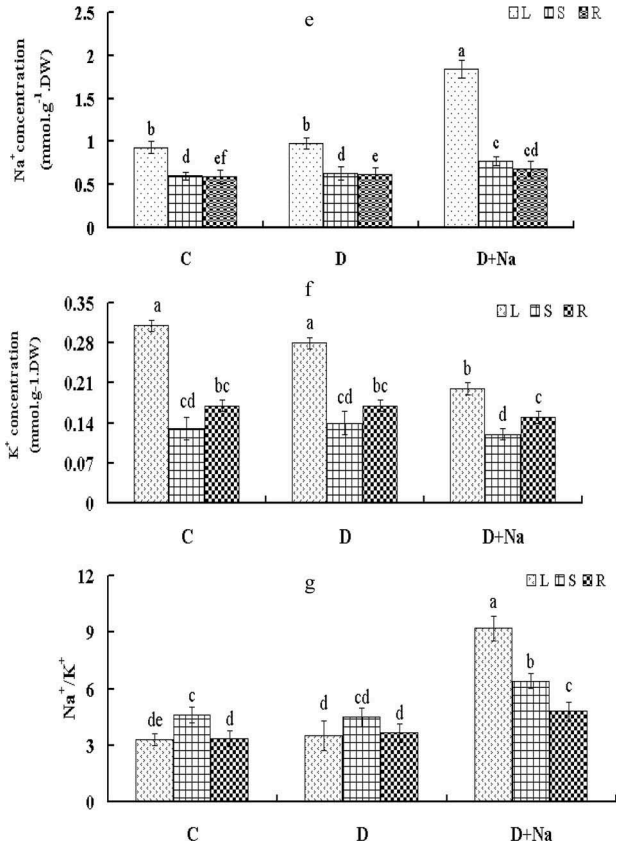

Figure 6. Na^+ (E) and K^+ (F) concentrations and Na^+/K^+ ratio (G) of leaves, stems and roots of *Nitraria tangutorum* grown under irrigation as the control (C), drought stress (D) or drought with Na compound fertilizer (D+Na) for 20 d under pot experiments in greenhouse conditions (October to December 2014). Values are means ± SD (n = 8). Columns with different letters indicate significant difference at $P < 0.05$.

The distribution characteristics (field experiment) of the total Na^+ (162 mg kg^{-1} dry soil) in the NaCF were that the Na^+ concentrations 121 mg kg^{-1} (74.6%) remained in the field soil (30 cm depth), and the soil could continue to be cultivated with *N. tangutorum* and improve fertilizer use efficiency in the following years (Table 5).

6. Discussion

6.1. The mechanical restriction of seed coat is the main reason for seed dormancy of N. tangutorum

It has been reported that the seed dormancy was strongly induced by the pericarp and/or seed coat for many species, which can be effectively overcome by mechanical and acid scarification (Baskin and Baskin 1998; Mackay *et al*. 2001; Phartyal *et al*. 2005). *N. tangutorum* seeds are tightly wrapped with a layer of bone and thick coat, and the wax layer, cork layer, palisade tissue and bone-shaped stone cells of the coat can cause seed dormancy (Tang *et al*. 2004; Zhang *et al*. 2004). Several possible mechanisms involved in the coat-imposed dormancy have been identified, including mechanical constraint, prevention of water and gaseous exchange and germination inhibitors (Adkins *et al*. 2002). Acid scarification is proposed to be an effective way to overcome coat-imposed dormancy for many species (Baker *et al*. 2005; Phartyal *et al*. 2005; Devillalobos *et al*. 2007). Our results showed that the seed dormancy of *N. tangutorum* was significantly broken by 98% H_2SO_4 scarification (Fig. 1), indicating that *N. tangutorum* seeds have the characteristic of coat-imposed dormancy. Although the 98% H_2SO_4 scarification had no effect on water uptake percentage of *N. tangutorum* seeds (Fig. 2), it had a substantial capacity to promote seed germination by thinning and weakening the hard endocarp. In addition, the mechanical scarification also failed to break the dormancy of *N. tangutorum* seeds. Similar results have been reported for some other species and genera such as *Empetrum hermaphroditum*, *Sambucus* and *Symphoricarpos* (Hidayati *et al*. 2000, 2001; Baskin *et al*. 2002). Therefore, we think that the impermeability of *N. tangutorum* endocarp to water uptake and gaseous exchange is not the main reason for the seed dormancy. We propose that the seed dormancy of *N. tangutorum* is at least partly caused by the constraint of radical protrusion of endocarp.

