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Research Note

Effect of prechilling and exogenous gibberellin on seed germination of *Primulina eburnea*: a calcium-rich vegetable

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Abstract

Calcium-rich food and calcium supplements are expensive, especially for people in developing countries. *Primulina eburnea* is a calcium-rich vegetable with a high soluble calcium content in its leaves. In this study, we investigated the effects of prechilling and exogenous gibberellin on *P. eburnea* seed germination. Our results showed that both prechilling time and exogenous gibberellin affect *P. eburnea* seed germination. Three days prechilling increased germination percentage by 20.7-40.6%; mean germination time and time to first germination were reduced, indicating faster germination. A longer cold treatment enhanced the germination percentage but delayed the germination time. Prechilled *P. eburnea* seeds germinated 17.3-40.6% more when they were treated with GA_3, but high GA_3 did not shorten the germination time. Combination of three days prechilling and 50 ppm GA_3 is the optimal treatment for *P. eburnea* seed germination. This research provides a powerful tool to accelerate breeding and cultivating this calcium-rich vegetable.

Keywords: endemic, germination, gibberellic acid, stratification

Experimental and discussion

Calcium is an important mineral and one of the most abundant minerals in the human body. The National Institute of Health (USA) recommends an optimal calcium intake of more than 1000 mg per day for an adult (Glade *et al*., 1997). There are many countries in which calcium intake is suboptimal, especially in most Asia and African countries (< 400 mg per day) (Balk *et al*., 2017). Long-term insufficient dietary calcium can decrease bone mass and thus results in skeletal diseases such as fractures or osteoporosis

(Weaver *et al*., 2016; Yao *et al*., 2020). In western countries, the major source of dietary calcium includes milk or milk products, yogurt, and calcium supplements (Haas, 2015). However, the high cost of these food sources means they are not feasible for many people, especially in developing countries. Calcium-rich vegetables are also considered to be alternative candidate sources for dietary calcium. However, low calcium content and poor bioavailability reduce the calcium uptake efficiency in the body. Thus, it is worth trying to domesticate and develop cheap but efficient calcium-rich vegetables.

Primulina is the largest genus of the Old World Gesneriaceae and is widely distributed throughout the lowland karst regions of southern China (Wang *et al*., 2011; Weber *et al*., 2011). Of this genus, *Primulina eburnea* (Hance) Yin Z. Wang has a great capacity for growth and survival in different environments and has the widest distribution range (Wang *et al*., 2017). In addition, *P. eburnea* contains calcium in its leaves, stored as pectate calcium and soluble calcium (Qi *et al*., 2013). This species is a perennially evergreen herb and produces a large amount of flowers and seeds (Zhang *et al*., 2017). These make *P. eburnea* an ideal candidate for calcium-rich vegetable development.

Seed germination is a crucial step in the development and breeding of crops. Seed germination and dormancy often control plants growth in harsh environments such as arid or salinity regions (Alouani and Bani-Aameur, 2004; Greenwood and MacFarlane, 2006). Germination is influenced by a number of factors, including hormone balance, temperature, water and biotic and/or abiotic stresses (Gorai and Neffati, 2007; Tanaka-Oda *et al*., 2009; Ribeiro and Costa, 2015). For vegetables, rapid seed germination will shorten the lifecycle and be helpful for the cultivation. Low temperature stratification (prechilling) and exogenous gibberellin treatment has been widely used to improve germination in many crops (e.g. Chen *et al*., 2021; Cha *et al*., 2022; Negishi and Ito, 2022). However, no research has been reported on *P. eburnea* seed germination. The present study aimed to investigate the effect of prechilling and exogenous gibberellin on *P. eburnea* seed germination. This study was designed to improve our understanding of factors that could help shorten the process of calcium-rich vegetable cultivation.

P. eburnea seedlings were collected manually from a wild population (25°41'45.7" N, 112°52'31.6" E and elevation 372 m a.s.l) in November 2021 in a karst region, a village in north Nanling Mountains of China. The seedlings were cultivated in the greenhouse at Nanchang Research Center of Lushan Botanical Garden (LSBG). In May 2022, an F_1 family including 15 progeny was generated from selfing single plants and from crosses between different individuals. Our previous observation found that seeds from either selfed or crossed flowers germinate at least two weeks after sowing. Here, we mixed seeds from all progeny evenly (figure 1) and used them for the subsequent research.

We adopted a two-factor randomised complete block design with three replicates. The factors were gibberellin (0, 50, 100, 200 mg L^{-1} GA_3 water solutions) and prechilling (0, 3, 6, 9 days). For each replicate of each treatment, 50 uniform and healthy seeds were selected and cultivated in 90 mm-diameter Petri dishes. A double layer of filter paper infused with 5 ml treatment solution was placed in each Petri dish. Petri dishes were then covered with lids and placed at 4°C in dark for stratification. After the stratification treatment, Petri dishes were moved to germinators with a 10 hour-light/14 hour-darkness diurnal cycle, constant temperature 25°C and humidity 70%. Seeds were considered

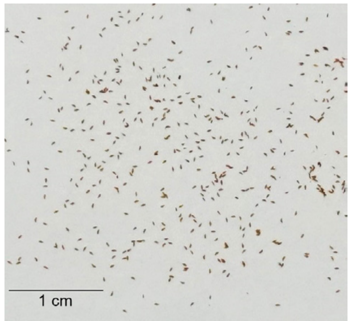

Figure 1. *Primulina eburnea* seeds.

to be germinated with the emergence of the radicle. Germinated seeds were counted and removed daily for 30 days. Three characteristics of germination were determined: germination percentage (GP), first germination day (delay of germination) corresponding to the days between sowing and the day of the first germination (FGD) and the mean germination time (MGT) (Gorai and Neffati, 2007). After transformation, data were analysed by two-way ANOVA. The Duncan test was performed for multiple comparisons to determine whether differences between individual treatments were significant ($P <$ 0.05).

