ORIGINAL PAPER

Hormonal and temperature regulation of seed dormancy and germination in *Leymus chinensis*

Xiao Wen Hu · Xiao Hui Huang · Yan Rong Wang

Abstract Fluctuating temperature plays a critical role in determining the timing of seed germination in many plant species. However, the physiological and biochemical mechanisms underlying such a response have been paid little attention. The present study investigated the effect of plant growth regulators and cold stratification in regulating *Leymus chinensis* seed germination and dormancy response to temperature. Results showed that seed germination was less than 2 % at all constant temperatures while fluctuating temperature significantly increased germination percentage. The highest germination was 71 % at 20/30 °C. Removal of the embryo enclosing material of *L. chinensis* seed germinated to 74 %, and replaced the requirement for fluctuating temperature to germinate, by increasing embryo growth potential. Applications of GA_{4+7} significantly increased seed germination at constant temperature. Also, inhibition of GA biosynthesis significantly decreased seed germination at fluctuating temperatures depending upon paclobutrazol concentration. This implied GA was necessary for non-dormant seed germination and played an important role in regulating seed germination response to temperature. Inhibition of ABA biosynthesis during imbibition completely released seed dormancy at 20/30 °C, but showed no effect on seed germination at constant temperature, suggesting ABA biosynthesis was important for seed dormancy maintenance but may not involve in seed germination response to temperature. Cold stratification with water or GA_3 induced seed into secondary dormancy, but this effect was reversed by exogenous FL, suggesting ABA biosynthesis during cold stratification was involved in secondary dormancy. Also, cold stratification with FL entirely replaced the requirement of fluctuating temperature for germination with seeds having 73 % germination at constant temperature. This appears to be attributed to inhibition of ABA biosynthesis and an increase of GA biosynthesis during cold stratification, leading to an increased embryo growth potential. We suggest that fluctuating temperature promotes seed germination by increasing embryo growth potential, mainly attributed to GA biosynthesis during imbibitions. ABA is important for seed dormancy maintenance and induction but showed less effect on non-dormant seed germination response to temperature.

Abbreviations
ABA Abscisic acid
GA Gibberellin
FL Fluridone
PA Paclobutrazol

X. W. Hu (✉) · X. H. Huang · Y. R. Wang (✉)
State Key Laboratory of Grassland Agro-ecosystems,
College of Pastoral Agriculture Science and Technology,
Lanzhou University, P.O.Box 61, Lanzhou 730020, China
e-mail: huxw@lzu.edu.cn

Y. R. Wang
e-mail: yrwang@lzu.edu.cn

Introduction

In many wild species, seeds are dormant at maturity and will not germinate until dormancy release after dispersal even when subjected to optimal conditions (Baskin and Baskin 1998; Chen et al. 2009). Temperature is a major environmental factor responsible for changes in dormancy

states of seeds, and dormancy can be broken by fluctuating temperature or warm/cold stratification (Baskin and Baskin 1998). Many researches (Baskin and Baskin 1998; Benech-Arnold et al. 1995) found that temperature fluctuation is an absolute requirement for germination of many species. In some cases, the requirement for temperature fluctuation could be substituted by light (Fenner and Thompson 2005) or cold stratification (Brändel 2004) depending upon the species. This phenomenon has important ecological significance associated with the strategies of seed to detect canopy gaps, depth of burial in soil or position under water (Fenner and Thompson 2005). While seed responses to fluctuating temperature are widespread among many species, the physiological and biochemical mechanisms underlying such responses are still largely unknown (Huarte and Benech-Arnold 2010).

The plant hormones gibberellins (GAs) and abscisic acid (ABA) are involved in seed dormancy and germination. It has been shown that abscisic acid (ABA) plays a role in the induction and maintenance of seed dormancy, whereas gibberellins (GAs) are associated with dormancy breaking and germination (Kucera et al. 2005). Dormant seeds treated with fluridone (a compound which inhibits carotenoid and, thus, ABA synthesis) often have similar germination characteristics to non-dormant seeds, indicating that the continued synthesis of ABA is required for dormancy maintenance in imbibed seeds of several species (Yoshioka et al. 1998; Kucera et al. 2005; Feurtado et al. 2007). Also, application of paclobutrazol(an inhibitor of GA biosynthesis) was found depressed seed germination indicated that a de novo biosynthesis of GAs is required during imbibition (Karssen et al. 1989; Bradford and Nonogaki 2007). Benech-Arnold et al. (1995) reported that fluctuating temperature stimulated germination of immature dormant sorghum caryopses by reducing embryo sensitivity to ABA. More recently, Huarte and Benech-Arnold (2010) found that fluctuating temperature decreased ABA concentration prior to radical emergence, and exogenous GA$_3$ enhanced seed germination at constant temperature. This implied that ABA and GA were involved in seed germination and the dormancy response to fluctuating temperature.

