Nutrient Medium Composition Optimization to Obtain Seed Progeny of Phalaenopsis (*Phalaenopsis × Hybridum* Blume)

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Abstract. Seed propagation of phalaenopsis is carried out by sowing seeds on artificial nutrient media. In the work, the influence of the basic composition of the nutrient medium, the addition of liquid endosperm of coconut, activated carbon, growth regulators and sucrose concentration on the germination of seeds and the effectiveness of the formation of seed progeny plants was studied. The best result was obtained by cultivating seeds on a B5 nutrient medium supplemented with 35 g/l of sucrose and 7 g/l of agar and amounted to 92.1% of germination and 34.4% of mature plants.

1. Introduction
Phalaenopsis (*Phalaenopsis × hybridum* Blume) is one of the most widespread and popular orchids, is used as a houseplant, as well as in flower arrangements. Phalaenopsis has considerable economic importance in floriculture. There is a great diversity of phalaenopsis varieties, including hybrid ones, with high decorativeness, differing in color, size and flower fragrance, compactness of the rosette. The method of microclonal propagation allows achieving a high rate of reproduction of hybrid varieties. Phalaenopsis seed progeny is obtained for breeding purposes, but this process includes some challenges. Phalaenopsis seeds are tiny, dust-like, do not have endosperm and require a symbiotic fungus for germination [1, 2]. Also, there is a sufficient number of publications which contain a description of the technology of germination of orchid seeds on in vitro nutrient media. However, when studying the publications, there are often recommendations to use organic additives that reduce the cost and simplify the preparation of nutrient media in the region where they can be easily obtained, but practically cannot be used in other countries, for example, the endosperm of fresh coconut or unripe banana homogenate [3, 4]. The authors, who study in detail the development, structure, and germination of orchid seeds, including those after cryopreservation [5, 6], pay more attention to species belonging to other genera. In this regard, it is necessary to optimize the composition of nutrient media to obtain seed progeny of phalaenopsis plants.

2. Materials and methods
Phalaenopsis plants purchased from garden centers and chain stores were cultivated at home in transparent plastic containers in Combo Sana orchid soil. The plants were watered by immersion once a week and were fed with complex fertilizers every 2 months. The variety names were determined from
catalogs freely available on the Internet. The flowering plants were self-pollinated (Pandora, Pasadena, Malta and Multiflora White varieties) and hybridized (Malta × Multiflora White, Multiflora White × Malta, Malta × Pasadena). One or two flowers of each plant were pollinated. Two-three months after pollination, immature fruits were cut off, transferred to the laboratory within 1–2 days, superficially sterilized for 10 minutes in 2% sodium hypochlorite (NaOCl) with the addition of 2 drops of Tween 20 and were washed three times in autoclaved water. The fruits were cut and washed in 10 ml of autoclaved water. The obtained suspension of seeds was evenly distributed over the surface of nutrient media in Petri dishes with a diameter of 9 cm.

To study the effect of adding coconut endosperm and activated carbon, the seeds obtained from self-pollination of plants of Malta and Multiflora white varieties and Malta × Multiflora white and Multiflora white × Malta hybrid combinations were germinated on the nutrient media 1) 1/2 MS [7, 8, 9] with the addition of 30 g/l of sucrose, 7 g/l of agar (1/2 MS); 2) ½ MS with the addition of 30 g/l of sucrose and 30% of liquid endosperm from coconut “Genuin coconut” (manufacturer: Greenfields-Logistics LLC, country of origin: Thailand), purchased in a chain store in Moscow (1/2 MS coconut); 3) ½ MS with the addition of 30 g/l of sucrose and 30% of the liquid endosperm of green coconut and 1 g/l of activated carbon (1/2 MS coconut, activated carbon). When studying the influence of the composition of the medium and the addition of growth regulators on germination, the seeds obtained from self-pollination of Pasadena plants were cultivated on the nutrient media 1) 1/2 MS with the addition of 30 g/l of sucrose, 7 g/l of agar (1/2 MS); 2) 1/2 MS with the addition of 30 g/l of sucrose, 7 g/l of agar and 2 mg/l gibberellic acid (1/2 MS + 2 GA); 3) 1/2 MS with the addition of 30 g/l of sucrose, 7 g/l of agar and 3 mg/l of thidiazuron (1/2 MS + 3 TDZ); 4) B5 [10] with the addition of 30 g/l of sucrose, 7 g/l of agar (B5 30). To determine the optimal concentration of sucrose, seeds of the Malta x Pasadena combination were germinated on the nutrient media 1) B5 with the addition of 25 g/l of sucrose, 7 g/l of agar (B5 25); 2) B5 with the addition of 30 g/l of sucrose, 7 g/l of agar (B5 30); 3) B5 with the addition of 35 g/l of sucrose, 7 g/l of agar (B5 35). The pH of the nutrient media was adjusted to 5.7 before autoclaving [11, 12]. Depending on the number of allocated seeds, the number of replicates in each variant of the experiment was 3–7 Petri dishes.

The seeds were cultivated on a rack in a light room at a temperature of 24–25 °C, with a photoperiod of 16 hours light/8 hours dark [13]. After 4 weeks, seed germination was determined by counting the number of seedlings in three fields of view of a Zeiss Stemi 2000-C stereomicroscope (Suzhou co., Ltd.) at 20x magnification in each Petri dish.

As seeds were germinating, the emerging protocorms were transplanted into plastic containers to the fresh media of the same composition. Further, developing seedlings were transplanted to fresh media every 3 weeks, plants with 3–4 leaves and roots were washed from the nutrient medium and adapted in vermiculite.