Germination improved the final germination of *P. eburnea* seeds (figure 2A). There was 42.7% germination of seeds that were not cold-treated or soaked in GA_3. Seeds germinated the most (90.7%) when they were prechilled for six days and incubated in 200 ppm GA_3. When seeds were pretreated with cold, the germination percentages were statistically similar to each other (81.3-90.7%) for seeds incubated in 50, 100 or 200 ppm GA_3 $P > 0.05$. This indicates that gibberellin concentration did not affect germination percentage significantly for prechilled seeds. For seeds without prechilling treatment, 200 ppm GA_3 even inhibits the germination compared to the lower concentration GA_3.

For many species in the temperate zone, seeds with intermediate physiological dormancy require a 12-16 weeks moist-cold stratification for germination (Baskin and Baskin, 2022). It looks different in *P. eburnean*: the seeds germinated faster after prechilling for only three days compared with other treatments (figure 2B). Seeds prechilled for six or nine days germinated later than the untreated seeds. Similar to germination percentage, the mean germination time (MGT) was also influenced by both prechilling time and gibberellin concentration. Indeed, the best MGT values (11.07 days) were obtained when the seeds were prechilled for three days and incubated in 50 ppm GA_3, even though the differences among various GA_3 treatments were not significant ($P > 0.05$) when the seeds were prechilled for three days (figure 2B). This shortened the germination time by 3.9 days (26.2%) compared to the untreated seeds.

The results of first germination day analysis showed that short cold-treated seeds germinated faster than those incubated in cold condition for a longer time (6 or 9 days) or without any prechilling treatment (figure 2C). Long cold treatment delayed germination.

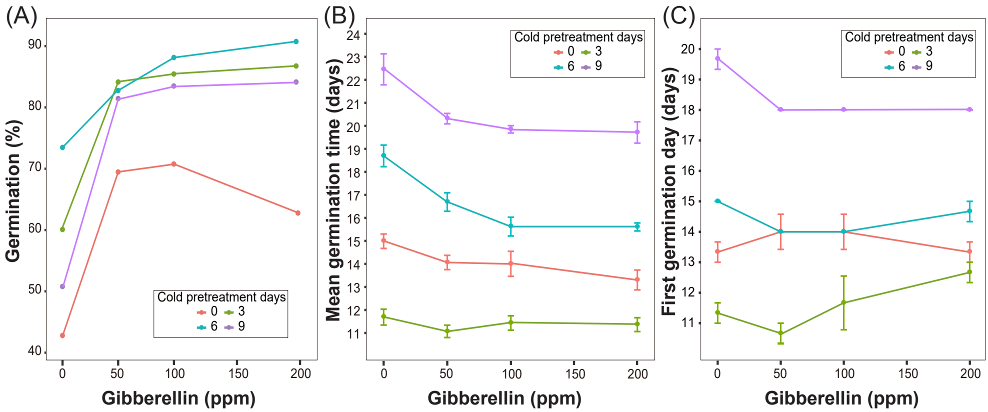

Figure 2. Germination percentage (**A**), mean germination time (**B**), and first germination day (FGD) (**C**) of *Primulina eburnea.*

This trend is consistent with the MGT analysis. For the seeds prechilled for three days, a low concentration of GA_3 (50 ppm) accelerated germination. In general, the combination of three days prechilling and incubating in 50 ppm GA_3 is the most efficient and economic method to promote and accelerate the germination of *P. eburnea* seeds based on this study.

Seed germination for *Achillea erba-rotta* subsp. m*oschata* (Wulfen) I. Richardson, which also grows in a harsh wild environment, was improved by 50, 75 and 100 ppm GA_3 (Aiello *et al*., 2017). In the semi-domesticated crop, *Rheum khorasanicum* B. Baradaran & A. Jafari, seeds germinated the most when they were soaked in 500 ppm GA_3 (Darrudi *et al*., 2015), while *Echinacea angustifolia* DC. seed germination percentage was not sensitive to GA_3 (Macchia *et al*., 2001). This indicates that the optimum concentration of GA_3 for seed germination is different among species. These differences may depend on the level and type of dormancy (Hamdaoui *et al*., 2021; Tippins, 2021). In addition to GA_3, prechilling treatment has also been demonstrated to be an efficient method for increasing seed germination in some species (reviewed by Balouchi and Sanavy, 2006). Low temperature pretreatments accelerates *P. eburnea* seed germination (figure 2B, C). These indicated that *P. eburnea* seeds dormancy is the non-deep type.

In conclusion, both low temperature prechilling and exogenous gibberellin affect *P. eburnea* seeds germination rate and speed. The combination between a suitable prechilling period (three days) and an effective level of GA_3 (50 ppm) would considerably enhance *P. eburnea* seed germination.

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