Similarly, cold stratification has been widely used to break dormancy and maximize the percentage and rate of seed germination (International Seed Testing Association 2009). This effect was attributed to a decrease in ABA concentration in dormant seeds or embryo sensitivity to ABA (Chien et al. 1998; Singh and Browning 1991). Also, an increase in GA concentration during cold stratification or an increase to GA sensitivity may induce seed germination in many dormant species (Karssen et al. 1989; Bewley and Black 1994). Brändel (2004) reported that

Bidens tripartite seed germination requirement for temperature fluctuation could be entirely substituted by cold stratification. Furthermore, previous research (Bewley and Black1994; Brändel 2004) have shown stratification is a temperature-dependent process. Bewley and Black (1994) reported that 15 °C was a threshold temperature of relief and induction of dormancy for barley, lettuce and wheat. This suggested an interaction among temperature, plant hormones and cold stratification existed in the regulation of seed dormancy and germination.

Leymus chinensis (Trin.) Tzvel. is a dominant perennial grass species in the eastern region of the Eurasian steppe, the northern and eastern parts of the People's Republic of Mongolia and the northern and northeast grasslands in China. The plant is an important forage grass with high yield and high palatability. It is also highly tolerant to arid environments, saline-alkali soils and low fertility (Liu et al. 2004; Xu and Zhou 2005). In recent decades, China has suffered severe degradation and desertification due to unsuitable human activities such as overgrazing. *Leymus chinensis* has been considered as one of the most promising grass species for grassland restoration (Liu and Han 2007). However, seed dormancy in *L. chinensis* has prevented many strategies from attaining expected ecological and economical goals.

Previous studies on seed dormancy and germination of *L. chinensis* have mainly focused on dormancy breaking methods(Yi 1994; He et al. 2010), ABA content in the caryopses(Yi et al. 1997) and mechanical resistance of different parts of the seed on seed germination (Ma et al. 2008; Zhang et al. 2006). Ma et al. (2010) found that *L. chinensis* seed germination has a strict requirement for fluctuating temperature. However, there was no research conducted to investigate the interaction effects of plant hormones and temperature on seed dormancy and germination.

In the present study, we aimed to investigate the role of ABA, GA, temperature, cold stratification and their interaction in regulating *L. chinensis* seed germination and dormancy.

Materials and methods

Seed collection

Leymus chinensis seeds for the present study were harvested in 2009 and provided by Jilin *L. chinensis* Propagation Center, Jilin Province, China. All seed samples were stored at 4 °C until used. Seed viability was determined according to the Rules for Forage Seed Testing (International Seed Testing Association 2009).

Seed germination response to temperature

Seeds were placed in temperature controlled chambers in the darkness either at constant temperature or fluctuating temperature (12 h/12 h). The six constant temperature treatments ranged from 5 to 30 °C with a 5 °C interval between temperatures. Fluctuating temperature regimes consisted of five treatments set up as follows: Treatment 1 had five fluctuating temperatures with a 5 °C interval, or amplitude, between temperatures (5/10–25/30 °C). Treatment 2 comprised four fluctuating temperatures with a 10 °C interval in amplitude (5/15–20/30 °C). Treatment 3 consisted of 3 fluctuating temperatures with a 15 °C interval in amplitude (5/20–15/30 °C). Treatment 4 comprised two fluctuating temperatures with a 20 °C interval in amplitude (5/25 and 10/30 °C). Treatment 5 had one fluctuating temperature with a 25 °C interval in amplitude (5/30 °C).

To test if the seed germination response to fluctuating temperature was controlled by the embryo or its enclosing materials, isolated embryos were used to test the germination at constant temperature (25 °C) and fluctuating temperature (20/30 °C) (12 h/12 h). The temperature combination was chosen for this test because 20/30 °C is the optimal temperature for seed germination (see results). Each embryo was isolated by immersing the seed in distilled water for 60 min, followed by removal of the embryo using a dissecting needle and Pasteur pipette under a dissecting microscope (100×).

Effect of plant growth regulators on seed germination

To detect the effects of plant hormones on seed germination response to temperature, seeds were incubated at either constant temperature (25 °C) or fluctuating temperature (20/30 °C, as described above) with 8 ml GA_3, GA_{4+7}, ABA, paclobutrazol(PA) or fluridone(FL) solution respectively. For all solutions, three different concentrations (10, 50, 200 μM) were used. Water was used as the control. Paclobutrazol (an inhibitor of GA biosynthesis) and fluridone (an inhibitor of ABA biosynthesis) solutions were prepared by dissolving the compounds in 0.1 % acetone until completely dissolved, followed by dilution with water (8 ml). Control experiments showed no acetone effect on germination.

To determine the interaction effect of plant hormones on seed dormancy and germination, mixed hormone solutions with three combinations of (1) randomly two hormones (all at 200 μM); (2) GA_3 plus PA(at 10, 50 and 200 μM) and (3) FL plus PA(at 10, 50 and 200 μM) were applied to test seed germination at optimal temperature(20/30 °C, 12 h/12 h, see Results). For each treatment, four replicates with 50 seeds each were used. All plant growth regulators used in this experiment were purchased from Sigma-Aldrich Trade Co., Ltd (Shanghai, China).

Stratification treatment

Seeds were placed onto two filter papers saturated with 8 ml distilled water or hormone solution (200 μM) and then transferred into the darkness for 1, 2, 4 and 8 weeks at 5 °C. The reason of 200 μM used in the stratification experiment is all hormones, except GA_3, showed significant effect on seed germination only at 200 μM (see Results). After stratification, the seeds were washed with distilled water and germinated at constant temperature (25 °C) or fluctuating temperature (20/30 °C). Each treatment comprised four replicates of 50 seeds each.

