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Seed germination demonstrates inter-annual variations in alkaline tolerance: a case study in perennial *Leymus chinensis*

Dandan Zhao^{1,2}, Hongyuan Ma^{1*}, Shaoyang Li¹ and Wenwen Qi¹

Abstract

Background and aims The escalating issue of soil saline-alkalization poses a growing global challenge. *Leymus chinensis* is a perennial grass species commonly used in the establishment and renewal of artificial grasslands that is relatively tolerant of saline, alkaline, and drought conditions. Nonetheless, reduced seed setting rates limit its propagation, especially on alkali-degraded grassland. Inter-annual variations have an important effect on seed yield and germination under abiotic stress, and we therefore examined the effect of planting year on seed yield components of *L. chinensis*.

Methods We grew transplanted *L. chinensis* seedlings in pots for two (Y_2), three (Y_3), or four (Y_4) years and collected spikes for measurement of seed yield components, including spike length, seed setting rate, grain number per spike, and thousand seed weight. We then collected seeds produced by plants from different planting years and subjected them to alkaline stress (25 mM Na_2CO_3) for measurement of germination percentage and seedling growth.

Results The seed setting rate of *L. chinensis* decreased with an increasing number of years in pot cultivation, but seed weight increased. Y_2 plants had a higher seed setting rate and more grains per spike, whereas Y_4 plants had a higher thousand seed weight. The effects of alkaline stress (25 mM Na_2CO_3) on seed germination were less pronounced for the heavier seeds produced by Y_4 plants. Na_2CO_3 caused a 9.2% reduction in shoot length for seedlings derived from Y_4 seeds but a 22.3% increase in shoot length for seedlings derived from Y_3 seeds.

Conclusions Our findings demonstrate significant differences in seed yield components among three planting years of *L. chinensis* under pot cultivation in a finite space. Inter-annual variation in seed set may provide advantages to plants. Increased alkalinity tolerance of seed germination was observed for seeds produced in successive planting years.

Keywords Planting year, Seed setting rate, Thousand seed weight, Alkaline stress, Germination

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Introduction

Grasslands, which cover over 40% of the earth's surface, provide crucial ecosystem services [1, 2]. Approximately 49.3% of the world's grasslands face degradation problems, which pose significant threats to animal husbandry, ecological security, and sustainable development [3–6]. Saline-alkaline soils are representative of degraded regions throughout the world [7, 8]. Soil salinization and alkalization have been recognized as major environmental threats to agricultural systems, significantly affecting plant growth, physiology, and metabolism [9]. Alkalization reduces community stability in grasslands by reducing species asynchrony and soil nutrient levels, thereby accelerating grassland degradation [10, 11]. To date, research on plant responses to salinity stress induced by NaCl has focused primarily on maintenance of osmotic and ion homeostasis through rapid perception of osmotic and ionic signals and corresponding physiological adjustment [12–14]. However, fewer studies have specially addressed the effects of alkaline stress. Alkaline stress, characterized by high pH and the presence of alkaline salts, has more complex and destructive effects on plants than salinization caused by neutral salts [8, 15–17].

The Songnen grassland, situated in northeast China (121°27'–128°12' E, 43°36'–49°45' N), is currently experiencing degradation [18]. Saline-alkalization is leading to significant soil degradation in this region, posing a serious environmental threat with negative effects on sustainable development [19]. *Leymus chinensis* (Trin.) Tzvel, a clonal wild ryegrass with vigorous belowground rhizomes, is the dominant perennial grass species in the Songnen grassland [20]. Because it develops strong rhizomes and adapts well to saline, alkaline, and drought conditions, this species plays an important role in the establishment and renewal of artificial grasslands and in environmental protection [21–25]. *L. chinensis* initiates its inflorescence in the autumn of the year before bloom, this is followed by regrowth in April, heading in May, flowering in June, and seed maturation in July and August. *L. chinensis* experiences the early-vegetative and mid-vegetative stages in August and September, and its growing season ends in late October [22, 26]. *L. chinensis* in the Songnen grassland faces challenges associated with low germination percentage, heading rate and seed setting rate, which hamper its propagation and severely exacerbate the degradation of grassland vegetation. In natural grasslands, *L. chinensis* relies heavily on vegetative propagation for spatial expansion and population renewal [27]. However, rhizomes of *L. chinensis* have a maximum lifespan of four years, and their numbers gradually decrease over successive years. This reduction in rhizome numbers has a significant effect on seed yield [28].

Plants exhibit significant variability in seed production and germination behaviors that can be attributed to various factors, including plant age, growth habitat, and variation among individuals [29, 30]. Thirty-three years of data from *Dacrydium cupressinum* in New Zealand did not support a strong relationship between seed production and variation in environmental factors [31]. Witkowski and Wilson [32] reported that seed production of *Chromolaena odorata* increased over the first 10 years but declined markedly after 15 years. Hampton et al. [28] found that tiller production of perennial ryegrass was optimal at 18–24°C but that higher temperatures depressed tiller production and seed yield. Pol et al. [33] suggested that perenniality could enable perennial grasses to make large reproductive investments despite harsh environmental conditions. However, little is known about the year-to-year variation in seed yield of *L. chinensis* growing in a finite space within the same habitat [34, 35].

Seed germination and the seedling stage are two critical developmental periods in the plant life cycle during which plants exhibit heightened sensitivity to environmental stresses [36]. Numerous studies have focused on the tolerance of seed germination to saline-alkaline stress [37–39]. Soil salinization delays seed germination process because high soil salt concentrations exceed the critical limit of plant osmotic tolerance, suppressing imbibition and germination [40, 41]. Likewise, alkalinity stress not only triggers the same osmotic stress but also increases the pH, leading to more severe osmotic damage [42]. Giménez-Benavides et al. [43] reported that germination of high-mountain Mediterranean species was highly variable among altitudes, populations, and years, but the results differed among species. However, there is little detailed information on the effects of alkaline stress on germination of *L. chinensis* seeds from different planting years.

In the present study, we measured the seed yield components of *L. chinensis* cultivated in pots for 2–4 years and examined differences in germination, shoot length, and root length of seeds/seedling, with and without alkaline stress. We hypothesized that (1) the seed yield components of the 2nd year plants would be higher than those of the 4th year; and that (2) alkaline tolerance would vary among seeds derived from different planting years. Our results have implications for the effective utilization of *L. chinensis* and the development of appropriate management practices.

Materials and methods

Experimental design and sampling

The pot experiment was performed in Changchun, Jilin Province (124°18'–127°02'E, 43°05'–45°15'N) from 2016 to 2019 to study the effect of planting year on seed yield

components of *L. chinensis*. This region has a temperate continental climate with a mean annual temperature of 4.9°C and average annual precipitation of 498 mm.

