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The Effect of Pollination Time and Gibberellic Acid (GA_3) on the Production and Seed Germination of *Phalaenopsis* Orchids

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Abstract

The germination power of orchids (*Orchidaceae* family) seems to be too weak due lack of albumen. The study carried out with various treatments including pollination time and GA_3 for breaking dormancy and increasing seed germination of orchids. The effect of pollination time (8 periods from January to August) and gibberellic acid (0, 500, 1000 and 1500 mg L^{-1}) were studied on germination of *Phalaenopsis* orchids. Capsules containing seeds with 2, 4, 8 and 10% hypochlorite sodium were disinfected. In order to grow seedlings the culture medium of cocopeat and coal with the ratio of 1:5, and cocopeat, coal, industrial shell, and polystyrene with the ratio of 1:1:2:4 was used. Results indicated that the most appropriate concentration of sodium hypochlorite in order to disinfect the capsules was 2%. The best month for pollination of flowers was January. The highest yield from one capsule obtained 15.3 seedlings in the medium of 1/2 MS containing 1000 mg L^{-1} gibberellic acid. The produced seedlings were transferred to greenhouse in order to hardening. The highest rate of viability was obtained through the medium of cocopeat, coal, industrial shell, and polystyrene particles.

Keywords: Culture medium, *Orchidaceae* species, Seed treatment, Viability.

INTRODUCTION

Orchids have 800 types as well as 2500 species within Orchidaceae family and they are considered as the largest plant families. Orchids have very small seeds as well as defective embryo and no albumen, hence, requiring symbiosis with fungi for germination in the nature. It should be noted that seed germination and seedling growth of orchids are very slow. There has been research conducted in order to stimulate and increase the rate and power of seed germination and seedling growth of orchids. Stimulation of seed germination and seedling growth within the medium containing growth regulators has been more common.

Mahendran and Narmatha Bai (2012) found the highest rate of germination and seedling growth of *Cymbidium bicolor* within a semi-solid MS medium containing 1 mg L^{-1} BA and 2 mg L^{-1} 2,4-D. Cooling as well as other treatments including seeding with gibberellic acid were applied to increase the percentage of seeds germination (Najafi *et al*., 2006). In another study, Sharma and Tandon (2010) looked into the effect of flower age and capsules as well as banana and potato juice on the seed germination rate of *Dendrobium tosaense* within MS medium. It has also been found that pollination time plays an important role in ovule growth and seed germination (Nadeau *et al.*, 1996).

Hence, the present study aims at determining the most appropriate time of pollination, optimized temperature, and different concentrations of gibberellic acid (GA_3) for production and seed germination of *Phalaenopsis* orchids.

MATERIALS AND METHODS

The flowers of *Phalaenopsis* orchids were prepared from a greenhouse in Chalous, Mazandarn, Iran. It should be noted that artificial pollination was done in the greenhouse and seed capsules were provided from there as well. Study treatments include pollination time in 8 levels (from January to August), GA_3 in 4 levels (0, 500, 1000, and 1500 mg L^{-1}) and disinfection method of seeds in 2 levels (30 seconds in alcohol 70% and 30 seconds in sodium hypochlorite with concentration of 2, 4, 8, and 10%). Artificial pollination and seed germination were conducted within 1/2 MS medium. The study was carried out with 48 treatments with 3 replications. Evaluated characters of the experiment were capsule production rate for pollinated flowers in different months, seed germination percentage, seed germination rate, and seedling production yeild.

The experiment was done as factorial arrangement based on RCD. The studied factors include pollination time, different concentrations of GA_3, temperature, and disinfection method. Data analysis was done SAS software and means compared with DMRT within 5% probability level.

RESULTS AND DISCUSSION

Analysis of variance regarding the effect of pollination time on the rate of seed production and petals wilting of *Phalaenopsis* orchids indicated that pollination time significantly affected seed production and petals wilting (Table 1).