Germination test

Four replicates of 50 seeds for each of above treatments were placed in 11 cm diameter Petri dishes with two layers of filter paper saturated with 8 ml distilled water or hormone solution. The dishes were placed in an incubator for 28 days. To maintain the hormone solution at a relative constant level, water was added as necessary to supplement the evaporation loss. A seed was considered to have germinated when the radical extension was at least 2 mm.

The speed of germination, which expressed as germination index (GI), was calculated using the following formula(Wang et al. 2004):

$$GI = \Sigma\,(Gt/Tt)$$

where Gt is the number of seeds germinated on tth day, and Tt is the days of seed germination.

Statistical analysis

A one way ANOVA at a significance level $P < 0.05$ was performed using SPSS 15.0 software. Duncan's multiple range tests was used to compare means of germination percentage between treatments when significant differences were found. Germination percentage data were arcsine transformed before analysis.
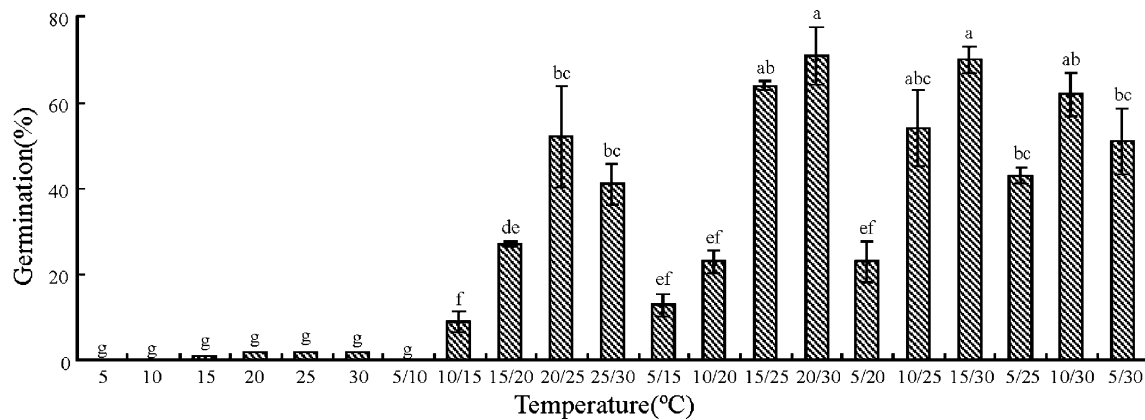
Results

Seed viability

Seed viability was 93 %.

Seed germination response to constant temperature and fluctuating temperature

As shown in Fig. 1, seed germination was less than 2 % at all constant temperatures, whereas fluctuating temperature significantly increased seed germination. Also, increased

Fig. 1 Effect of constant and fluctuating temperature on seed germination of *Leymus chinensis*. Different *letters* indicate significant differences between temperatures with Duncan's multiple range tests

amplitude (difference between the two temperatures) positively affected seed germination. The highest germination (71 %) was observed at 20/30 °C (Fig. 1).

Germination of isolated embryos germinated to 74 % at 25 °C, which was significantly higher than intact seeds. However, germination percentage and germination index of isolated embryos at 25 °C (74 %, 23) were significantly less than these embryos germinated at 20/30 °C (94 %, 55) (Fig. 2).

Role of GA and ABA in regulation of seed germination and dormancy response to temperature

Seed germination was not affected by GA_3 at 20/30 or 25 °C (Fig. 3a). In contrast, GA_{4+7} significantly increased seed germination depending on concentration (Fig. 3b). At the highest concentration (200 μM), GA_{4+7} increased seed germination from 71 to 88 % at 20/30 °C and from 2 to 21 % at 25 °C. Abscisic acid (ABA) showed no effect on seed germination at 20/30 °C at the lowest concentrations (10 and 50 μM). However, seed germination was significantly reduced to 4 % at the highest concentration (200 μM) (Fig. 3c). Paclobutrazol application significantly reduced seed germination at 20/30 °C with germination decreasing from 71 % (control) to 9 % (200 μM) with increased concentration (Fig. 3d). Fluridone application

significantly increased seed germination from 71 to 94 % at 20/30 °C, but showed less effect on seed germination at 25 °C (Fig. 3e).

Combinations of GA_3 plus ABA, GA_3 plus FL, and ABA plus FL showed no effect on seed dormancy and germination to temperature fluctuation, indicating that any ABA inhibitory effect was reversed by GA_3 and FL (Fig. 4a). Also, the FL dormancy release effect was reversed both by GA_3 and ABA. Combinations of PA plus GA_3 significantly reduced seed germination at 20/30 °C regardless of the hormones ratios (Fig. 4b). Combinations of PA plus FL reduced or did not affect seed germination depending on the ratio of the two hormones used (Fig. 4c). GA_3, PA and ABA application did not affect seed germination at 25 °C (Data not shown).