Plastic pots (diameter and height, 30 cm) were filled with 10 kg of sieved soil that had a pH of 7.12, electrical conductivity of 0.73 dS/m, and concentrations of soil organic carbon, nitrogen, and phosphorus at 2.83%, 1.37 g/kg, and 0.67 g/kg, respectively [44]. *L. chinensis* ‘Dongdi 4’ seedlings of uniform size without offspring ramets were excavated to a depth of 15 cm from the same field site (Figure S1) and transplanted into the plastic pots on 15 May 2016, 15 May 2017, and 15 May 2018 (Fig. 1). Fifteen seedlings were transplanted into each pot, with eight replicates pots per planting year, for a total of 24 pots. Regular watering and weeding were carried out to ensure the normal growth of *L. chinensis*. All plants were grown in the field beneath a transparent polyvinylchloride roof. Belowground irrigation was applied once every two days at 193 ml·pot⁻¹, which was calculated on the basis of 498 mm mean annual precipitation [44]. Concentrations of N, P, and K in the irrigation water were below the limits of detection. Temperature data from Changchun City during the experimental period are provided in Table S2.

Seed yield components were measured for all plants in 2019. At this time, plants transplanted in 2016 were 4 years old (Y₄), those from 2017 were 3 years old (Y₃), and those from 2018 were 2 years old (Y₂) (Table S1). At the stage of seed maturity on 16 August 2019, two spikes were randomly selected from each plot, placed into separate bags, and air-dried in the lab for 3 weeks. Spike length (SL), grain number per spike (GN), and deflated grain number per spike (DN) were measured. Dried plump seeds with glumes from each treatment were divided into 5 groups, each containing 100 grains. Each

group was weighed on an electronic, semi-analytical balance (Sartorius AG, Goettingen, Germany). Thousand seed weight (TSW) was calculated as the average weight of the ten groups multiplied by 10. The seed setting rate (SSR) was calculated using the equation [44]:

$$SSR = \frac{GN}{GN + DN} \times 100\%$$

where GN is the grain number per spike and DN is the deflated grain number per spike.

Na₂CO₃ treatment and seed germination

In late August 2019, mature seeds of *L. chinensis* were collected from plants of each planting year, air-dried at room temperature, and stored in paper bags at 4°C. The seeds were surface sterilized using 0.1% HgCl₂ for 10 min and rinses multiple times with distilled water before use. Groups of 20 seeds were sown in 9-cm Petri dishes containing 0.7% (w/v) water agar supplemented with or without (control) 25 mM Na₂CO₃; there were three replicate dishes per planting year and treatment. The dishes were incubated in a growth chamber with a 12-h dark, 16°C/12-h light, 28°C cycle; fluorescent and incandescent white light were provided at 54 μmol·m⁻²·s⁻¹. Seed germination was monitored until no new germination occurred over a span of five days. After the germination experiments, five seedlings were randomly selected from each Petri dish for measurements of root and shoot length.

The germination percentage was calculated using the equation:

$$\text{Germination percentage} = \frac{n}{N} \times 100\%$$

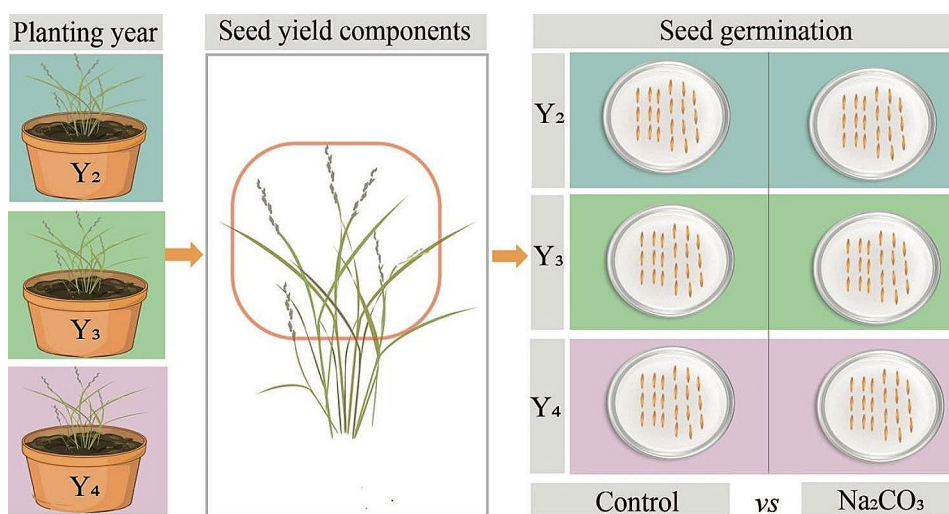


Fig. 1 Diagram illustrates the experimental design. Y₂: transplanted in 2018, 2nd year; Y₃: transplanted in 2017, 3rd year; Y₄: transplanted in 2016, 4th year

where n is the number of germinated seeds at the end of the test and N is the total number of seeds tested.

Statistical analyses

All data were analyzed using R statistical software (R4.2.2). Principal component analysis (PCA) was performed on all traits measured at harvest using the factoextra and factoMinR packages to visualize overall differences in trait variation among the three planting years. From the PCA results, we extracted the explained variance and loadings for each component, which provided insights into the contribution of each variable to its respective component. We repeated the PCA on bootstrapped datasets and then computed the standard deviation from the bootstrapped outputs to obtain the bootstrap standard errors for explained variance, contributions, and loadings. The bootstrapping was performed to estimate the uncertainty of explained variances, loadings, and contributions in the PCA output.

We also used (generalized) linear models to examine the variation in each seed yield component across planting years and to evaluate the effect of planting years on the alkaline tolerance of seed germination. We used (generalized) linear models with a binomial distribution for germination percentage and seed setting rate; a normal distribution for spike length, thousand seed weight, shoot length and root length; and a Poisson distribution for grain number per spike. ANOVA followed by Tukey’s honestly significant difference (HSD) *post hoc test* was used for pairwise multiple comparisons of mean values among planting years and between alkaline stress conditions (R package emmeans).

Results

Effect of planting year on seed yield components of *L. chinensis*

Together, the first two retained principal components (PCs) explained 79.5% of the variance in the seed yield component dataset (Fig. 2). The first principal component (PC1), explained 55.1% of the variance; it exhibited high

