Effects of Temperature, Melatonin and Gibberellin on Kiwi Seed Germination

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Abstract. To study the influence of temperature and growth regulating substances on seed germination, kiwi seeds stored at 4℃ refrigerated for 55 days were used as experimental materials. The treatments were set as below: (1) poikilothermal (25℃ 8h/4℃ 16h) or constant temperature (25℃ 24h) treatment; (2) treated with melatonin and gibberellin under constant temperature; (3) treated with melatonin and gibberellin under poikilothermal. The germination rate and germination potential of the treated seeds were analyzed statistically. The results showed that the germination rate of seeds could be increased by poikilothermal treatment, especially for the variety MW2, and the germination rate reached to 86.86%. Under constant temperature, gibberellin had a significant effect on the germination of kiwi seeds, while melatonin had no effect. Under the condition of poikilothermal, gibberellin and melatonin treatment alone can obviously promote the germination of kiwi seeds, while the application of gibberellin and melatonin at the same time can promote the germination of kiwi seeds, but there was no synergistic effect.

1. Introduction

Mature kiwi seeds need cold storage to break down dormancy before germination. Melatonin (MT) is a small molecule of indoles, chemically known as n-acetyl-5-methoxytryptamine. MT plays an important role in plant growth and reproduction. Studies have shown that melatonin content in various organs of plants generally conforms to the following rules: seed > leaf > root > flower > fruit [1]. In addition, the content of melatonin in lupine leaves was expressed as follows: leaf tip > leaf middle > leaf base [2]. The distribution gradient of melatonin is very similar to that of auxin, indicating that these two indoleamine compounds may have similar or synergistic physiological functions in plants [3], and the regulation effect of auxin IAA on seeds is as follows: low concentration promotes seed germination, while high concentration inhibits seed germination. Therefore, it is speculated that MT can promote seed germination at a certain concentration. Posmyk et al. found that the germination rate of red cabbage seeds was higher than that of the control group. When cucumber seeds were treated with water and osmotic initiation, adding melatonin (25 ~ 100mol/L) could significantly improve the germination rate.

To date, there are few studies on the effect of melatonin on kiwi seed germination. Therefore, in this experiment, kiwi seeds were treated with different concentrations of melatonin and gibberellin in order to study the effect of melatonin on the germination of kiwi seeds.
2. Materials and methods

2.1. Material preparation and treatment
About 10,000 seeds of kiwi MW1 and MW2 were collected and soaked with 5% sodium hypochlorite solution for 5 min for disinfection, then cleaned by distilled water for several times. After placed in petri dishes (15 cm in diameter) putted two layers of filter paper at 4℃ refrigerator for 55 days, then treated as below: (1) temperature treatment, the seeds of two varieties MW1 and MW2 were placed in constant temperature at 25℃ or poikilothermal treatment at 25℃ 8h/4℃ 16h for 2 weeks; (2) Under 25℃ condition, seeds of MW1 were treated with different growth regulating substance, 800mg/L GA, 50μmol/L MT, 800mg/L GA + 50μmol/L MT and water as control; (3) Under poikilothermal condition at 25℃ 8h/4℃ 16h, seeds of MW1 were treated with different growth regulating substance, 800mg/L GA, 50μmol/L MT, 800mg/L GA + 50μmol/L MT and water as control. During treatment, germination rate (GR) and germination potential were observed daily.

2.2. Determination of germination rate (GR) and germination potential (GP) of seeds
The n is the number of seeds that germinate normally, m is the number of seeds when the number of seeds germinated reaches the highest peak every day during the germination process, and N is the number of seeds were sown.

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GR (%) = \left(\frac{n}{N}\right) \times 100\% \quad GP (%) = \left(\frac{m}{N}\right) \times 100\%
\]

2.3. Data analysis
Using the software Excel2016 to calculate the test data and draw a diagram to calculate the germination rate and germination potential of the seeds processed.