Results showed that GA_3 plays a very important role in seed germination (Bewley 1997; Baskin and Baskin 1998). More importantly, application of exogenous GA_3 promoted the seed germination rate with stony endocarp in *E. hermaphroditum* (Baskin *et al*. 2002) and cupule in *Tripsacum dactyloides* (Tian *et al*. 2003). In the paper, GA_3 further markedly increased the germination rate of optimal H_2SO_4-treated seeds (Fig. 4). Previous studies showed that the seed dormancy of *N. tangutorum* was effectively overcome by stratification (Zhao 1991; Ji *et al*. 2004). Stratification contributes to increase the biological activity of GA_3 within the embryo (Foley 2001; Corbineau *et al*. 2002; Yamauchi *et al*. 2004). It is suggested that GA_3 can release the seed coat dormancy by increasing the embryo growth potential and/or by reducing the mechanical constraint (Finch-Savage and Leubner-Metzger 2006). This indicates that embryo dormancy does not occur in at least 69% of *N. tangutorum* seeds (Fig. 4). Therefore, mechanical restriction by coat is the main reason for the seed dormancy, suggesting that the

Table 4. Na^+ and K^+ concentrations and Na^+/K^+ ratio of aboveground and root of *Nitraria tangutorum* in desert conditions.

2015	Field						Pot					
Treatments	D+C		D+DP		D+Na		D+C		D+DP		D+Na	
Na^+ (mmol g^{-1})	Aboveground	Root	Aboveground	Root	Aboveground	Root	Aboveground	Root	Aboveground	Root	Aboveground	Root
	2.9 (0.4)c	0.71 (0.03)e	3.3 (0.7)b	0.8 (0.05)d	4.7 (0.9)a	0.9 (0.06)d	2.6 (0.6)c	0.64 (0.05)ef	3.0 (0.6)b	0.69 (0.06)e	4.6 (0.7)a	0.7 (0.04)e
Total Na^+ (mmol g^{-1})	3.61 (0.5)bc		4.1 (0.6)b		5.6 (0.6)a		3.24 (0.5)c		3.69 (0.4)bc		5.31 (0.6)a	
Total K^+ (mmol g^{-1})	0.72 (0.02)a		0.59 (0.03)b		0.48 (0.03)c		0.67 (0.02)a		0.56 (0.02)b		0.47 (0.01)c	
Na^+/K^+	5.01 (0.4)e		6.95 (0.8)c		11.67 (1.2)a		4.84 (0.4)ef		6.71 (0.5)cd		10.62 (0.9)ab	

Plants were treated with drought with control (D+C), drought with diammonium phosphate (D+DP) and drought with Na compound fertilizer (D+Na) and then were subjected to water deficit (30% of FWC) for 6 months (Apri1 to September 2015) under field and pot experiments in desert conditions. Values are means ± SD (n = 8). Columns with different letters indicate significant difference at $P < 0.05$.

seed dormancy of *N. tangutorum* belongs to the type of comprehensive dormancy.