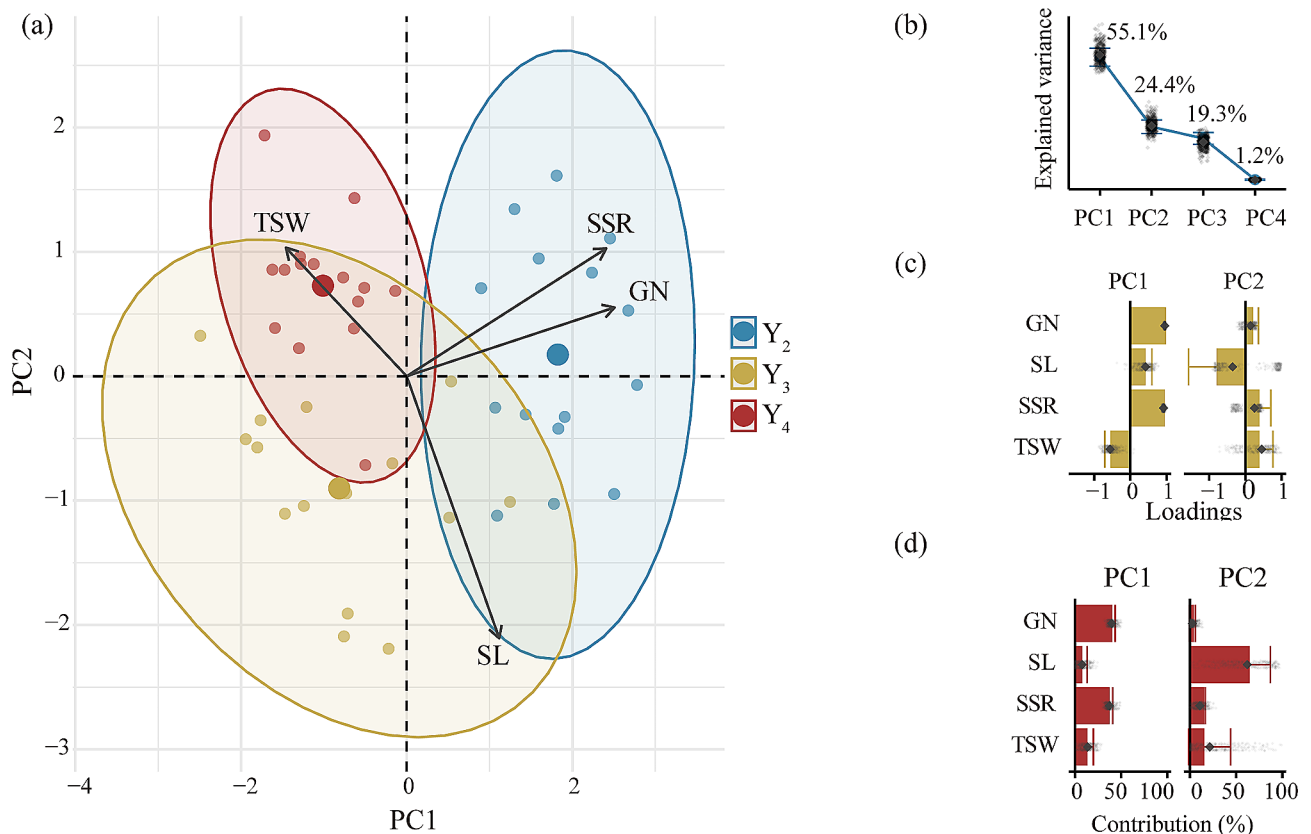


Fig. 2 Principal component analysis of seed yield components in different planting years. **(a)** PCA biplot; different colors represent different planting years (Y_2 , transplanted in 2018, 2nd year; Y_3 , transplanted in 2017, 3rd year; Y_4 , transplanted in 2016, 4th year). SL, spike length; GN, grain numbers per spike; TSW, thousand seed weight; SSR, seed setting rate. The three larger points represent the centroids of the distribution for each planting year. **(b)** Percentage of variance explained by the retained principal components (PCs). **(c-d)** Bar plot of the loadings **(c)**, and **(d)** contributions **(d)** of each variable to PC1 and PC2. The circular symbols in **(b)** and the bars in **(c)** and **(d)** show the pertinent estimates based on the full dataset. In **(b-d)** error bars are centered on the estimates and represent the standard error estimated with the bootstrap procedure ($n=500$ bootstrap iterations); the small gray diamonds show the estimates of each bootstrap iteration, and the large gray diamonds represent the median of all bootstrap iterations

positive loadings for GN and SSR and a substantial negative loading for TSW. The second principal component (PC2) explained 24.4% of the variance and had a high negative loading for SL. As shown in Fig. 2a, Y₂ and Y₄ plants were clearly separated along the first principal component. The Y₂ plants had positive values of PC1 associated with higher SSR and GN. By contrast, the Y₄ plants tended to have negative values of PC1 association with higher TSW.

Spike length was significantly shorter in Y₄ plants than in Y₂ and Y₃ plants (Fig. 3a; Table S3). Grain number per spike was significantly lower in Y₃ and Y₄ plants than in Y₂ plants, with reductions of 82.6% and 75.1%, respectively (Fig. 3b). Y₄ plants had significantly higher thousand seed weight, 17.9% higher than that of Y₂ plants (Fig. 3c). Seed setting rates were significantly lower in the Y₃ plants and Y₄ plants by 72.9% and 52.9%, respectively, compared with the Y₂ plants (Fig. 3d). Tillers per pot were significantly higher (44.1%) in the Y₂ plants than in the Y₃ plants (Figure S2).

Effects of planting year and alkaline stress on germination and seedling growth of *L. chinensis*

Seeds were harvested from Y₂, Y₃, and Y₄ plants for measurement of germination and seedling growth. Both planting year and alkaline stress treatment (25 mM Na₂CO₃) had significant effects on the germination percentage of *L. chinensis* seeds (Fig. 4). In the absence of Na₂CO₃ stress, the germination percentage was 32.1% lower for seeds from Y₂ plants than for those from Y₄ plants. Germination percentage was lower in the presence of Na₂CO₃ across all three planting years. Under Na₂CO₃ stress, germination percentage was again highest for Y₄ seeds (88.3%), followed by Y₃ seeds (73.3%) and Y₂ seeds (55.0%).

Shoot lengths of *L. chinensis* seedlings were significantly affected by both planting year and the planting year × alkaline stress interaction (Fig. 5a). Shoots of seedlings derived from Y₄ seeds were longer than those of seedlings derived from Y₂ seeds at the same Na₂CO₃