Role of stratification in regulating seed germination and dormancy response to temperature

The effect of cold stratification on seed dormancy and seed germination response to temperature varied with stratification time and hormone solution. Except for FL, which significantly increased seed germination, all other treatments showed no effect on seed germination at constant temperature (Fig. 5a). Stratification with FL showed no changes at fluctuating temperature regardless of the stratification

Fig. 2 Final germination (%) (**a**) and germination index (**b**) of intact seeds and isolated embryos of *Leymus chinensis* at constant (25 °C) and fluctuating temperature (20/30 °C). Different *letters* indicate significant differences between treatments with Duncan's multiple range tests
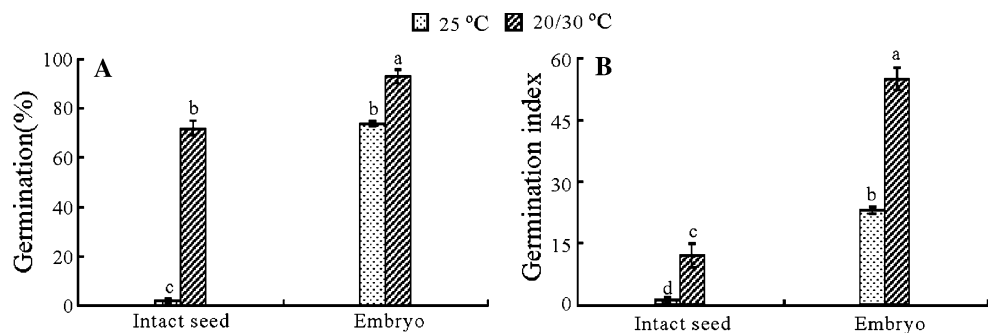
Fig. 3 Final germination (%) of *Leymus chinensis* seeds incubated in different plant hormone solutions at constant temperature (25 °C) and fluctuating temperature (20/30 °C). **a** GA₃, **b** GA₄₊₇, **c** ABA, **d** PA, **e** FL. *PA* paclobutrazol; *FL* fluridone. Different *letters* indicate significant differences between treatments within the same hormone solution with Duncan's multiple range tests
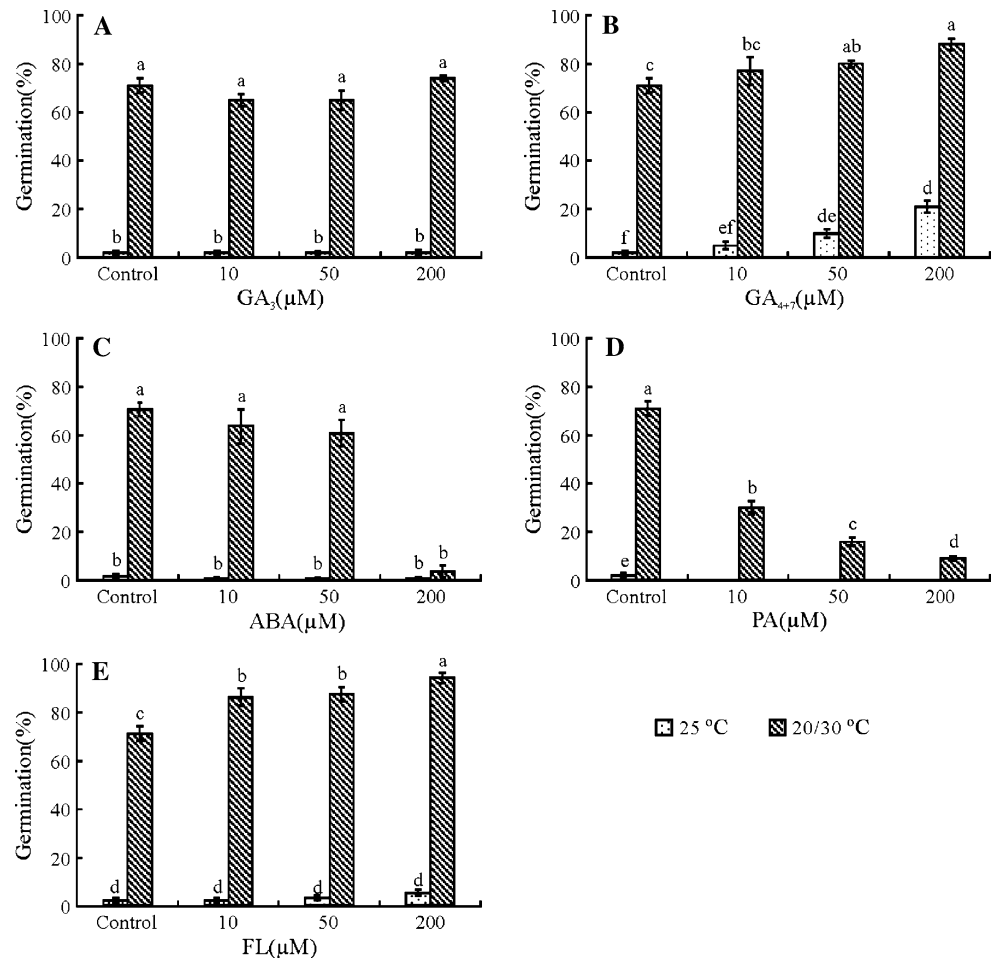
duration (Fig. 5a, b). After 8 weeks stratification in water, GA₃, and PA there was a significant decrease in seed germination although the size of the decrease varied (Fig. 5b).

