



The establishment of chilling model for rhizome bud dormancy releasing in *Polygonatum kingianum*

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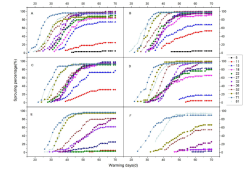
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Abstract

Polygonatum kingianum Collett & Hemsl. is an important traditional Chinese medicine [1]. However, several factors can inhibit the production of *P. kingianum* following seed germination. One of these issues derives from the stages of early rhizome development, wherein the young germinated seed quickly develops a small rhizome that passes into a prolonged stage of bud dormancy, requiring a sustained period of cold for the initiation of additional growth and development.

The current protocol is conducted to explore specific chill models for rhizome bud dormancy break in *Polygonatum kingianum*. In this study, rhizome buds were set at various chilling temperatures for different durations and then transferred to warm conditions for germination and subsequent evaluation of the temperature efficiency and chill requirements.

Guidelines

1.For each treatment temperature (-2, 0, 2, 4, 6, 8, 10, 12, 14°C), rhizome bud was exposed for 5, 11, 15, 18, 22, 27, 32, 36, 52, 61, 71, or 81 days in the dark condition. After cold treatment, the rhizome buds were moved to 25°C condition for sprouting.

2.To verify the utility of the chilling models under more environmentally realistic variability, forty rhizome buds were placed in a controlled environment with a temperature increase from 0 to 10°C at the increment of 1°C per day, and the increased temperature was maintained for 88 days. One hundred and twenty rhizome buds were moved to 25°C for sprouting.

Before start

To obtain the rhizome bud, seeds were cultivated in a dark growth chamber at 25°C for 50 days and watered every two days. All rhizome buds used from that point forward which had elongated to 3-6 mm and entered the dormant state were chosen for this protocol.

- 1 Prepare a seedling tray with substrate of peat soil: vermiculite =3:1
- 2 Twenty rhizome buds were seeding the seedling tray
- 3 The seedling tray was placed in controlled temperature environment (-2, 0, 2, 4, 6, 8, 10, 12, 14) and for each temperature, the rhizome buds were exposed for 5, 11, 15, 18, 22, 27, 32, 36, 52, 61, 71 and 81 days in the dark condition and watered every two days respectively.
- 4 After each cold treatment, the rhizome buds were moved to 25°C condition for sprouting with humidity 60%, light intensity 15000Lx and a light:dark photoperiod of 14:10 h. The sprouting percentage under each treatment group was recorded daily for 70 days.
- 5 The chill unit, which can describe the efficiency for each cold temperature on releasing bud dormancy, was calculated based on the average slope of all the chilling-response curves (**Fig 1**) for each chilling temperature
- 6 We used the chill unit for each temperature relative to the chill unit for 0°C, and then, the equation for the relationship between temperature and chilling unit was fitted. The resulting model was named "CU_{kingianum}".
- 7 We define that the chilling accumulation for *P. kingianum* rhizome bud in an hour at a certain temperature is equal to the chill unit for that temperature that we get from the above
- 8 we substituted chill unit into all of the above chilling treatments to obtain the chilling accumulation under each treatment, and then the function between chilling accumulation and sprouting average was fitted. This model was named "CAS_{kingianum}"
- 9 To verify the utility of the chilling models under more environmentally realistic variability, forty rhizome buds, which were seeded in a seedling tray with substrate (peat soil: vermiculite =3:1) and keeping the sowing depth same, were placed in a controlled environment with a temperature increase from 0 to 10°C at the increment of 1°C per day, and the increased temperature was maintained for 88 days.
- 10 One hundred and twenty rhizome buds were moved to 25°C for sprouting with humidity 60%, light intensity 15000 Lx and a light:dark photoperiod of 14:10 h every 11 days, and their sprouting percentage was checked daily for 70 days. The observed values were compared with the predicted sprouting percentages drawn from the above "CAS_{kingianum}" model.