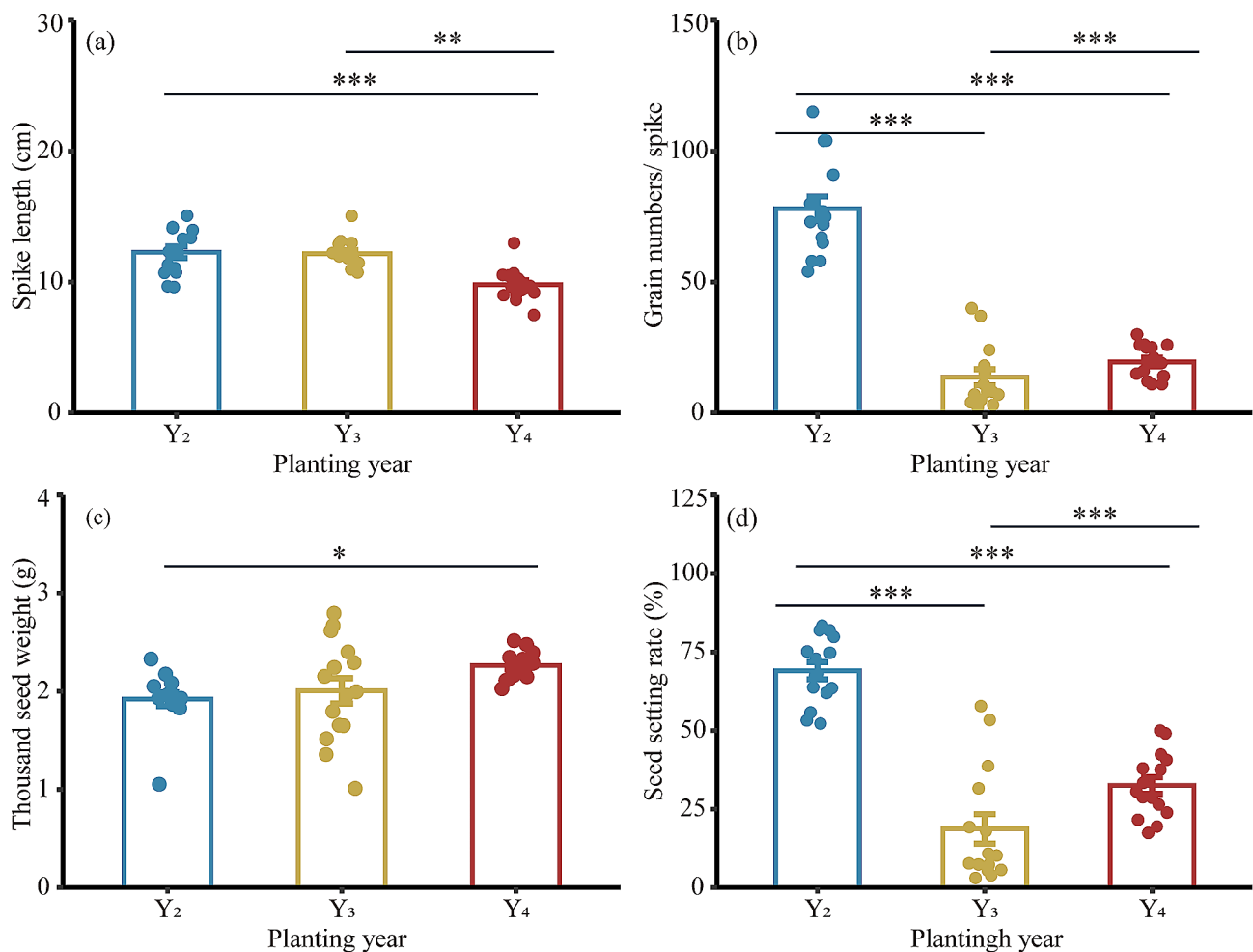


Fig. 3 Inter-annual variations in seed yield components of *L. chinensis*. (a) Spike length; (b) Grain number per spike; (c) Thousand seed weight; (d) Seed setting rate. Y₂: transplanted in 2018, 2nd year; Y₃: transplanted in 2017, 3rd year; Y₄: transplanted in 2016, 4th year. Asterisks indicate significant differences determined by Tukey's test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

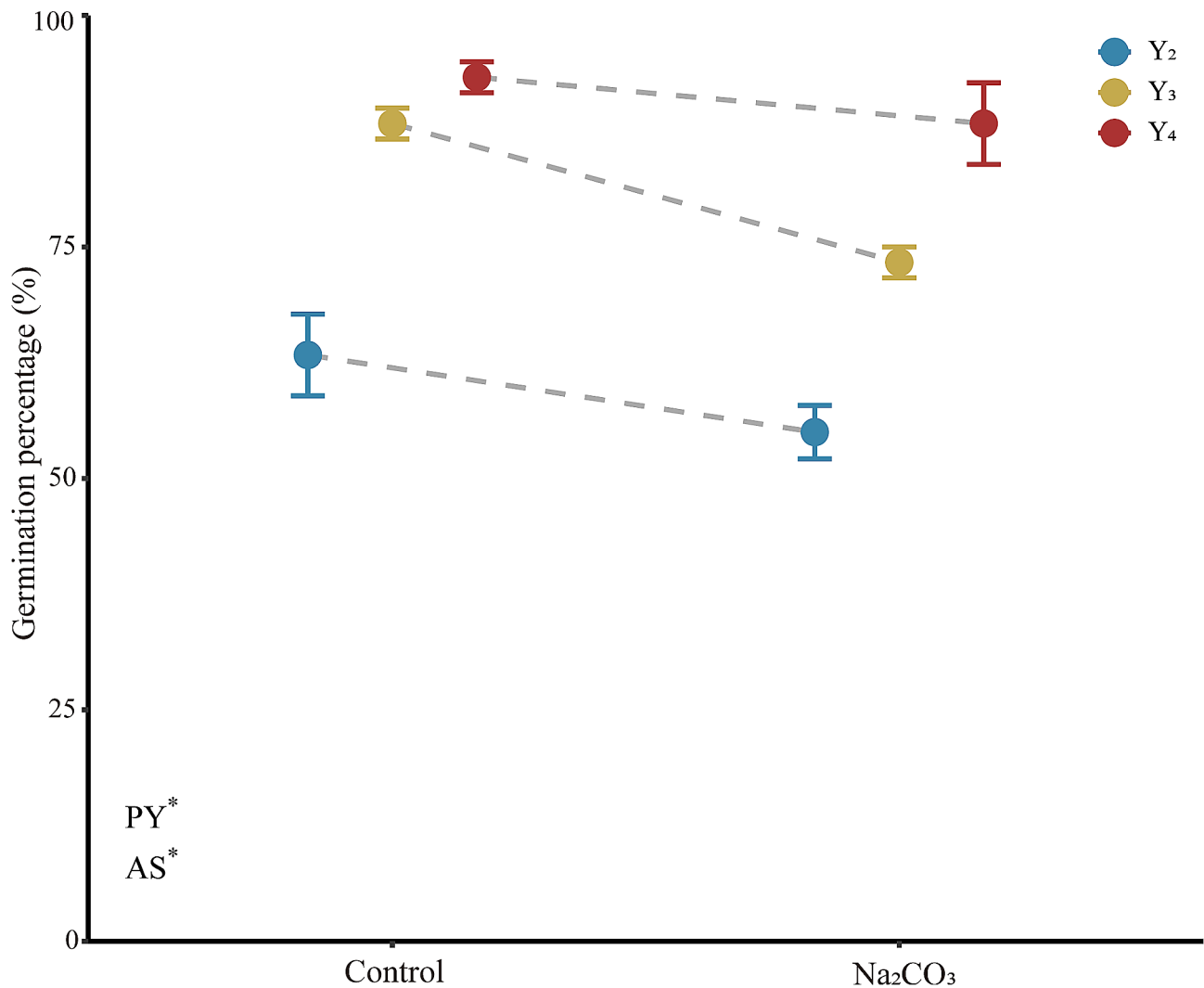


Fig. 4 Mean values (± SE) of germination percentages of *L. chinensis* seeds from plants of three planting years (Y₂, Y₃, Y₄) in the presence and absence of 25 mM Na₂CO₃. Asterisks indicate that the main effects of both model terms (PY, planting year; AS, alkali stress) were significant at *P* < 0.05. Y₂: transplanted in 2018, 2nd year; Y₃: transplanted in 2017, 3rd year; Y₄: transplanted in 2016, 4th year

concentration. The effects of alkaline stress differed among planting years. Alkaline stress caused a 9.2% reduction in shoot length of the Y₄-derived seedlings. By contrast, alkaline stress caused a 22.3% increase in the shoot length of Y₃-derived seedlings but had little effect on that of Y₂-derived seedlings (6.3 cm vs. 6.5 cm). Planting year, alkali stress, and their interaction all had significant effects on seedling root length (Fig. 5b). Alkaline stress reduced root length of seedlings derived from Y₂, Y₃ and Y₄ seeds by 84.1%, 9.5%, and 71.9%, respectively.

