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NaOH Scarification and Stratification Improve Germination of *Iris lactea* var. *chinensis* Seed

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***Abstract. Iris lactea* seed is characterized mainly by physiological dormancy. Two experiments were conducted to investigate the effect of NaOH treatment and stratification on *Iris* seed germination. In Experiment 1, seeds were treated with 14.38 M NaOH for 0 to 28 hours. In Experiment 2, NaOH treated and nontreated seeds were stratified under 7 °C and moistened condition for 0 to 40 days. As results, NaOH treatment for 20 hours effectively removed seedcoat and improved germination percentage from 0% to 56% compared to control (0 hours). However, germination percentage started to decrease after 20 hours. Stratification for 40 days further improved germination percentage of NaOH treated seeds to >80%, but did not affected seeds without NaOH treatment. Results demonstrate that combination of NaOH treatment and stratification is an effective practice to break *Iris* seed dormancy and improve germination percentage.**

Iris lactea Pall. var. *chinensis* (Fisch.) Koidz., a native herbaceous perennial, is preferably used in gardening and vegetation regeneration for its beautiful and easy planting traits and high tolerance of drought, salt, cold and trampling (Liu et al., 1998; Xu, 1989; Xu et al., 2003). Its seed germination pattern is similar to other Iris species and hybrids which dormancy persists for several months (Randolph and Cox, 1943; Stoltz, 1968). By examining structure and measuring mechanical strength of seedcoat in the micropylar area of the Iris seed, Blumenthal et al. (1986) found that the main cause for *I. lorteti* dormancy was the mechanical resistance of the integument. Excised Iris embryos grew *in vitro* showed the embryos' absorbing ability of water was low (Stoltz, 1971), and the growth potential increased while growth regulators were added in agar nutrient medium (Stoltz, 1977). It was confirmed that the dormancy was mainly caused by physiological dormancy that the embryo was low growth potential and could not overcome mechanical restrain of covering layers (Baskin and Baskin, 2003). Blumenthal et al. (1986) and Wu et al. (1998) also found that the outer integument of *I. lorteti* and *I. confuse* seeds contained a compound toxic to the germinated embryo. A method to improve seed germination was used to cut off the integument at the micropylar area (Blumenthal et al., 1986; Lee et al., 2002; Xu et al., 1990), but seed was rigid and the micropylar area was not easy to recognize. Therefore, a better method of improving *Iris* seed germination percentage is needed. The objective of the present study was to investigate the effectiveness of a combination of NaOH treatment and stratification on seed germination of *Iris lactea*.

Materials and Methods

Plant material. Iris lactea seeds were collected from wild stands growing in the suburb of Beijing in September 2003. Capsules were air dried for 10 d to release seeds and seeds were stored in paper bags at room temperature (about 20 °C) for 10 d before experiment.

Experiment 1: Effect of NaOH. 150 g Seeds were immersed in 32 °C 500 mL 14.38 M NaOH solution, placed in a growth chamber at a constant temperature of 32 °C for 28 h, and stirred every 30 min. During the period, about 500 seeds were taken out every 4 h, washed under tap water for 10 min with a little force to peel off the decayed seedcoat, and washed in sterile water for another 5 min. Seeds were then dried in air and surface sterilized by immersion for 30 min in a solution containing 2.94 M H_2O_2, 10^{-4} M CuSO4 and 5.1×10^{-4} M Na ascorbate and then rinsed five times with a large volume of sterile distilled water (Blumenthal et al., 1986) and then transferred to germinate in growth chamber.

Experiment 2: Effect of NaOH and stratification. Seeds were immersed in 14.38 M NaOH solution for 20 h and then washed, dried and surface sterilized as in Expt. 1. The treated seeds were sown in 12-cm petri dishes containing 100g quartz sand (about 0.70 mm in size) and 26 mL sterile water. Petri dishes were kept in 7 °C for 0 (control), 10, 20, 30, and 40 d and then transferred to germinate in growth chamber. *I. lactea* seeds without NaOH treatment were surface sterilized and sown in petri dishes and put under 7 °C for 0, 10, 20, 30 and 40 d in the same way and transferred to germinate in above growth chamber.