Discussion

Seed germination and dormancy response to temperature

In the field, seeds are exposed to alternating temperatures that are more favorable for germination than constant temperatures (Baskin and Baskin 1998). Ma et al. (2008) showed that a fluctuating temperature regime was necessary for *L. chinensis* seed germination. Consistent with this, results of the present study showed that *L. chinensis* had minimal germination at all constant temperatures, whereas temperature amplitudes >10 °C fluctuation could significantly increase seed germination. This requirement maybe controlled by the embryo enclosing material as isolated embryos germinated to 74 % at 25 °C similar to germination of intact seeds at 20/30 °C. Furthermore, isolated embryos germinated to 94 % at 20/30 °C. Ma et al. (2008) and He et al.

(2010) reported that mechanical resistance of glumes and endosperm was the main cause for seed dormancy of *L. chinensis*. Two antagonistic forces (1) embryo growth potential, (2) mechanical resistance of embryo enclosing material determine whether a seed will germinate. In the present study, fluctuating temperature significantly increased the embryo growth potential of *L. chinensis*, with isolated embryos showing a significantly higher germination percentage and germination rate at 20/30 °C compared to 25 °C. This is consistent with Huarte and Benech-Arnold (2010) who showed that fluctuating temperature enhanced *Cynara cardunculus* seed embryo capacity to overcome physical constraint. However, the effect of temperature fluctuation on the reduction of the physical constraint of embryo enclosing material requires further research.

Role of ABA and GA in regulating seed germination and dormancy response to temperature

Several studies have investigated seed dormancy and germination regulation by ABA and GA₃ (Kucera et al. 2005; Chen et al. 2009). Huarte and Benech-Arnold (2010) found that exogenous GA₃ could entirely substitute for

Fig. 4 Final germination (%) of *Leymus chinensis* seeds incubated at fluctuating temperature (20/30 °C) with **a** combinations of hormones at 200 μM, **b** combinations of GA_3 and PA at different concentrations and **c** combinations of FL and PA at different concentrations. Concentrations of hormones (μM) in *brackets*. *FL* fluridone; *PA* paclobutrazol. Different *letters* indicate significant differences between treatments within each combination with Duncan's multiple range tests
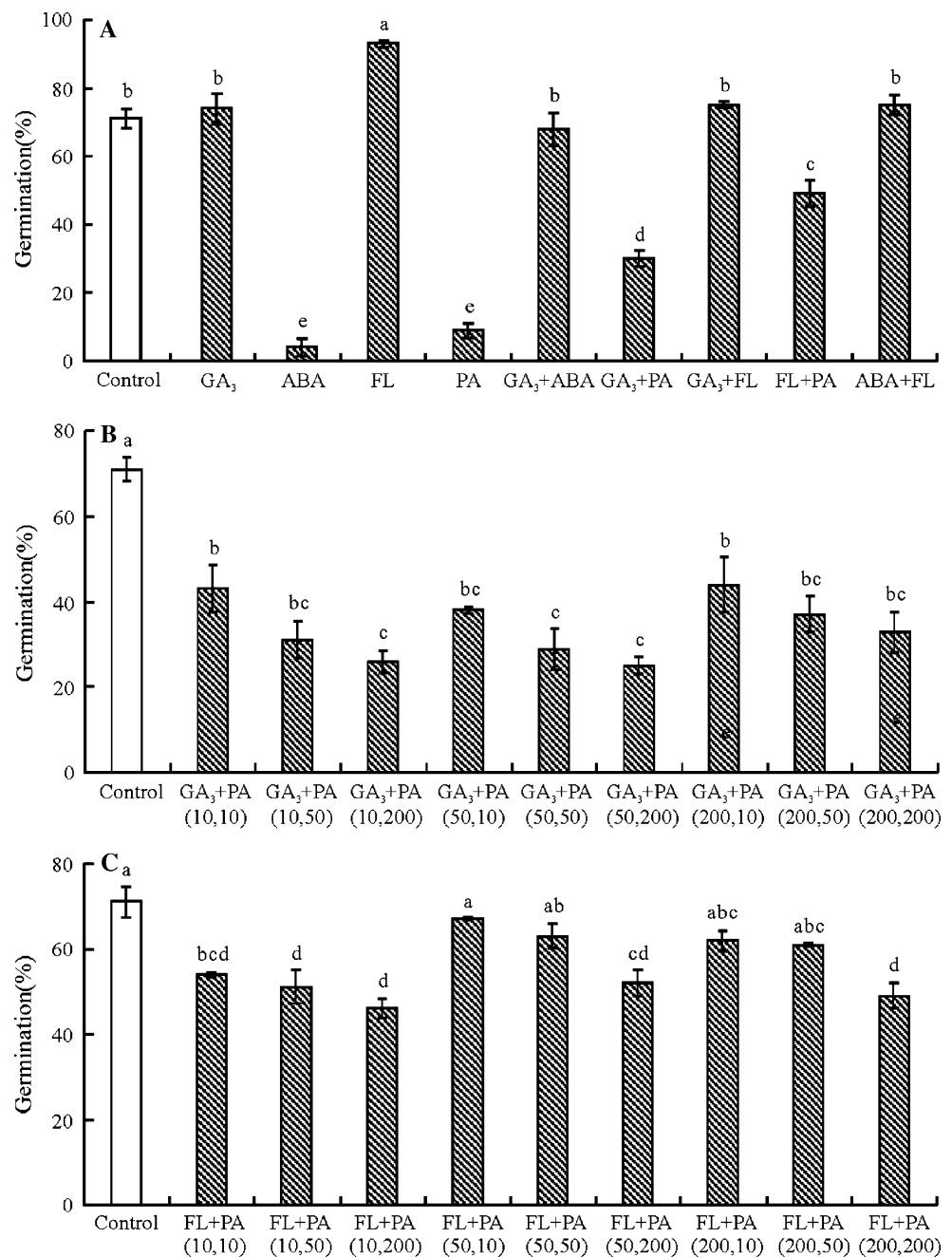
temperature fluctuation for *C. cardunculus* seed germination. This suggests that temperature fluctuation may increase GA biosynthesis during imbibition and increase embryo growth potential to overcome physical constraint. Consistent with this, GA_{4+7} but not GA_3 improved seed germination at 25 °C dependent upon concentration. GA_3 showed no effect on seed germination at fluctuating temperature (20/30 °C), whereas GA_{4+7} significantly enhanced seed germination and released seed dormancy. This may be due to the embryo enclosing structures (glumes, seed coat, endosperm) being less permeable to GA_3 than to GA_{4+7} (Takahashi et al. 1986;