The first successful sign of pollination has been the wilting of petals. As to the treatments, the interval between pollination and petals wilting has been rather long, in a sense that the longest interval (17 days) was related to flowers pollinated in February, while the shortest (4 days) belonged

Table 1. Analysis of variance regarding the effect of pollination time on the rate petals wilting of *Phalaenopsis* orchids.

Treatments	df	Mean Square
Pollination time	7	25.31**
Error	12	0.53
cv (%)		25

**: significant difference at 1% probability level.

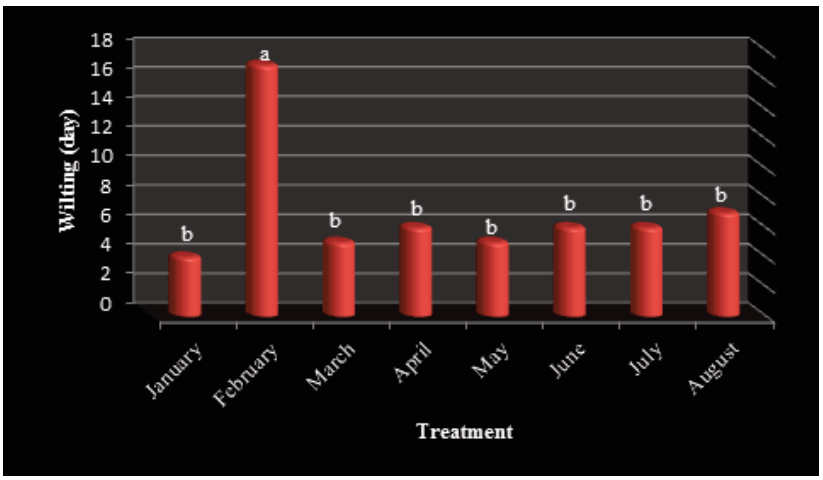

Fig. 1. Effects of time on the petals wilting of *Phalaenopsis* orchids.

Table 2. Analysis of variance regarding the effect of pollination time on the production of seed capsules of orchid flower. *Phalaenopsis* orchids.

Treatments	df	Mean Square
Production of seed capsules	6	23.43**
Error	107	0.49
cv (%)		21

**: significant difference at 1% probability level.

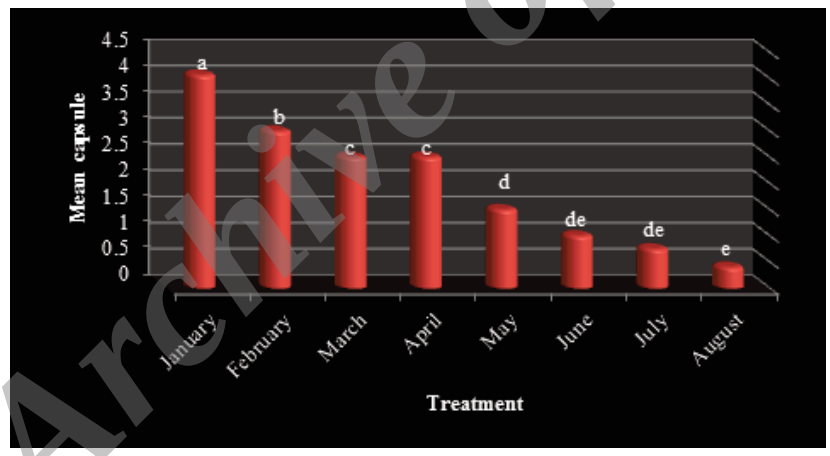

Fig. 2. Effect of pollination time on the production of seed capsules.

to pollinated flowers in January (Fig. 1).

Analysis of variance (Table 2) showed that there was a significant relationship (1% probability) between pollination time and the production of seed capsules of orchid flower. Fig. 2 shows different means of capsule production among pollination times highlighting the point that the most capsule production (4.07) among pollination times was found to be in January, while the least one (0.4) observed in August.

