A Multi-year Beneficial Effect of Seed Priming with Gibberellic Acid-3 (GA₃) on Plant Growth and Production in a Perennial Grass, *Leymus chinensis*

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Seed priming is a widely used technique in crops to obtain uniform germination and high-quality seedlings. In this study, we found a long-term effect of seed priming with gibberellic acid-3 (GA₃) on plant growth and production in *Leymus chinensis*. Seeds were germinated on agar plates containing 0–200 μM GA₃, and the germinated seedlings were transplanted to clay planting pots and grown for about one year. The clonal tillers grown from the mother plants were transplanted to field conditions in the second year. Results showed that GA₃ treatment significantly increased seed germination rate by 14–27%. GA₃ treatment also promoted subsequent plant growth and biomass production, as shown by a significant increase in plant height, tiller number, and fresh and dry weight in both pot (2016) and field (2017) conditions. It is particularly noteworthy that the growth-promoting effect of a single seed treatment with GA₃ lasted for at least two years. In particular, GA₃ treatment at 50 μM increased aboveground fresh and dry weight by 168.2% and 108.9% in pot-grown conditions, and 64.5% and 126.2% in field-grown conditions, respectively. These results imply a transgenerational transmission mechanism for the GA-priming effect on clonal offspring growth and biomass production in *L. chinensis*.

Seed quality is the basis of adequate plant establishment and is associated with the productive success of crops. Therefore, a variety of strategies are employed in improving seed germination, seedling growth, and productivity. Seed priming, a low-cost and low-risk tool, is considered to be the most effective of these methods. It comprises a pre-soaking treatment of soaking seeds in a specified solution, allowing some metabolic activities to proceed before germination, and can increase germination percentage, shorten germination time, and improve seedling establishment. Based on the priming agents, seed priming can generally be classified into four groups: hydropriming, osmopriming, halopriming, and hormone priming.

In hormone priming, plant growth regulators such as gibberellic acids (GAs), abscisic acid (ABA), or salicylic acid (SA) have been widely used to increase synchronized seed germination, seedling growth, and also the yield of a variety of crop species, such as rice, corn, safflower, wheat, beet, and sunflower. However, most studies have focused on the life stages of seed germination and seedling growth, and little attention has been given to priming effects over a long-time span. Moreover, there have been scarce reports of studies on hormone priming in grass species, especially perennial grass species.

GAs play important roles in many essential plant growth and development processes, including seed germination, stem elongation, leaf expansion, flower and fruit development, and floral transition. They are often used to overcome seed dormancy, and can significantly improve seed germination in many species, mainly through the activation of embryo growth, mobilization of reserves, and weakening of the endosperm layer. It has also been reported that seed priming with GAs improves germination and the growth parameters of shoot length, root
centrations of 10 and 50 with GA 3 at all concentration levels promoted subsequent plant height (df = 5, F = 8.360, p < 0.001) during the whole growth period (Fig. 2). Plant height was highest at 50 μM, and tiller number was greatest at 5 μM and 10 μM GA 3, respectively (Fig. 2).

Grass production was also markedly enhanced by seed treatment with GA 3 (Fig. 3). Both the fresh (df = 5, F = 4.570, p = 0.017) and dry weight (df = 5, F = 4.428, p = 0.019) of shoots were significantly affected by GA 3 concentrations, with a GA 3 treatment of 50 μM showing the highest promoting effect. No significant effect on grass production was observed when GA 3 concentrations was >100 μM (Fig. 3).

Transgenerational effects of seed priming with GA 3 treatment on clonal offspring growth in field conditions (2017). Seed treatment with GA 3 at all concentration levels promoted clonal offspring plant growth during the whole growth period, as shown by the increased plant height and tiller number per plant (Fig. 4). Values of plant height and tiller number were highest at 50 μM GA 3 (Fig. 4A). Plant height (df = 5, F = 19.458, p < 0.001) of L. chinensis was significantly affected by GA 3 treatment. Significant promotion of tiller number by GA 3 concentration (df = 5, F = 11.083, p < 0.001) was observed, especially at a concentration of 50 μM GA 3 (Fig. 4B).