Chen et al. 2007, 2009). This is consistent with He et al. (2010) and He (2010) who showed a semi-permeable layer existed in the seed coat of *L. chinensis*, and GA_3 application showed no effect on intact seeds but significantly improved seed germination when the seed coat was pierced. Moreover, PA application significantly reduced seed germination at 20/30 °C, suggesting that GA biosynthesis is necessary for non-dormant seed germination. This was further confirmed by combinations of PA plus GA_3 or FL treatments, which showed that the PA inhibitory effect could be partly reversed depending upon the concentration.

Fig. 5 Final germination (%) of *Leymus chinensis* seeds at **a** constant temperature (25 °C) and **b** fluctuating temperature (20/30 °C) after different periods of stratification in various hormone solutions. *PA* paclobutrazol; *FL* fluridone. Different *letters* indicate significant differences between treatments within same germination temperature with Duncan's multiple range tests
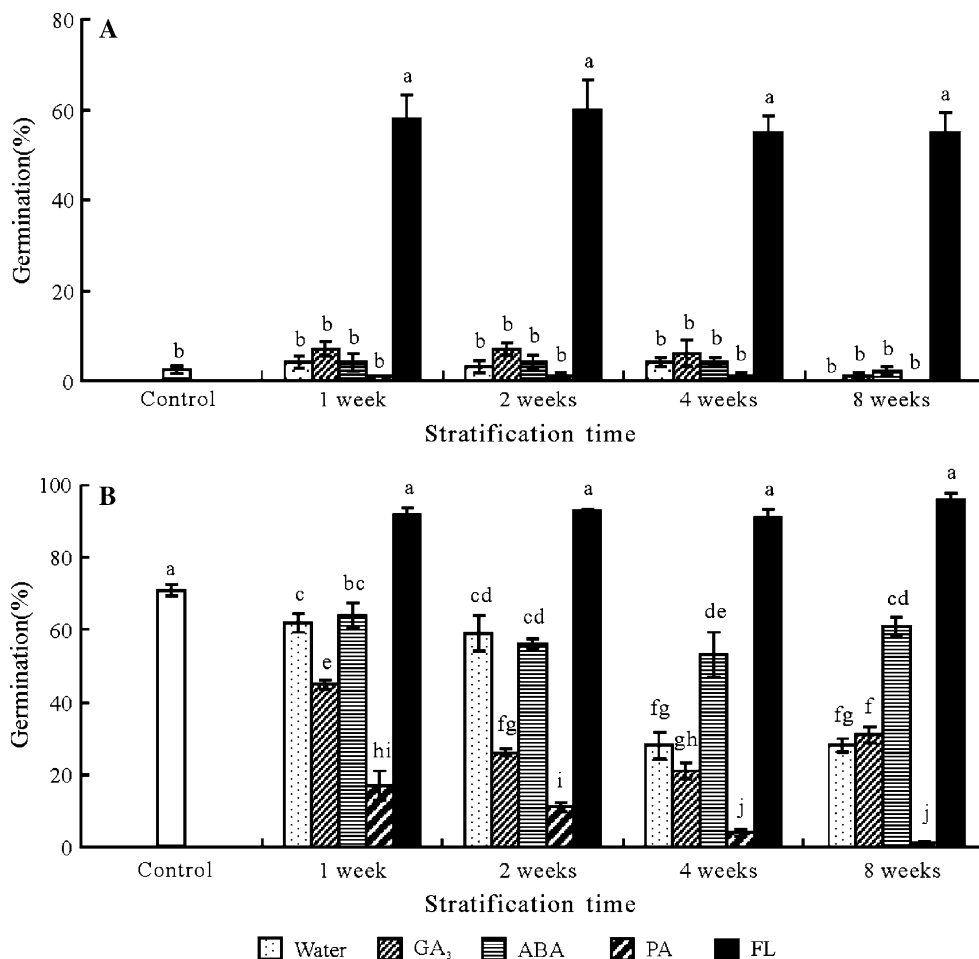
Many studies (Chen et al. 2009; Baskin and Baskin 2004) have shown that ABA is one of the most important inhibitors of seed germination. Huarte and Benech-Arnold (2010) reported that fluctuating temperature significantly reduced embryo sensitivity to ABA, and possible contributed to dormancy release. Results of the present study showed that an ABA inhibitor, fluridone, significantly increased seed germination at 20/30 °C, suggesting that ABA biosynthesis during imbibition play a critical role in seed dormancy maintenance (Baskin and Baskin 2004). This is consistent with Yi (1994) and Yi et al. (1997) who attributed the dormancy of *L. chinensis* seeds mainly to ABA in caryopses, glumes and endosperm. Moreover, fluridone increased germination could be completely reversed by exogenous ABA. Interestingly, fluridone increased germination could be reversed both by GA$_3$ and PA addition at fluctuating temperature. PA inhibits GA$_3$ biosynthesis and decrease non-dormant seed germination. However, GA$_3$ overcoming the FL effect is somewhat contradictory to the GA$_3$ promoting effect of germination on seed coat peircing (He et al. 2010). A possible explanation is that ABA and GAs share the initial steps of the terpenoid pathway for their biosynthesis in plants. Thus, the inhibition of ABA biosynthesis by fluridone might not only have decreased ABA content, but also increased GA content in the seed (Crozier et al. 2000).Thus, FL increased endogenous GA content that changed the sensitivity of the embryo to exogenous GA$_3$. Therefore, a relatively lower concentration of exogenous GA$_3$ could suppress seed germination. This is consistent with Ma et al. (2008) who reported that high exogenous GA$_3$ concentration decreased seed germination of *L. chinensis*.