The present study indicated that if pollination carried out in cool months of the year (e.g. winter), capsule production rate is possibly high, while in spring and summer, in which the weather seems to be hot, the least production rate of capsules can be seen. There are two external factors (i.e. high temperature and ethylene) and physiological basis (such as short lifetime of ovule and pollen grains as well as ovary growth failure) affecting capsule production.

According to the ANOVA, it was found that capsule disinfection treatment significantly

Table 3. Analysis of variance capsule disinfection treatments.

Treatments	df	Mean Square
Disinfection treatment	3	The sample was contaminated (2.16** The sample Defunct (2.31**)
Error	396	0.09
cv (%)	15.85	19.22

**: significant difference at 1% probability level.

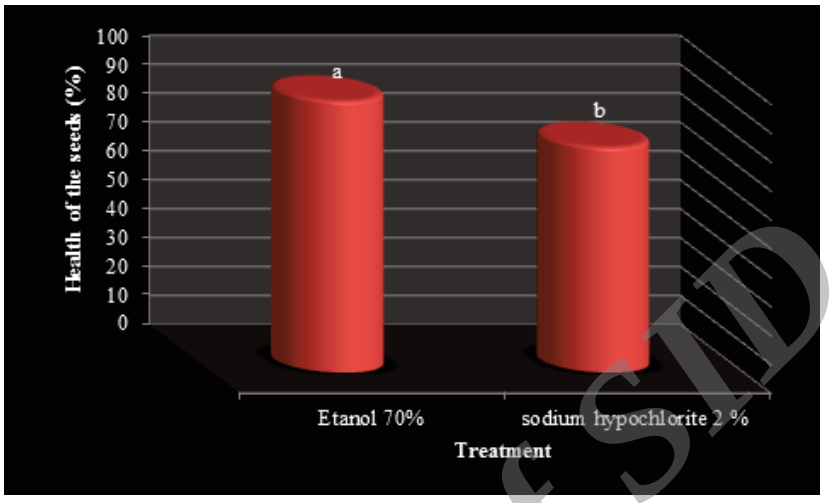

Fig. 3. Compare effects of sodium hypochlorite 2% and ethanol 70% on disinfect of seed.

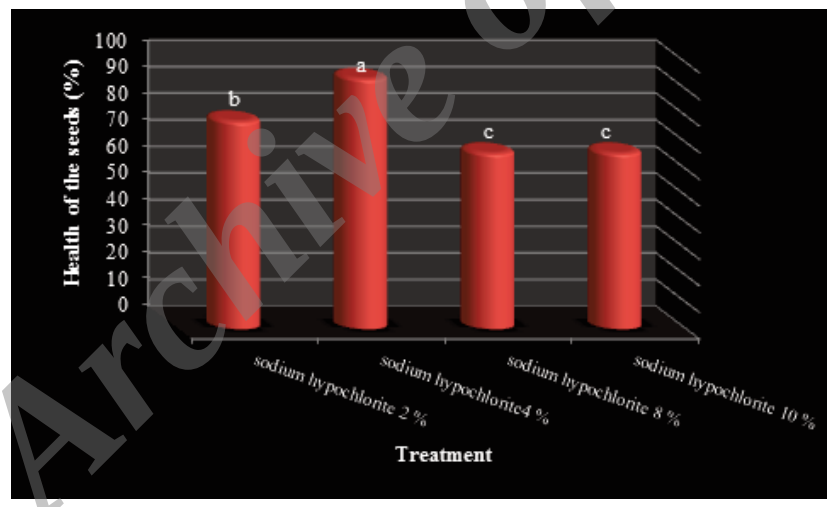

Fig. 4. Effects of sodium hypochlorite concentration on disinfect of seed.

(1% probability) affected *Phalaenopsis* orchids (Table 3).

Findings regarding capsule disinfection highlighted that if the concentration of sodium hypochlorite is lower, pollution rate is high, and to the extent that sodium hypochlorite concentration is high, pollution is reduced. However, high concentration causes physical harms leading to making the seeds black and useless. As to the treatments, the best performance was attributed to alcohol 70% within 30 seconds and 94% percent of cultivated samples were healthy (Fig. 3 and 4), which was in agreement with studies done by Arditi (1993) and Chung *et al*. (2009) in terms of disinfecting the capsules of *Phalaenopsis amabilis*.