Discussion

Thousand seed weight of *L. chinensis* increased as the number of years after transplant increased from 2 to 4, although the number of grains per spike decreased. The heavier seeds produced by the Y₄ plants also showed less pronounced inhibition of germination in the presence

of 25 mM Na₂CO₃, suggesting that prolonging the duration of pot cultivation had a mitigating effect on alkaline stress in the resulting seeds. These findings align with previous research in which heavier seeds tended to exhibit higher germination percentages than lighter seeds [45]. Our results provide further evidence in support of this notion, as the lighter Y₂ and Y₃ seeds tended to have lower germination and reduced shoot and root lengths. However, our findings contrast with those of Fernández-Pascual et al. [46], who found no significant differences in germination between heavy and light seeds of alpine plants. These discrepancies could be attributed to variations in experimental conditions that influence germination behavior. Consistent with previous studies [47, 48], our results demonstrated that seedlings derived from heavier seeds had greater biomass than those derived from lighter seeds, likely due to the presence of greater

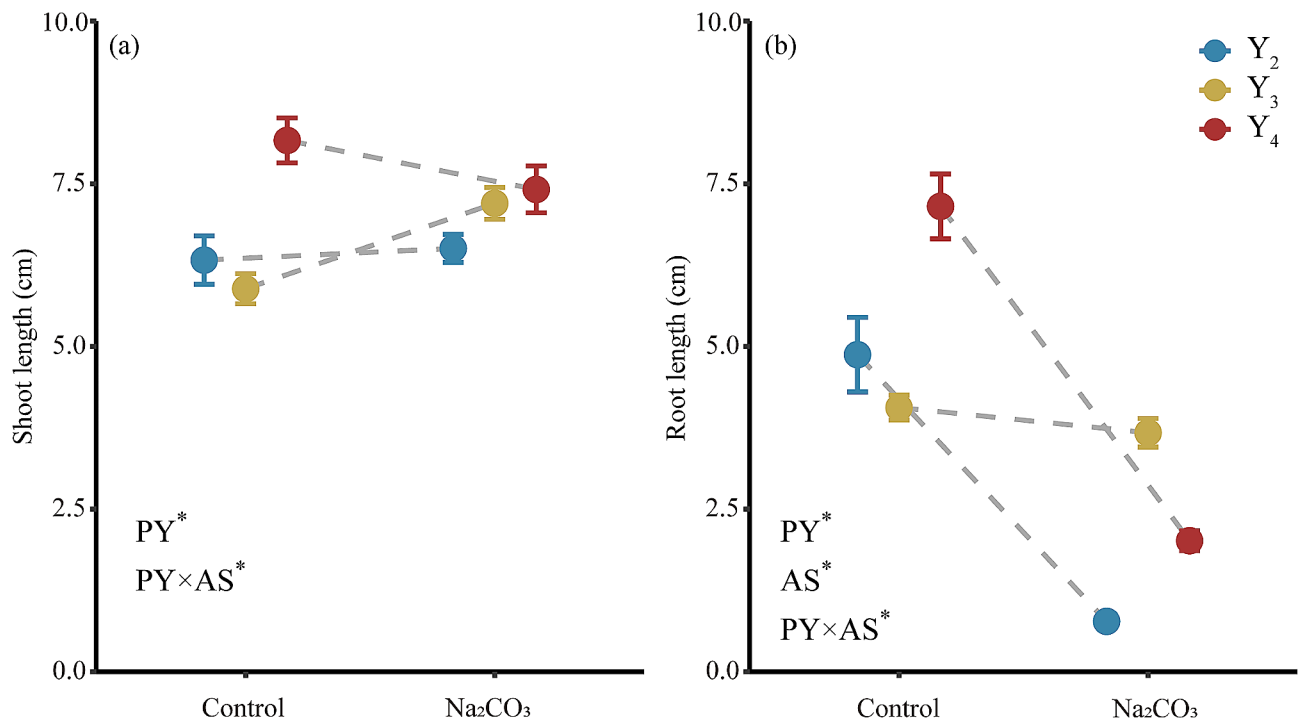


Fig. 5 Mean values (\pm SE) of shoot length (a) and root length (b) of *L. chinensis* seedlings derived from seeds produced by plants from three planting years (Y_2, Y_3, Y_4), in the presence and absence of 25 mM Na_2CO_3 . Asterisks indicate main effects and interactions that were significant at $P < 0.05$. Y_2 : transplanted in 2018, 2nd year; Y_3 : transplanted in 2017, 3rd year; Y_4 : transplanted in 2016, 4th year

seed reserves in their cotyledons [49, 50]. Intuitively, heavier seeds would seem to be advantageous because seed mineral reserves should increase as a function of total seed mass [51, 52].

The Y_2 and Y_4 plants were clearly separated along the first principal component in Fig. 2. Spike length, grain number per spike, and seed setting rate were all lower in Y_4 plants than in Y_2 plants. This result may reflect changes in resource allocation caused by increased plant density and decreased soil nutrient content over multiple years in a limited space [53, 54]. For example, Lou et al. [55] found that seed production gradually decreased in or after the second year of plant growth in the perennial herb *Saussurea nigrescens*. As a plant grows, its demands for nutrients and energy increases, and seed yield declines when supplied nutrients and energy can no longer meet these demands [56, 57]. The planting year of the mother plant has been shown to influence various aspects of seed development and seedling growth in perennial plants species [58]. Here, we observed high inter-annual variation in seed setting rate of *L. chinensis*, consistent with an adaptive seed production strategy. Previous research has suggested that *L. chinensis* exhibits strong plasticity in its reproductive characteristics, particularly in response to inter-annual variations in resource availability [59]. This plasticity enables clonal plants to regulate population stability and promote ecological balance through quantitative adjustments [60, 61].

We observed a decline in the germination percentage of *L. chinensis* under alkaline stress, consistent with previous findings [62, 63]. The detrimental effects of alkaline stress on germination can be attributed to the effect of osmotic pressure and ion toxicity. The presence of added chlorine ions exacerbates the osmotic stress experienced by the seeds. Moreover, uptake of Na^+ during seed germination can result in cell Na^+ toxicity, further inhibiting or delaying the germination process [62–64]. Although alkaline stress inhibited *L. chinensis* seedling growth in the present study, this inhibition was generally less severe for the heavier seeds produced by Y_4 and Y_3 plants. The reduced shoot and root lengths under Na_2CO_3 stress can be explained by the detrimental effects of high Na^+ concentrations and high pH stress. Elevated pH levels, particularly in the presence of high sodic salt concentrations, interfere with ion uptake, disrupt intracellular ion balance, damage root cell structure, and ultimately reduce seedling elongation [65, 66]. Variability in germination characteristics has been suggested to be one of the most important survival strategies for species growing under unpredictable environmental conditions [67, 68] and can reduce the risk of seedlings being subjected to poor growing conditions due to the establishment of intense competition hierarchies [69]. This variability helps to reduce the risk of seedlings being subjected to unfavorable growth conditions as a result of intense competition hierarchies.