3. Results and discussion

3.1. Effects of temperature treatment on the germination in kiwi seeds
After breaking dormancy by storing at 4℃ for almost 2 months, neither MW1 nor MW2 seeds didn't germinate when cultured at constant temperature 25℃. While under poikilothermal condition, the GR of MW1 was increased to 15.36%, the GP was increased to 9.29%; The GR of MW2 was increased to 86.85%, and the GP was increased to 80.25% (Table 1). The result proved that poikilothermal treatment can promote seed germination and has more obvious effect on MW2 seeds.

| Varieties | The temperature | Germination rate | Germination potential |
|-----------|-----------------|-----------------|-----------------------|
| MW1       | 25℃             | 0               | 0                     |
|           | poikilothermal  | 15.36           | 9.29                  |
| MW2       | 25℃             | 0               | 0                     |
|           | poikilothermal  | 86.85           | 80.25                 |

3.2. Effects of MT and GA on kiwi seed germination under constant temperature
Under constant temperature at 25℃, the seeds in CK group (treated with water) and MT group treated with 50μmol/L MT solution alone did not germinate, and failed to improve the GR and GP of kiwi seeds MW1. While when treated with GA solution at 800mg/L, the GR and GP of MW1 seeds increased to 10.96% and 10.96% respectively. In addition, when treated with 800mg/L GA solution and 50μmol/L MT, some MW1 seeds began to germinate, but the GR of the seeds was low, and the GR and GP of MW1 seeds increased to 10.83% and 9.83% respectively. (Table 2). The result suggesting that under the constant temperature, GA can promote seed germination, while MT does not play a role in promoting seed germination.
Table 2. Effects of hormone treatment under constant temperature.

| Varieties | The temperature | The treatment       | Germination rate | Germination potential |
|-----------|-----------------|---------------------|------------------|-----------------------|
| MW1       | 25℃ water       |                     | 0                | 0                     |
| MW1       | 25℃ 800mg/L GA  | 10.96               | 10.96            |
| MW1       | 25℃ 50μmol/L MT | 0                   | 0                |
| MW1       | 25℃ 800mg/L GA + 50μmol/L MT | 9.83 | 10.83 |

3.3. Effects of MT and GA on kiwi seed germination at poikilothermal condition

Under poikilothermal condition at 25℃ 8h/4℃ 16h, germination of seeds was observed in all groups. The GR and GP of seeds in CK group increased to 78.22% and 67.02% respectively. After treated with GA solution at 800mg/L, the GR and GP of MW2 seeds increased to 89.46% and 79.00%, which was somewhat improved compared with CK group. And after treated with MT solution at 50μmol/L, the GR and GP of MW2 seeds increased to 91.80% and 90.09% respectively. Compared with the control group, the GR and GP of seeds in GAMT group were improved to some extent, which increased to 87.44% and 74.67%, but it was lower than that in MT group. The result suggesting that under the poikilothermal condition, both MT treatment at 50μmol/L and GA treatment at 800mg/L can promote seed germination. While under the combined effect of the GA and MT, the GR of the seeds was higher than that of the control group, but there is no synergistic effect.

Table 3. Effect of hormone treatment at variable temperature.

| Varieties | The temperature | The treatment       | Germination rate | Germination potential |
|-----------|-----------------|---------------------|------------------|-----------------------|
| MW2       | poikilothermal  | water               | 78.22            | 67.02                 |
| MW2       | poikilothermal  | 800mg/L GA         | 89.46            | 79.00                 |
| MW2       | poikilothermal  | 50μmol/L MT        | 91.80            | 90.09                 |
| MW2       | poikilothermal  | 800mg/L GA + 50μmol/L MT | 87.44 | 74.67 |

4. Conclusions

Mature kiwi seeds need certain cold accumulation to break dormancy, and the germination rate of seeds can be effectively increased by poikilothermal treatment. In this experiment, it was found that under constant temperature, MT treatment had no obvious effect on GR and GP of seed MW1. While under poikilothermal treatment, MT treatment significantly improved the GR and GP of seeds MW2, which indicated that only after the poikilothermal treatment did MT play a role in promoting the germination of kiwi seeds. In this experiment, the mixed treatment of MT and GA had no obvious effect on seed germination, so it was speculated that MT and GA had no synergistic effect. Many studies have shown that MT can improve seed stress resistance and promote seed germination under stress conditions [4,5], and the poikilothermal treatment in this experiment can also be regarded as a stress condition. Therefore, the germination rate of seeds treated with poikilothermal was higher. To sum up, both melatonin treatment and gibberellin treatment at a certain concentration can promote the germination of kiwi seeds under the condition of variable temperature.

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