Grass production was also markedly enhanced by seed treatment with GA 3 (Figs 5, 6). Both fresh (df = 5, F = 5.279, p = 0.021) and dry (df = 5, F = 8.552, p < 0.001) shoot weights were significantly affected by GA 3 concentrations, with the 50 μM GA 3 treatment having the highest promoting effect.

Figure 1. Effect of the application of gibberellic acid-3 (GA 3) on seed germination in Leymus chinensis. Seeds were germinated in agar plates containing 0 (control), 5, 10, 50, 100, and 200 μM of GA 3, for 28 d under an alternating cycle of 12/12 h of light (fluorescent and incandescent white light of 54 μmol m −2 s −1) at 28/16 °C. Values are mean ± s.e.
Discussion

Native perennial species in natural grassland plays a very important role in the broad-scale restoration of degraded ecosystems, where grass reseeding technology has great potential for restoring ecosystem functionality. *Leymus chinensis* previously dominated native perennial grass species on the eastern Eurasian Steppe and is considered to be the most attractive grass in the restoration of artificially established grasslands. In this study, seed priming with GA3 significantly enhanced seed germination and subsequent plant growth (Figs 2, 4), and grass production (Figs 3, 5) in *L. chinensis*. In particular, GA3 priming at a concentration of 50 μM enhanced germination rate by 27.0%, and grass production in fresh and dry matter by 168.2% and 108.9% in pot (Fig. 3), and 64.5% and 126.2% in field (Fig. 5) conditions, respectively. It is noteworthy that the significant improvement in grass production for at least two years was obtained by just a single GA3 seed treatment (priming). These results strongly demonstrated that seed priming with GA3 is a simple but effective method for enhancing grass production in *L. chinensis*, especially in artificial grasslands where seeding is necessary.

The poor seed germination of *L. chinensis* has been considered an obstacle to the establishment of artificial grasslands. Several strategies for improving seed germination have been suggested, for example, cold stratification, removal of glumes, and exogenous hormone treatments. Seed priming with GA3 has been demonstrated to be a useful tool for activating metabolic germination processes and facilitating increments in physiological processes during seed germination, especially for grass seeds exhibiting physiological dormancy (PD), e.g., *Leymus arenarius*, *Setaria viridis*, *Tripsacum dactyloides*, and some *Triodia* species (Poaceae). In *L. chinensis*, we previously proved a positive relationship (p > 0.05) between seed germination and endogenous hormone content during seed development. In this study, exogenous GA3 treatment at a range of 5–200 μM enhanced germination rate, with the highest effect recorded at 50 μM (Fig. 1). High GA3 concentrations of ≥100 μM showed
a less beneficial effect on seed germination compared to concentrations of 10–50 μM. These results are somewhat inconsistent with previous reports that GA₃ concentrations as high as 300 μM or 2.89 mM showed higher promoting effects than other concentrations in L. chinensis seed germination. This discrepancy may be ascribed to different degrees of dormancy in the seed material used in the experiments.

GA-priming has been demonstrated to promote seedling growth in various crop plants; Seed priming using GA₃ at appropriate concentrations leads to high germination rates and better seedling growth; however, the beneficial concentration differs among plant species. GA₃ treatment showed the highest promoting effect on seed germination and seedling growth in Capparis spinosa at 360.9 μM, Trigonella foenum-graecum at 180.4 μM, and 721.8–1443.5 μM for Parthenium argentatum Gray. The yield attributes of Helianthus annuus L. and Triticum aestivum L. were also increased by seed treatment with 10–100 μM GA₃ for 8 h. In previous studies on L. chinensis, GA spraying at various growth stages remarkably promoted plant growth and grass production. In this study, we showed that seed priming with GA₃ significantly promoted plant growth (Figs 2, 4) and enhanced grass production (Figs 3, 5) in L. chinensis, in both pot and field experiments. Similar to the effect on seed germination (Fig. 1), seed treatment with GA₃ at 50 μM yielded the highest promoting effect on plant growth (Figs 2–6), and GA₃ levels above 50 μM were less beneficial to plant growth than in the range of 5–50 μM (Figs 2–6). This is in accordance with observations that phytohormones only function within a threshold range of concentration levels. However, the most promoting effects of GA₃ concentrations on production of the first, second, and following generations in L. chinensis needs further study.