Conclusion

Our study revealed clear differences in seed yield components between plants grown for two years after transplant and those grown for four years. The Y_2 plants had higher seed setting rate and grain number per spike, but the Y_4 plants had higher thousand seed weight. Seeds obtained from plants grown for four years after transplant showed somewhat less inhibition of seed germination under alkaline stress (25 mM Na_2CO_3). A substantial proportion of the inter-annual variation in seed yield components and germination of *L. chinensis* might be due to changes in plant density and/or soil nutrient availability. Further investigations will be required to fully clarify the physiological and molecular mechanisms of the inter-annual variations in germination under alkaline stress.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-024-05112-6>.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

D.Z. and H.M. conceived the study. W.Q. and S.L. conducted the pot experiment, sample collection and analyzed the data. D.Z. wrote and revised the manuscript. All authors reviewed the manuscript.

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Data availability

Data is provided within the supplementary information files.

Declarations

Ethics approval and consent to participate

Samples for this study were cultivated and transplanted from the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences in Changchun, Jilin, China. The plant materials used in this study are all in compliance with institutional, national, and international rules and protocols.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Wang K, Zhang Y, Tang Z, Shanguan Z, Chang F, Jia F, et al. Effects of grassland afforestation on structure and function of soil bacterial and fungal communities. *Sci Total Environ*. 2019;676:396–406. <https://doi.org/10.1016/j.scitotenv.2019.04.259>
- Apellaniz M, Burnside NG, Broly M. Temperate grassland afforestation dynamics in the aguapey valuable grassland area between 1999 and 2020: identifying the need for protection. *Remote Sens*. 2021;14(1):74. <https://doi.org/10.3390/rs14010074>
- Horion S, Cornet Y, Ericum M, Tychon B. Studying interactions between climate variability and vegetation dynamic using a phenology based approach. *Int J Appl Earth Obs*. 2013;20:20–32. <https://doi.org/10.1016/j.jag.2011.12.010>
- Yan Z, Eziz A, Tia D, Li X, Hou X, Peng H, et al. Biomass allocation in response to nitrogen and phosphorus availability: insight from experimental manipulations of *Arabidopsis thaliana*. *Front Plant Sci*. 2019;10:598. <https://doi.org/10.3389/fpls.2019.00598>
- Lyu X, Li XB, Dang DL, Dou HS, Xuan XJ, Liu SY, et al. A new method for grassland degradation monitoring by vegetation species composition using hyperspectral remote sensing. *Ecol Indic*. 2020;114:106310. <https://doi.org/10.1016/j.ecolind.2020.106310>
- Zhang Y, Wang Q, Wang Z, Yang Y, Li J. Impact of human activities and climate change on the grassland dynamics under different regime policies in the Mongolian Plateau. *Sci Total Environ*. 2020;698:134304. <https://doi.org/10.1016/j.scitotenv.2019.134304>
- Horie T, Kaneko T, Sugimoto G, Sasano S, Panda SK, Shibasaki M, et al. Mechanisms of water transport mediated by PIP aquaporins and their regulation via phosphorylation events under salinity stress in barley roots. *Plant Cell Physiol*. 2011;52(4):663–75. <https://doi.org/10.1093/pcp/pcr027>
- Ma H, Yang H, Lü X, Pan Y, Wu H, Liang Z, et al. Does high pH give a reliable assessment of the effect of alkaline soil on seed germination? A case study with *Leymus chinensis* (Poaceae). *Plant Soil*. 2015;394:35–43. <https://doi.org/10.1007/s11104-015-2487-4>
- Ye T, Wang Y, Feng YQ, Chan Z. Physiological and metabolomic responses of Bermuda grass (*Cynodon dactylon*) to alkali stress. *Physiol Plant*. 2021;171(1):22–33. <https://doi.org/10.1111/pp.13209>
- Chen Q, Xie HS, Wei GY, Guo XR, Zhang J, Lu XY, et al. Metabolic differences of two constructive species in saline-alkali grassland in China. *BMC Plant Biol*. 2022;22(1):53. <https://doi.org/10.1186/s12870-021-03401-y>
- Liu K, Liu ZC, Zhou N, Shi XR, Lock TR, Kallenbach RL, et al. Diversity-stability relationships in temperate grasslands as a function of soil pH. *Land Degrad Dev*. 2022;33(10):1704–17. <https://doi.org/10.1002/ldr.4259>
- Xiao F, Zhou HP. Plant salt response: perception, signaling, and tolerance. *Front Plant Sci*. 2023;13:1053699. <https://doi.org/10.3389/fpls.2022.1053699>
- Hao SH, Wang YR, Yan YX, Liu YH, Wang JY, Chen S. A review on plant responses to salt stress and their mechanisms of salt resistance. *Horticulturae*. 2021;7(6):132. <https://doi.org/10.3390/horticulturae7060132>
- Nie H, Xu L, Zhao QC, Wang NN, You CG, Zhang FG. Mechanisms of plant response to salinity stress: current understanding and recent progress. *Pak J Bot*. 2020;52(5):1879–83. [https://doi.org/10.30848/PJB2020-5\(3\)](https://doi.org/10.30848/PJB2020-5(3))
- Shi D, Wang D. Effects of various salt-alkaline mixed stresses on *Aneurolepidium chinense* (trin.) Kitag. *Plant Soil*. 2005;271:15–26. <https://doi.org/10.1007/s11104-004-1307-z>
- Acosta CA, Dominguez AM, Llobet LG, Rodriguez AMG. Effects of NaCl and NaHCO_3 stress on morphological growth and nutrient metabolism on selected avocados (*Persea americana* Mill.). *J Plant Nutr*. 2019;42(2):1–14. <https://doi.org/10.1080/01904167.2018.1551490>
- Fang SM, Hou X, Liang XL. Response mechanisms of plants under saline-alkali stress. *Front Plant Sci*. 2021;12:667458. <https://doi.org/10.3389/fpls.2021.667458>
- Zhao DD, Ma HY, Li SY, Qi WW, Li Y, Li YX, et al. Divergent germination responses to fire-related cues of *Suaeda glauca* dimorphic seeds under salinity stress. *Seed Sci Technol*. 2023;51(3):311–7. <https://doi.org/10.15258/sst.2023.51.3.04>
- Wang S, Sun L, Ling N, Zhu C, Chi FQ, Li WQ, et al. Exploring soil factors determining composition and structure of the bacterial communities in saline-alkali soils of Songnen Plain. *Front Microbiol*. 2020;10:2902. <https://doi.org/10.3389/fmicb.2019.02902>