6.2. Positive effects of NaCF on the growth and drought tolerance of N. tangutorum

It has been confirmed that Na^+ is known as an important physiological osmotica for succulent xerophytes against stressful environments (Wu *et al*. 2011; Yue *et al*. 2012; Ma *et al*. 2012, 2014). In this study, we successfully applied NaCF on *N. tangutorum* after breaking seed dormancy, and significantly promoted the growth of *N. tangutorum* seedlings in a pot experiment in a greenhouse, and in field and pot experiments in desert conditions (Fig. 5A, B; Tables 2, 3). Similar studies have been reported for some other halophytes, for instance *Atriplex halimus, Suaeda salsa, Kosteletzkya virginica, Z. xanthoxylum* and *H. ammodendron* (Ma *et al*. 2012; Kang *et al*. 2013, 2014), which indicates that biomass production is closely related to Na^+ accumulation (Debez *et al*. 2006; Kang *et al*. 2013). Leaf area acts as an important function in plant production since it affects the amount of radiation intercepted and carbon assimilated, which is closely linked to plant growth and biomass production (Gifford and Evans 1981; Gutierrez-Boem and Thomas 1998). Our study indicates that under water deficit, NaCF significantly increased leaf area and improved photosynthesis of *N. tangutorum* by accumulating Na^+ concentration in leaves (Table 4; Fig. 6). Results have also shown that Na^+ may influence the hormonal balance, and induce many changes in hormone synthesis and translocation within plant tissues, which may play an important role in plant growth and biomass accumulation (Ghanem *et al*. 2008; Albacete *et al*. 2010).

Our result also showed that the NaCF significantly improved the ability of *N. tangutorum* to resist water deficit by increasing the Na^+ more than the K^+ concentration (Fig. 6E–G; Table 4), resulting in a significant increase in RGR, PR and WUE (Tables 2, 4; Fig. 6). In particular, the significant increase of biomass is indicative of the net accumulation of photosynthate in *N. tangutorum* photosynthetic capacity under water deficit (Tables 2, 3; Fig. 5). Chlorophyll content is also an important physiological parameter for photosynthesis of plants, which is closely related to plant growth and biomass production under water deficit (Ma *et al*. 2012; Luo *et al*. 2013). Our result showed that the increase of Chl a content in leaves of *N. tangutorum* in the present of NaCF was significant, indicating that the increase of Chl a content in leaves of *N. tangutorum* improved the photosynthesis of *N. tangutorum* under water stress (30% of FWC; Table 2). Therefore, it can be concluded that the NaCF stimulated growth and mitigated deleterious impacts in *N. tangutorum* by improving Pn and WUE, which is one of the main factors resulting in the increase of biomass accumulation and improvement of drought resistance under water deficit (Ma *et al*. 2012). Reports indicate the salt-accumulating halophyte plant *S. salsa* grows well in high-salinity medium where the available Na^+ content (272.9 μmol g^{-1} dry soil) is about 66 times more than that in the natural habitat of succulent xerophyte plants (4.1 μmol g^{-1} dry soil) *H. ammodendron* and *Z. xanthoxylum* growing in a low-salt environment (Wang *et al*. 2004; Kang *et al*. 2013). But *H. ammodendron* and *Z. xanthoxylum* have a strong ability to obtain large amounts of Na^+ from arid and poor soils and gather it in PB of *H. ammodendron* and leaves of *Z. xanthoxylum* which were equal to *S. salsa*, and even more than that of other halophytes grown in high-salinity conditions (Tobe *et al*. 2000; Wang *et al*. 2004; Wu *et al*. 2011; Kang *et al*. 2013). Like *H. ammodendron* and *Z. xanthoxylum*, we propose that *N. tangutorum* should be considered a xero-halophyte species. Interestingly, why succulent xerophytes in saline conditions continue to assimilates and gather large amount of Na^+ without any detectable signs of NaCl toxicity is unclear; this may be due to the Na^+ compartmentalization from cells into vacuoles regulated by the vacuolar Na^+/H^+ antiporter (NHX), therefore maintaining cytosolic Na^+ content at low toxic levels (Blumwald *et al*. 2000; Zeng *et al*. 2009; Dennis and Andrea 2012; Wu *et al*. 2011; Yuan *et al*. 2015).

6.3. The NaCF can be popularized and applied in desert areas

Our study indicates that in comparation with the pot experiment, the use efficiency of NaCF was relatively lower in the field experiment (Table 5). However, the remaining Na^+ concentration of 121 mg kg^{-1} (74.6%) in the field soil (30 cm depth) could continue to be cultivated with *N. tangutorum* and improve fertilizer use efficiency in the following years (Table 5). Similarly, the remaining Na^+ concentrations 330 mg (50.9%) in the pot experiment in the greenhouse, and 848.8 mg (52.4%) in the pot experiment in desert conditions, would be further absorbed by *N. tangutorum* in the following growth periods. It is worth mentioning that only 60 mg (9.3%) of Na^+ concentrations in the pot experiment in

Table 5. Distribution characteristics of Na^+ in Na compound fertilizer (NaCF).