The most significant and unexpected finding in this study was that the beneficial effect of seed priming with GA₃ was passed on to clonal offspring for at least two years in L. chinensis (Figs 2–6). The fact that the priming...
effect was also observed in the next generation plants (Figs 4–6) implies a transgenerational transmission mechanism for GA-priming effects in this species. Transgenerational effects have been observed in many species in passing on to offspring maternal stress responses including responses to drought\textsuperscript{43}, salinity\textsuperscript{44}, and light\textsuperscript{45,46}. Hartmann et al.\textsuperscript{45} report that far-red irradiated seeds of Chenopodium album and Stellaria media showed a significantly reduced emergence for two years, demonstrating the influence of the maternal far-red-absorbing seed phytochrome Bfr over time\textsuperscript{45}. Very recently, Ren et al.\textsuperscript{47} have reported that long-term overgrazing-induced memory decreased the photosynthesis of clonal offspring in L. chinensis by decreasing leaf chlorophyll content and Rubisco enzyme activity, and downregulating a series of key genes that regulate photosynthetic efficiency, stomata opening, and chloroplast development\textsuperscript{47}. It would be very interesting to observe further through how many generations GA priming effects can succeed. The molecular and physiological mechanisms underlying the transgenerational effects of GA-priming remain to be explored, although DNA methylation changes induced by environmental cues have been implicated in many studies of transgenerational effects\textsuperscript{43,48}.

**Conclusion**

In this study, we showed that seed priming with GA\textsubscript{3} can significantly enhance seed germination rate and subsequent plant growth and grass production in a perennial grass species, L. chinensis. The GA priming effect was transgenerational, with the clonal offspring also showing enhanced plant growth and grass production. Our findings provide a new practical method for improving perennial grass productivity, especially in artificial grasslands, in which seeding is necessary. On the other hand, some questions remain unresolved, such as how long or through how many generations can the GA-priming effect be preserved, and what molecular and physiological mechanisms underpin the transgenerational transmission of GA-priming effects to clonal offspring.

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**Figure 4.** Effect of seed priming with gibberellic acid-3 (GA\textsubscript{3}) on plant growth of Leymus chinensis in the field experiment (2017). Leymus chinensis seeds were germinated in the presence of GA\textsubscript{3} at different concentrations, as indicated (0–200\textmu M), and the seedlings were transplanted to and grown in clay pots in 2016. The tillers grown from the mother plants were separated individually and transplanted to the field in 2017. (A) Plant height, (B) tillers per plant. The columns in light grey and grey represent the measurements made on July 20 and August 10, 2017, respectively. Data are means ± s.e.
Figure 5. Effect of seed priming with gibberellic acid-3 (GA₃) on fresh and dry weight of *Leymus chinensis* in the field experiment (2017). *Leymus chinensis* seeds were germinated in the presence of GA₃ at different concentrations, as indicated (0–200 μM). The seedlings were transplanted to and grown in clay pots in 2016. The tillers grown from the mother plants were separated individually and transplanted to the field in 2017. (A) Plant height, (B) tillers per plant. The columns in light grey and grey represent the measurements made on July 20 and August 10, 2017, respectively. Data are means ± s.e.

Figure 6. Image of the effect of priming with gibberellic acid-3 (GA₃) on plant growth in transgenerational *Leymus chinensis* offspring in field conditions (2017).
Materials and Methods

A schematic of the overall experimental design is shown in Fig. 7.