Compared to 20/30 °C, inhibition of ABA biosynthesis only showed a small increase in germination at 25 °C. This implies that fluctuating temperature promotes seed germination but not through decreasing ABA concentration in the seed. This supports the idea that fluctuating temperature increased embryo growth potential maybe attributed to endogenous GA increasing without a concomitant decrease in ABA concentration. Also, the inhibition of ABA biosynthesis by fluridone, while increasing ABA concentration, may have also increased GA content in the seed, and thus increased seed germination at constant temperature as discussed above (Crozier et al. 2000).

Role of stratification in regulating seed germination and dormancy response to temperature

Cold stratification has been shown to be a useful tool to release seed dormancy in many species (Baskin and Baskin 1998). Gu et al. (2005) reported that low temperature could improve seed germination of *L. chinensis*. However, Fan et al. (2005) found that a 1 week pre-chill treatment did not affect *L. chinensis* seed germination. In contrast with these results, our study showed that the effect of cold stratification on seed germination varied with stratification time and hormone solution. More than 2 weeks cold stratification with water significantly reduced seed germination at 20/30 °C, implying cold stratification induced seed dormancy. This may be attributed to ABA concentration increasing during cold stratification, because this effect could be completely reversed by exogenous FL. Moreover, previous researches(Bewley and Black 1994; Brändel 2004) have shown that stratification treatment is a temperature-dependent process, e.g. Brändel (2004) showed that the minimum temperature for dormancy induction in *Bidens tripartite* was 7 °C. This implies stratification treatment release or induce seed dormancy mainly depends on stratification temperature used. This may, at least in part, explain various interpretations about the role of stratification treatment on seed germination (Gu et al. 2005; Fan et al. 2005).

Stratification in FL solution increased germination to 75 % at constant temperature, indicating that cold stratification with FL could entirely substitute for temperature fluctuation for germination. As discussed above, GA_{4+7} but not ABA enhanced seed germination at constant temperature. A possible explanation for this effect is that both ABA and GA biosynthesis are involved in cold stratification. Chen et al. (2007) reported that cold stratification increased GA concentration in *Prunus campanulata* seed, but ABA concentration remained high in the seed endocarp. Based on our results and previous, we suggest that cold stratification increases GA concentration in the seed, but also increases ABA concentration which induces seed into dormancy. Thus, inhibition of ABA biosynthesis during cold stratification plays a critical role in regulating the germination response to temperature. To better understand the underlying mechanisms, further work involving hormone concentration is necessary.

Conclusion

In brief, fluctuating temperature are required for optimum germination of *L.chinensis* seed, which was mainly attributed to GA biosynthesis during imbibitions. On the other hand, ABA is important for seed dormancy maintenance and induction but showed less effect on non-dormant seed germination response to temperature.