Concerning Fig. 4, the treatment containing sodium hypochlorite 2% resulted in 78% health of the seeds. When the concentration of sodium hypochlorite reached 8%, it was found that despite the reduction of pollution, 23% of the cultivated samples within the medium of 1/2 MS turned to

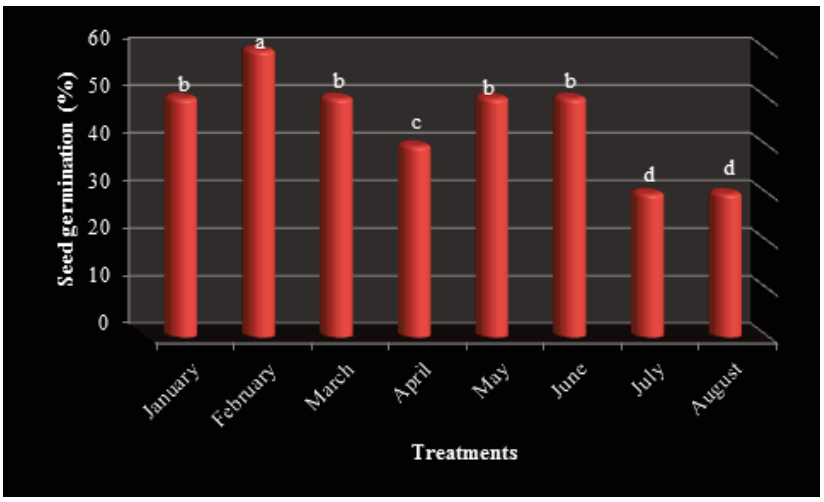

Fig. 5. Effect of time on seed germination on medium ½ MS.

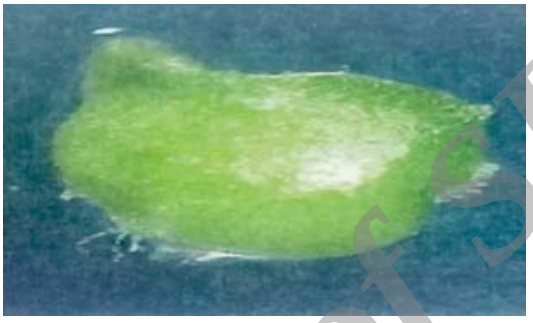
Fig. 6. Protocorm and white-colored rhizoids around these protocorms.

black in the second week and only 66% of the samples were healthy.

Fungal pollutions emerged after 7 days of cultivation, and the samples harmed by disinfection process were visible after 9 days. As to the treatment, the highest rate of seed germination was obtained in February while the lowest rate was found to be in July. Data of seed germination showed that the best time for capsule pollination is winter because winter seeds of pollinated flowers showed the highest rate of germination. On the other hand, findings also concluded that summer has been the most inappropriate season for pollination because there were a few produced capsules as well as the least amount of seed germination (Fig. 5).

The first sign of seed germination was the production of green-colored protocorm. There were white-colored rhizoids around these protocorms, which were in contact with the medium surface and acted as the root. After many months, true leaves and roots formed and plantlet emerged completely (Fig. 6). Pierick (1990) found that the required time from seeding to seedling growth can be estimated as 6 months, although nothing presented with respect to the size of seedlings.

The highest treatment (15.3 seedlings) was obtained within the medium of 1/2 MS containing 1000 mg L^{-1} gibberellic acid (Fig. 7). The least number of seedlings was found in the controlled seeds. The number of active buds in the medium containing GA_3 in relation to the controlled seeds indicated that this growth regulator has the potential to stimulate the buds in order to form the shoots and germinating the seeds. Kosir *et al.* (2004) argued that the best yeild was obtained through 8.53 seedlings for each seed within the commercial medium of Sigma P 6793 containing 2 mg L^{-1} BAP and 0.5 mg L^{-1} NAA.