Plant seeds. Matured seeds of *L. chinensis* were collected from the Da’an Sodic Land Experiment Station (45°35′58″–45°36′28″N, 123°50′27″–123°51′31″E), in the western part of the Songnen Plain, northeast China, in late July, 2015. The collected seeds were air-dried at room temperature, placed in a paper bag, and stored at 4 °C until November 2015, just before the germination experiments were undertaken.

**GA₃ treatment and seed germination (2015).** A 10 mM GA₃ stock solution was prepared by adding 0.0346 g GA₃ to a 10 ml volumetric flask and dissolved by a drop of 95% ethyl alcohol, after which distilled water was added to make a total solution of 10 ml. Seeds of *L. chinensis* were surface-sterilized in 0.1% HgCl₂ for 10 min and then washed with distilled water several times before being used in the experiments. Approximately 25 seeds were sown in a Petri dish (diameter 9 cm) containing 0.7% (w/v) water agar supplemented with 0 (control, ethylalcohol at a concentration comparable to that in GA solutions), or 5, 10, 50, 100, 200 μM of GA₃, with four replicates for each treatment. The Petri dishes were incubated under an alternating cycle of 12/12 h light (fluorescent and incandescent white light of 54 μmol·m⁻²·s⁻¹) at 28/16 °C. Seed germination was measured every second day until no new germination occurred within three days.

**Plant growth in pot experiments (2016).** After determining the germination rate, seedlings were transplanted to clay pots (diameter 30 cm, and height 30 cm) each containing 10 kg of soil, in early January 2016. The clay loam soil was collected from a field based at the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China. Four seedlings were transplanted into each pot, with four replicates per treatment. Tap water was added to keep the soil moist. Plant height and tillers per plant were recorded during growth, and grass production (fresh and dry weight of shoots) was measured in late July. The aboveground *L. chinensis* plant material in each pot were cut separately and the fresh weight recorded immediately. Then, the plants were put in paper bags and dried in an oven at 105 °C for 2 h and 80 °C for another 48 h, and the dry weight of each treatment was recorded.

**Plant growth in field experiments (2017).** The *L. chinensis* tillers were separated individually from the mother plants grown in pots, and transplanted to a field with one individual clonal offspring in each hill at the Institute of Geography and Agroecology on April 24, 2017; the field was the same as that from which soil was collected for the pot experiment, and therefore the soil was of the same type. The row and line spacings were 60 cm and 50 cm, respectively. The plot was irrigated once, immediately after the transplantation. Height and tiller number of plants in each hill were recorded on June 20 and August 10, 2017, respectively, with 30 hills for each GA₃ concentration. Grass production (fresh and dry weight of shoots) was measured in late August, 2017 with 10 replicates for each GA₃ concentration treatments.

**Statistical analysis.** Generalized linear models (GLMs) with a binomial error structure and logit link function were used to compare proportional data relating to the final germination of *L. chinensis* in the GA₃ treatments. The differences in the tiller number and plant height among GA₃ treatments were analyzed by repeated measures ANOVA using a linear mixed effect model in the lme4 package in R. We treated sampling time as a random effect and allowing concentration to enter the model as a fixed effect. If necessary, data were log transformed to meet assumptions of normality and homogeneity of variance. In addition, data of fresh and dry weights among different treatments were compared separately by one-way analysis of variance (ANOVA). Before the analyses of ANOVA, the normality (shapiro-wilk test) and homoscedasticity (Levene's test) was conducted. A Tukey's test was used for multiple comparisons when the among treatments was significant. All of the analyses were carried out using the R statistical platform.

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**Author Contributions**

H.-Y.M., C.-J.J., Z.-W.L. designed the experiment, and H.-Y.M., D.-D.Z., Q.-R.N., J.-P.W., Y.L., M.-M.W., X.-L.L. conducted the experiments and collected the data. H.-Y.M. and Q.-R.N. analyzed the data. H.-Y.M. and C.-J.J. wrote the manuscript.

**Additional Information**

**Competing Interests:** The authors declare no competing interests.

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