Germination methods. Protocols by Holloway (1987) and Morgan (1990) were followed. Seeds were sown in 12-cm petri dishes that contained 100g quartz sand and 30 mL sterile water. Petri dishes were put in the growth chamber at 30 °C for 8 h (day) and 20 °C for 16 h (night) in darkness. Each treatment had 4 replications with 50 seeds per replication. The petri dish was opened to add 2 to 3 mL sterile water to keep proper moisture every 7 d. On the day 28, the number of germinated seeds (radicle >2 mm) was recorded.

Statistical analysis. Collected data on germination were analyzed with ANOVA. Multiple comparison was done when treatment effects were significant. Significance was declared at $P < 0.05$.

Results and Discussion

Experiment 1. As the time of seed immersion in NaOH solution increased, the seedcoat was gradually decayed. After 20 h of treatment, the seedcoat was almost decayed and the endosperm was exposed (Fig. 1). Treatment had a significant effect on germination within 12 h (Fig. 2). The germination percentage reached the maximum (56%) at 20 h and started to decline afterward. Blumenthal et al. (1986) found the resistance of the integument at the micropylar area was the main factor preventing germination in *Iris* species. Xu et al. (1990) and Lee et al. (2002) showed that removing of testa and excising the endosperm of micropyle area in *I. lactea* and *I. sanguinea* promoted seed germination to >80% at 20 d after sowing. All these demonstrated that seedcoat played an important role in *Iris* seed dormancy. Using NaOH solution to break seed dormancy was reported in the seed of *Zoysia japonica* (Han et al., 1996); and 1% (m/m) KOH used in the seed of *Chrysanthemum coronarium* L. (Chiang and Park, 1994). Scanning by the electron microscopy revealed that the wax on the cuticular surface of the seedcoat was reduced in thickness. In preparing experiment, seeds were conglutinated with each other and seedcoat did not decay when put into the concentrated H_2SO_4 (98% concentration). However, when put into the NaOH solution, the germination

Fig. 1. Seed of *Iris lactea* var. *chinensis* (the left was intact seed while the right is treated with 14.38 M NaOH for 20 h.)

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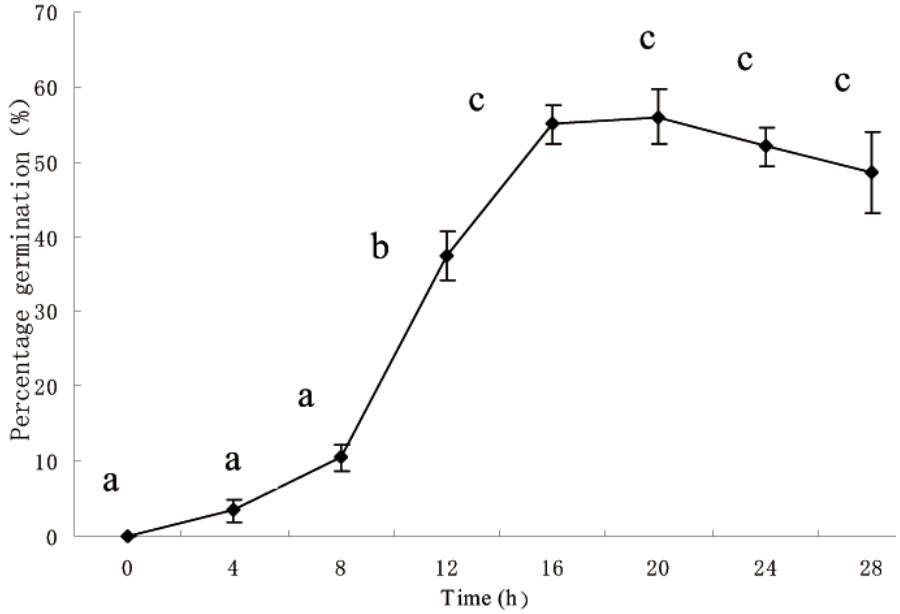