3. Results and discussions

The collection of fruits from phalaenopsis plants was carried out 2–3 months after pollination when the fruits stopped increasing in size. The authors of scientific publications recommend introducing phalaenopsis seeds in the culture after 120, 140 [14] or 150 days after pollination [9, 11, 15], when the seeds contain mature embryos [16]. However, in the cultivation conditions, with such a long development time, the fruits of plants not taken into account in this project became dry, revealed, and their seeds were scattered. In addition, it is known that in the more mature fruits of phalaenopsis, the seeds are dormant, their germination is less than when using seeds from younger fruits [14].

The seeds of the studied genotypes began to germinate after 2–2.5 weeks of cultivation (figure 1), the protocorms continued to be formed within a month. When transplanting to fresh nutrient media after 3 months of cultivation, there were plants with several leaves and roots (figure 2).
Figure 1. Protocorms formed after 3 weeks of cultivation of phalaenopsis seeds on B5 nutrient medium.

Figure 2. Plants formed 4 months after sowing phalaenopsis seeds on B5 nutrient medium.

The addition of 30% of the coconut endosperm to a nutrient medium led to a significant decrease in the germination of seeds of all 4 genotypes studied compared to cultivation on a medium without additives (table 1). It is probably due to a change in the composition of the endosperm of fresh coconut during storage and transportation. On the nutrient medium with the addition of 30% of coconut endosperm and 0.1% of activated carbon, only seeds obtained from self-pollination of the Malta variety plant germinated, which visually germinated better and on other variants of nutrient media, although no reliable effect of the genotype on seed germination was revealed.

Table 1. Influence of coconut endosperm and activated carbon in the nutrient medium on the germination of phalaenopsis seeds, %.

| Nutrient medium composition | Genotype          |          |          |          |
|----------------------------|-------------------|----------|----------|----------|
|                            | Malta             | Multiflora white | Malta × Multiflora white | Multiflora white × Malta |
| 1/2 MS                     | 23.5<sup>a</sup>  | 19.3<sup>a</sup> | 18.9<sup>a</sup> | 18.3<sup>a</sup> |
| 1/2 MS coconut             | 8.2<sup>b</sup>   | 0.0<sup>b</sup>  | 3.2<sup>b</sup> | 1.3<sup>b</sup>  |
| 1/2 MS coconut, activated carbon | 4.0<sup>b</sup> | 0.0<sup>b</sup>  | 0.0<sup>b</sup> | 0.0<sup>b</sup>  |

Note: values marked with the same letters do not differ at p<0.05

Cultivation of seeds obtained as a result of self-pollination of phalaenopsis of the Pasadena variety on nutrient media with the addition of 2 mg/l of gibberellic acid or 3 mg/l of thidiazuron did not lead to a significant increase in seed germination or the yield of formed plants.

When establishing this experiment, so many seeds were allocated from the phalaenopsis fruit of the Pasadena variety that an additional version of the experiment B5 30 was introduced. As a result, the germination of seeds on B5 30 medium did not differ from other variants of the experiment, however, a significantly larger number of plants were obtained on this medium as a percentage of the sown seeds (table 2), so this medium was used in further work.
Table 2. Influence of the nutrient medium composition on the germination of seeds obtained as a result of self-pollination of phalaenopsis of the Pasadena variety, %.

| Nutrient medium composition | Seed germination, % | Plant formation, % |
|-----------------------------|---------------------|--------------------|
| 1/2 MS                      | 30.7 \textsuperscript{a} | 3.4 \textsuperscript{b} |
| 1/2 MS+2 GA                 | 54.0 \textsuperscript{a} | 5.2 \textsuperscript{b} |
| 1/2 MS+3TDZ                 | 33.8 \textsuperscript{a} | 5.1 \textsuperscript{b} |
| B5 30                       | 53.9 \textsuperscript{a} | 22.1 \textsuperscript{c} |

Note: values marked with the same letters do not differ at p<0.05

When cultivating seeds of the hybrid combination Malta \times\ Pasadena on B5 media with a sucrose concentration of 25, 30 and 35 g/l of sucrose, no significant difference was found in germination and the number of plants forming (table 3).

Table 3. Influence of the nutrient medium composition on the germination of seeds obtained as a result of self-pollination of phalaenopsis of the combination Malta \times\ Pasadena, %.

| Nutrient medium composition | Seed germination, % | Plant formation, % |
|-----------------------------|---------------------|--------------------|
| B5 25                       | 66.4 \textsuperscript{a} | 65.0 \textsuperscript{a} |
| B5 30                       | 55.2 \textsuperscript{a} | 24.3 \textsuperscript{a} |
| B5 35                       | 92.1 \textsuperscript{a} | 34.4 \textsuperscript{a} |

Note: values marked with the same letters do not differ at p<0.05

The resulting plants with several leaves and roots were washed from the nutrient medium, placed in moistened vermiculite and covered with clean plastic containers. After 4 days, the containers were removed. The plants successfully acclimatized, formed an additional 1–2 leaves, but after 3–4 months after adaptation, 87% of the plants were necrotic. In the future, we plan to adapt plants in peat and soil for orchids.

4. Conclusion

The use of B5 nutrient medium with the addition of 35 g/l of sucrose and 7 g/l of agar for germination of phalaenopsis seeds obtained from pollination of a plant of the Malta variety by pollinia of a plant of the Pasadena variety resulted in 92.1% of germination, plants with several leaves and roots were formed from 34.4% of the seeds. When cultivating the seeds on the medium with a lower concentration of sucrose (20 and 25 g/l), these values were lower, although they did not differ significantly. The addition of 30% of liquid coconut endosperm to the nutrient medium significantly reduces the germination of phalaenopsis seeds.

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