Pot	Nursery soil (mg kg^{-1})	Fertilization (mg 4 kg^{-1})	Absorbed (mg, five plants)	Remained (mg 4 kg^{-1}, each pot)	Leached (mg kg^{-1})	Leached/nursery soil (%)
2014 October to December	363	648	258	330	15	
Percentage			(39.8)	(50.9)	(9.3)	4.1
Pot	Nursery soil (mg kg^{-1})	Fertilization (mg 10 kg^{-1})	Absorbed (mg, three plants)	Remained (mg 10 kg^{-1}, each pot)	Leached (mg kg^{-1})	Leached/nursery soil (%)
2015 Apri1 to September	363	1620	691.2	848.8	8	
Percentage			(42.7)	(52.4)	(4.9)	2.2
Field	Nursery soil (mg kg^{-1})	Fertilization (mg kg^{-1})	Absorbed (mg, one plant)	Remained (mg kg^{-1}, 30 cm in depth)	Leached (mg kg^{-1})	Leached/nursery soil (%)
2015 Apri1 to September	363	162	271	121		
Percentage				(74.6)		

Four-week-old plants were treated with Na compound fertilizer (NaCF) for 20 d under pot culture experiments in greenhouse conditions (October to December 2014); Plants were also treated with Na compound fertilizer (NaCF) and then were subjected to water deficit (30% of FWC) for 6 months (Apri1 to September 2015) under field and pot culture experiments in desert conditions.

the greenhouse, and 80 mg (4.9%) of Na^+ concentrations in the pot experiment in desert conditions, were leached, which accounted for 4.1 and 2.2% of the nursery soil, respectively (Table 5). These findings further suggest that NaCF has no obvious deleterious impact on the ecological environment and may be popularized and applied to restore and reconstruct degenerated vegetation in desert areas. However, in our study, only the cultivation of *N. tangutorum* in the presence of NaCF was studied by short-term pot experiment in a greenhouse and long-term field and pot experiments in a desert environment. How to transplant (method) and what is the transplanting effect under water deficit in desert environment? Which cultivation method (pot or field) has a good effect on transplanting? All of these questions are worth investigating deeply in the coming years.

7. Concluding remarks and ecological implications

In this paper, we successfully explored a method that can rapidly promote the seed germination of *N. tangutorum*. The seed germination of *N. tangutorum* reached a peak of 69% when seeds were soaked in 98% H_2SO_4 for 55 min and then treated with 150 mg L^{-1} GA_3 for 48 h under the fluctuating temperature of 25/5°C in darkness for 8 d. It is worth mentioning that NaCF significantly mitigated deleterious impacts of water deficit on the growth of *N. tangutorum* after breaking seed dormancy by accumulating a higher concentration of Na^+ than K^+ for osmotic adjustment. Thus, the rapid seed germination technology of *N. tangutorum* combined with NaCF could shorten the seed germination period and make seedling establishment much easier under water stress, which opens a new perspective for the improvement of *N. tangutorum* for biomass production and restoration in desert areas.

It is interesting that *H. ammodendron* and *N. tangutorum* could absorb and accumulate a great quantity of Na^+ as important osmotica, while still being able to accumulate silicon (Si) to resist water stress, and the coexistence of Na and Si at suitable levels can more effectively improve drought stress of the two species than Na or Si alone (Kang *et al*. 2014, 2015). These abovementioned studies may indicate that the rational use of Na and Si would also contribute to assist in the development of fertilizers which can further promote growth and improve drought tolerance of those succulent xerophyte species grown on barren desert soil.

Acknowledgments

The authors are very grateful to the anonymous reviewers and editors for their critical review and comments which helped to improve and clarify the manuscript.

Funding

This work was supported by the Major State Basic Research Development Program of China [973 Program No. 2013CB429903] and a grant from the National Natural Science Foundation of China [Program No. 31360086, 31101750].

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