Fig. 2. Percentage germination of *Iris lactea* seeds after different time (h) of immersion in 14.38 M NaOH solution. Germination was 8 h at 30 °C (day) and 16 h at 20 °C (night) in darkness for 28 d. Mean percentage germination was significantly different shown by different letters ($P < 0.05$). Error bars are SE.

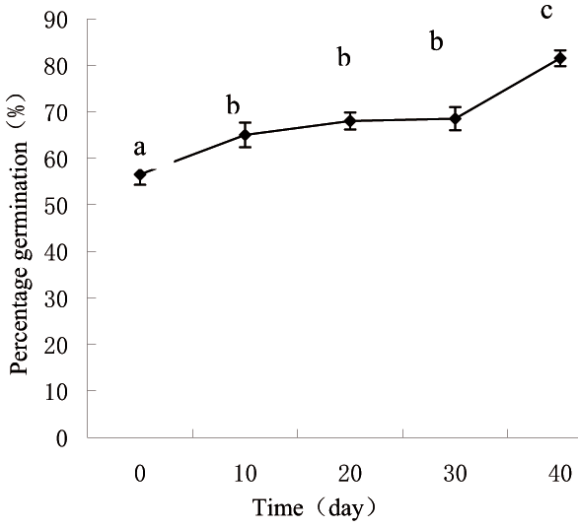

Fig. 3. Percentage germination of 14.38 M NaOH treated *Iris lactea* seeds after different d of stratification. Germination was 8 h at 30 °C (day) and 16 h at 20 °C (night) in darkness for 28 d. Mean percentage germination was significantly different shown by different letters ($P < 0.05$). Error bars are SE.

percentage decreased from 56.0% at 20 h to 48.5% at 28 h, possibly because the embryos were injuried by NaOH after seedcoat and endosperm decayed.

Expt. 2. As the stratification time increased from 0 to 40 d, germination percentage of seeds treated 20 h with NaOH dramatically increased from 58.5% to 81.5% (Fig. 3). Holloway (1987) showed that, when seeds of Alaska Iris were stratified in peat at 4 °C for 125 d, germination increased from 0% to 30%. Wees (2004) found the storing *I. versicolor* seeds in wet paper towels at 4 to 5 °C for 4 weeks increased germination percentage from 9% to 58% as compared to control. It was showed that stratification was an effective method in *Iris* seeds to improve germination via the growth potential of the embryo increased like seed of *Syringa reflexa* (Junttila,1973), or the pericarp (or endosperm) structure disintegrated like seed of *Camptotheca acuminate (*Chen et al., 2001). When the seedcoat or pericarp was decayed or removed, the stratification time of *Acer saccharum* (Web, 1969) and *Camptotheca acuminata* (Chen et al., 2001) could be reduced. This may be due to both reduced mechanical inhibition and improved gas and water exchange, making seed physiological mature quickly and germinate easily, as seed of *Syringa reflexa* (Junttila, 1973). In this experiment, Seeds of *I. lactea* without NaOH treatment failed to germinate in 40 d of stratification. Abdalla and Mckelvie (1980) reported that *I. mellita* seeds failed to germinate when seeds were stratified at 1.5 °C for 4 weeks. It seemed that a chilling period of 40 d for *I. lactea* or 4 weeks for *I. mellita* was not long enough to overcome dormancy, and more days could be applicable for dormancy broken as practised by Holloway (1987).

In conclusion, treatment of 14.38 M NaOH for 20 h could effectively remove the seedcoat of *Iris lactea* and improve seed germination percentage. Stratification of the NaOH treated seeds at 7 °C and under moistened condition for 40 d could further improve seed germination percentage to >80%.

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