20. Dong G, Guo JX, Chen JQ, Sun G, Gao S, Hu LJ, et al. Effects of spring drought on carbon sequestration, evapotranspiration and water use efficiency in the songnen meadow steppe in northeast China. *Ecohydrology*. 2011;4(2):211–24. <https://doi.org/10.1002/eco.200>
21. Yang YR, Cao YP, Li ZX, Zhukova A, Yang ST, Wang JL, et al. Interactive effects of exogenous melatonin and Rhizophagus intraradices on saline-alkaline stress tolerance in *Leymus Chinensis*. *Mycorrhiza*. 2020;30:357–71. <https://doi.org/10.1007/s00572-020-00942-2>
22. Wang YN, Lin JX, Yang F, Tao S, Yan XF, Zhou ZQ, et al. Arbuscular mycorrhizal fungi improve the growth and performance in the seedlings of *Leymus chinensis* under alkali and drought stresses. *Peer J*. 2022;10:e12890. <https://doi.org/10.7717/peerj.12890>
23. Zhang J, Shen X, Mu B, Shi Y, Wu X, Mu C, et al. Moderately prolonged dry intervals between precipitation events promote production in *Leymus chinensis* in a semi-arid grassland of Northeast China. *BMC Plant Biol*. 2021;21:1–11. <https://doi.org/10.1186/s12870-021-02920-y>
24. Mayel S, Jarrah M, Kuka K. How does grassland management affect physical and biochemical properties of temperate grassland soils? A review study. *Grass Forage Sci*. 2021;76(2):215–44. <https://doi.org/10.1111/gfs.12512>
25. Zhang Y, Wang L, Addrach ME, Lin K. *Fusarium incarnatum-equiseti* species complex causing root rot disease on *Leymus chinensis* in China. *Plant Dis*. 2022;106(2):762. <https://doi.org/10.1094/PDIS-06-21-1165-PDN>
26. Shi YJ, Gao S, Zhou DH, Liu MX, Wang JF, Knops JMHK, et al. Fall nitrogen application increases seed yield, forage yield and nitrogen use efficiency more than spring nitrogen application in *Leymus chinensis*, a perennial grass. *Field Crops Res*. 2017;214:66–72. <https://doi.org/10.1016/j.fcr.2017.08.022>
27. Chen HN, Tao LY, Shi JM, Han XR, Chen XG. Exogenous salicylic acid signal reveals an osmotic regulatory role in priming the seed germination of *Leymus chinensis* under salt-alkali stress. *Environ Exp Bot*. 2021;188:104498. <https://doi.org/10.1016/j.envexpbot.2021.104498>
28. Hampton JG, Conner AJ, Boelt B, Chastain TG, Rolston P. Climate change: seed production and options for adaptation. *Agriculture*. 2016;6(3):33. <https://doi.org/10.3390/agriculture6030033>
29. Poorter H, Fiorani F, Pieruschka R, Wojciechowski T, van der Putten WH, Kleyer M, et al. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytol*. 2016;212(4):838–55. <https://doi.org/10.1111/nph.14243>
30. Larson JE, Anacker BL, Wanous S, Funk JL. Ecological strategies begin at germination: traits, plasticity and survival in the first 4 days of plant life. *Funct Ecol*. 2020;34(5):968–79. <https://doi.org/10.1111/1365-2435.13543>
31. Norton DA, Kelly D. Mast seeding over 33 years by *Dacrydium cupressinum* Lamb. (rimu) (Podocarpaceae) in New Zealand: the importance of economies of scale. *Funct Ecol*. 1988;2:399–408. <https://doi.org/10.2307/2389413>
32. Witkowski ETF, Wilson M. Changes in density, biomass, seed production and soil seed banks of the non-native invasive plant, *Chromolaena odorata*, along a 15 year chronosequence. *Plant Ecol*. 2001;152:13–27. <https://doi.org/10.1023/A:1011409004004>
33. Pol RG, Pirk GI, Marone L. Grass seed production in the central Monte desert during successive wet and dry years. *Plant Ecol*. 2010;208:65–75. <https://doi.org/10.1007/s11258-009-9688-y>
34. Houle C, Filion L. Interannual variations in the seed production of *Pinus banksiana* at the limit of the species distribution in northern Quebec, Canada. *Am J Bot*. 1993;80(11):1242–50.
35. Guo J, Richards CL, Holsinger KE, Fox GA, Zhang Z, Zhou C. Genetic structure in patchy populations of a candidate foundation plant: a case study of *Leymus chinensis* using genetic and clonal diversity. *Am Jo Bot*. 2021;108(12):2371–87. <https://doi.org/10.1002/ajb2.1771>
36. Ambavaram MMR, Basu S, Krishnan A, Ramegowda V, Batlang U, Baisakh N, et al. Coordinated regulation of photosynthesis in rice increases yield and tolerance to environmental stress. *Nat Commun*. 2014;5(1):5302. <https://doi.org/10.1038/ncomms6302>
37. Guo R, Zhou J, Hao WP, Gong DZ, Zhong XL, Gu FX, et al. Germination, growth, photosynthesis and ionic balance in *Setaria viridis* seedlings subjected to saline and alkaline stress. *Can J Plant Sci*. 2011;91(6):1077–88. <https://doi.org/10.4141/cjps10167>
38. Ye XX, Wang H, Cao XL, Jin XJ, Cui FQ, Bu YY, et al. Transcriptome profiling of *Puccinellia tenuiflora* during seed germination under a long-term saline-alkali stress. *BMC Genomics*. 2019;20:1–17. <https://doi.org/10.1186/s12864-019-5860-5>
39. Chen HN, Shi JM, Tao LY, Han XR, Lin GL, Cheng XG. Exogenous spermidine priming mitigates the osmotic damage in germinating seeds of *Leymus chinensis* under salt-alkali stress. *Front Plant Sci*. 2021;12:701538. <https://doi.org/10.3389/fpls.2021.701538>
40. Zelm EV, Zhang YX, Testerink C. Salt tolerance mechanisms of plants. *Annu Rev Plant Biol*. 2020;71:403–33. <https://doi.org/10.1146/annurev-arplant-050718-100005>
41. Nikolić N, Ghirardelli A, Schiavon M, Masin R. Effects of the salinity-temperature interaction on seed germination and early seedling development: a comparative study of crop and weed species. *BMC Plant Biol*. 2023;23(11):446. <https://doi.org/10.1186/s12870-023-04465-8>
42. Hazman M, Hause B, Eiche E, Riemann M, Nick P. Different forms of osmotic stress evoke qualitatively different responses in rice. *J Plant Physiol*. 2016;202:45–56. <https://doi.org/10.1016/j.jplph.2016.05.027>
43. Benavides LG, Escudero A, García FP. Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. *Ecol Res*. 2005;20(4):433–44. <https://doi.org/10.1007/s11284-005-0059-4>
44. Zhao DD, Ma HY, Wang L, Li SY, Qi WW, Ma MM, et al. Effects of water and nitrogen addition on the seed yield and germination characteristics of the perennial grass. *Front Environ Sci*. 2021;9:704097. <https://doi.org/10.3389/fenvs.2021.704097>
45. Baskin CC, Baskin JM. *Seeds: Ecology, Biogeography, and evolution of Dormancy and Germination*. San Diego: Academic; 2014.
46. Pascual EF, Carta A, Mondoni A, Cavieres LA, Venn SRS, Satyanti A, et al. The seed germination spectrum of alpine plants: a global meta-analysis. *New Phytol*. 2021;229(6):3573–86. <https://doi.org/10.1111/nph.17086>
47. Ma Z, Willis CG, Zhang CH, Zhou HK, Zhao XQ, Dong SK, et al. Direct and indirect effect of seed size on seedling survival along an experimental light availability gradient. *Agric Ecosyst Environ*. 2019;281:64–71. <https://doi.org/10.1016/j.agee.2019.05.009>
48. Maes SL, Perring MP, Depauw L, Römermann MB, Blondeel H, Brümelis G, et al. Plant functional trait response to environmental drivers across European temperate forest understorey communities. *Plant Biol*. 2020;22(3):410–24. <https://doi.org/10.1111/plb.13082>
49. Tangney R, Merritt DJ, Callow JN, Fontaine JB, Miller BP. Seed traits determine species' responses to fire under varying soil heating scenarios. *Funct Ecol*. 2020;34(9):1967–78. <https://doi.org/10.1111/1365-2435.13623>
50. Smith TM, Sherman CDH, Cumming EE, York PH, Jarvis JC. Size matters: variations in seagrass seed size at local scales affects seed performance. *Hydrobiologia*. 2022;849(10):2335–52. <https://doi.org/10.1007/s10750-022-04873-1>
51. Hanley ME, Cordier PK, May O, Kelly CK. Seed size and seedling growth: differential response of Australian and British Fabaceae to nutrient limitation. *New Phytol*. 2007;174(2):381–8. <https://doi.org/10.1111/j.1469-8137.2007.02003.x>
52. Ao YN, Wang JF, Liu MX, Wang J, Knops JMH, Mu CS. Larger seed size shows less germination and seedling growth decline caused by seed ageing under Na₂CO₃ stress in *Leymus Chinensis*. *Agron J*. 2019;111(5):2326–31. <https://doi.org/10.2134/agronj2018.12.0757>
53. Richardson DM, Holmes PM, Esler KJ, Galatsetsch SM, Stromberg JC, Kirkman SP, et al. Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Divers Distrib*. 2007;13(1):126–39. <https://doi.org/10.1111/j.1366-9516.2006.00314.x>
54. Jahani A, Goshtasb H, Saffariha M. Tourism impact assessment modeling of vegetation density for protected areas using data mining techniques. *Land Degrad Dev*. 2020;31(12):1502–19. <https://doi.org/10.1002/ldr.3549>
55. Lou YX, Wang RL, Che PY, Zhao C, Chen YL, Yang YHS, et al. Nitrogen Addition affects interannual variation in seed production in a tibetan perennial Herb. *Biology*. 2023;12(8):1132. <https://doi.org/10.3390/biology12081132>
56. Muñoz VL, Balzarini M, Delard C, Del RR, Alvarez A. Inter-annual variability of *Pinus pinea* L. cone productivity in a non-native habitat. *New for*. 2020;51(6):1055–68. <https://doi.org/10.1007/s11056-020-09774-6>
57. Xue W, Li XY, Zeng FJ. Inter-annual variations of seed cotton yield in relation to soil organic carbon and harvest index in reclaimed desertified land. *Field Crops Res*. 2021;272:108267. <https://doi.org/10.1016/j.fcr.2021.108267>
58. Lembicz M, Olejniczak P, Żukowski W, Bogdanowicz AM. Effect of mother plant age on germination and size of seeds and seedlings in the perennial sedge *Carexsecalina*. (Cyperaceae) *Flora*. 2011;206(2):158–63. <https://doi.org/10.1016/j.flora.2010.09.006>
59. Li ZM, Wu JF, Han Q, Nie KY, Li YF, Wang XY, et al. Nitrogen and litter addition decreased sexual reproduction and increased clonal propagation in grasslands. *Oecologia*. 2021;195:131–44. <https://doi.org/10.1007/s00442-020-04812-8>
60. Yang P, Huang L, He S, Zeng XH, Chen YY, Wang HM. Adaptive strategies employed by Clonal Plants in heterogeneous patches. *Forests*. 2023;14(8):1648. <https://doi.org/10.3390/f14081648>