ANOVA also indicated that there was a significant relationship (1% probability) on the effects of seedlings hardening for produced seedling of Phalaenopsis orchids (Table 4).

The best performance was attributed to the plants produced within enriched medium of 1/2 MS containing 1000 mg L^{-1} gibberellic acid. In order to make the cultivated seedling compatible with the mentioned medium, the both media were distinguished as appropriate, in the sense that,

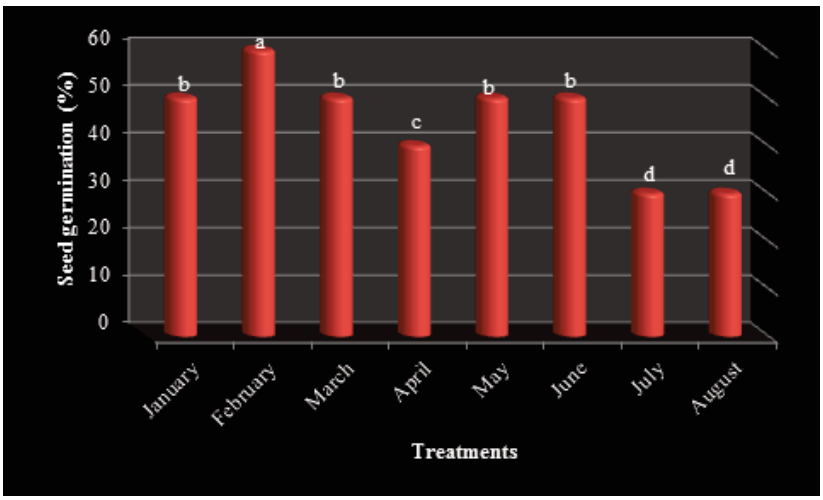

Fig. 7. Effects of different concentration GA_3 on seed germination.

Table 4. Analysis of variance effects of seedlings compatibility on the flowers of *Phalaenopsis* orchids.

Treatments	df	Mean Square
Seedlings	2	153.03**
Medium	1	50.7**
Seedlings* Medium	2	0.7 ns
Error	24	2.03
cv (%)	1.48	1.48

**: significant difference at 1% probability level, ns: Not significant.

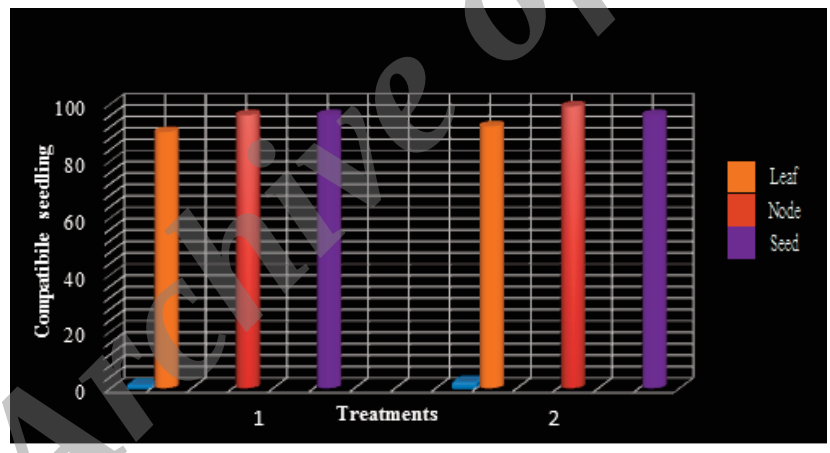

Fig. 8. Compatibility of seedlings on two mediums.

after 30 days, 99% (within the culture medium of cocopeat, coal, industrial shells, and polystyrene particles with the ratio of 1:1:2:4) (1) and 96% (within the culture medium of cocopeat and coal with the ratio of 1:5) (2) of seedlings were compatible with greenhouse conditions. It should be noted that these seedlings showed an acceptable compatibility due to having more and longer roots (Fig. 8).

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