References

Baskin CC, Baskin JM (1998) Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14:1–16

Benech-Arnold RL, Steinbach HS, Kristof G, Sánchez RA (1995) Fluctuating temperatures have different effects on embryonic sensitivity to ABA in *Sorghum* varieties with contrasting pre-harvest susceptibility. J Exp Bot 46:711–717

Bewley JD, Black M (1994) Seeds: physiology of development and germination, 2nd edn. Plenum Press, New York

Bradford KJ, Nonogaki H (2007) Seed development, dormancy and germination. Blackwell, Oxford

Brändel M (2004) The role of temperature in the regulation of dormancy and germination of two related summer-annual mudflat species. Aquat Bot 79:15–32

Chen SY, Chien CT, Chung JD, Yang YS, Kuo SR (2007) Dormancy-break and germination in seeds of *Prunus campanulata* (Rosaceae): role of covering layers and changes in concentration of abscisic acid and gibberellins. Seed Sci Res 17:21–32

Chen SY, Chien CT, Baskin CC, Baskin JM (2009) Storage behavior and changes in concentrations of abscisic acid and gibberellins during dormancy break and germination in seeds of *Phellodendron amurense* var. *wilsonii*(Rutaceae). Tree Physiol 30:275–284

Chien CT, Kuo-Huang LL, Lin TP (1998) Changes in ultrastructure and abscisic acid level, and response to applied gibberellins in *Taxus mairei* seeds treated with warm and cold stratification. Ann Bot 81:41–47

Crozier A, Kamiya Y, Bishop G, Yokota T (2000) Biosynthesis of hormones and elicitor molecules. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, pp 850–929

Fan TE, Gao LW, Guo YL (2005) Effects of environmental conditions on seed germination of *Leymus chinensis*. Inner Mong Pratacultural 17(4):60–62 (in Chinese)

Fenner M, Thompson K (2005) The ecology of seeds. Cambridge University Press, Cambridge

Feurtado JA, Yang J, Ambrose SJ, Cutler AJ, Abrams SR, Kermode AR (2007) Disrupting abscisic acid homeostasis in western white pine (*Pinus monticola* Dougl. Ex D. Don) seeds induces dormancy termination and changes in abscisic acid catabolites. J Plant Growth Regul 26:46–54

Gu AL, Yi J, Holubowiecz R (2005) Effects of low temperature on seed germination of *Leymus chinensis* and *Pascopyrum simithii*. Grassl China 27:50–54 (in Chinese)

He XQ (2010) Seed coat permeability and location of semi-permeable layer in seeds of several grass species. PhD Thesis, Lanzhou University, Lanzhou

He XQ, Hu XW, Wang YR (2010) Study on seed dormancy mechanism and breaking technique of *Leymus chinensis*. Acta Bot Boreal 30(1):0120–0125

Huarte HR, Benech-Arnold RL (2010) Hormonal nature of seed responses to fluctuating temperatures in *Cynara cardunculus* (L.). Seed Sci Res 20:39–45

International Seed Testing Association (2009) International rules for seed testing. Seed Sci Technol 27 (Supplement)

Karssen CM, Zagorski S, Kepczynski J, Groot SPC (1989) Key role for endogenous gibberellins in the control of seed germination. Ann Bot 63:71–80

Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15:281–307

Liu GX, Han JG (2007) Seedling establishment of wild and cultivated *Leymus chinensis* (Trin.) Tzvel. under different seeding depths. J Arid Environ 72(3):279–284

Liu GS, Qi DM, Shu QY (2004) Seed germination characteristics in the perennial grass species *Leymus chinensis*. Seed Sci Technol 32:717–725

Ma HY, Liang ZW, Wang ZC (2008) Lemmas and endosperms significantly inhibited germination of *Leymus chinensis* (Trin.)Tzvel. (Poaceae). J Arid Environ 72(4):573–578

Ma HY, Liang ZW, Wu HT, Huang LH, Wang ZC (2010) Role of endogenous hormones, glumes, endosperm and temperature on germination of *Leymus chinensis* (Poaceae) seeds during development. J Plant Ecol 3(4):269–277

Singh Z, Browning G (1991) The role of ABA in the control of apple seed dormancy re-appraised by combined gas chromatography mass spectrometry. J Exp Bot 42:269–275

Takahashi N, Yamaguchi I, Yamance H (1986) Gibberellins. In: Takahashi N (ed) Chemistry of plant hormones. CRC Press, Florida, pp 57–151

Wang YR, Zhang JQ, Liu HX, Hu XW (2004). Physiological and ecological responses of lucerne and milkvetch seed to PEG priming. Acta Ecol Sinica 24:402–408 (in Chinese)

Xu ZZ, Zhou GS (2005) Effects of water stress and nocturnal temperature on carbon allocation in the perennial grass, *Leymus chinensis*. Physiol Plant 123:272–280

Yi J (1994) Studies on dormancy of *Leymus chinense* seeds. Grassl China 16:1–6 (in Chinese)

Yi J, Li QF, Tian RH (1997) Seed dormancy and hormone control of germination in *Leymus Hochst*. Acta Agrestial Sinica 5:93–99 (in Chinese)

Yoshioka T, Endo T, Satoh S (1998) Restoration of seed germination at supraoptimal temperatures by fluridone, an inhibitor of abscisic acid biosynthesis. Plant Cell Physiol 39(3):307–312

Zhang WD, Bi JJ, Ning TY, Liu XL, He MR (2006) Effects of temperature, light and other treatments on seed germination of *Leymus chinensis*. Can J Plant Sci 86(1):143–148