61. Li XL, Hu NN, Yin JJ, Ren WB, Fry E. Historic grazing enhances root-foraging plasticity rather than nitrogen absorbability in clonal offspring of *Leymus Chinensis*. *Plant Soil*. 2021;466(1):65–79. <https://doi.org/10.21203/rs.3.rs-163508/v1>
62. Ren Y, Wang W, He J, Zhang L, Wei Y, Yang M. Nitric oxide alleviates salt stress in seed germination and early seedling growth of pakchoi (*Brassica chinensis* L.) by enhancing physiological and biochemical parameters. *Ecotoxicol Environ Saf*. 2020;187:109785. <https://doi.org/10.1016/j.ecoenv.2019.109785>
63. Kamran M, Wang D, Xie K, Lu YS, Shi CH, Sabagh AEL, et al. Pre-sowing seed treatment with kinetin and calcium mitigates salt induced inhibition of seed germination and seedling growth of choysum (*Brassica rapa* var. *Parachinensis*). *Ecotox Environ Safe*. 2021;227:112921. <https://doi.org/10.1016/j.ecoenv.2021.112921>
64. Ibrahim EA. Seed priming to alleviate salinity stress in germinating seeds. *J Plant Physiol*. 2016;192:38–46. <https://doi.org/10.1016/j.jplph.2015.12.011>
65. Nie W, Gong B, Chen Y, Wang J, Wei M, Shi Q. Photosynthetic capacity, ion homeostasis and reactive oxygen metabolism were involved in exogenous salicylic acid increasing cucumber seedlings tolerance to alkaline stress. *Sci Hortic*. 2018;235:413–23. <https://doi.org/10.1016/j.scienta.2018.03.011>
66. Jia XM, Wang H, Svetla S, Zhu YF, Hu Y, Cheng L, et al. Comparative physiological responses and adaptive strategies of apple *Malus halliana* to salt, alkali and saline-alkali stress. *Sci Hortic*. 2019;245:154–62. <https://doi.org/10.1016/j.scienta.2018.10.017>
67. Satyanti A, Guja LK, Nicotra AB. Temperature variability drives within-species variation in germination strategy and establishment characteristics of an alpine herb. *Oecologia*. 2019;189(2):407–19. <https://doi.org/10.1007/s00442-018-04328-2>
68. Phartyal SS, Rosbakh S, Ritz C, Posclod P. Ready for change: seed traits contribute to the high adaptability of mudflat species to their unpredictable habitat. *J Veg Sci*. 2020;31(2):331–42. <https://doi.org/10.1111/jvs.12841>
69. Klinerová T, Dostál P. Nutrient-demanding species face less negative competition and plant-soil feedback effects in a nutrient-rich environment. *New Phytol*. 2020;225(3):1343–54. <https://doi.org/10.1111/nph.16227>

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