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Reproduction technology of a unique orchid flower in the conditions of Namangan

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Abstract The unique orchid flower is said to be unique in that there are more than 25,000 species in the world, and many species are now endangered, and the reproduction process of this flower is much more difficult than other flowers. One orchid seed can grow from 1300 to 4000000 seeds, which makes the plant the leader among flowers in terms of seed yield, but it is much more difficult to fertilize this flower and seeds. Orchid and mushroom live in symbiosis, because they also have important conditions for germination or if there is a root coexisting fungal environment.

Keywords: Orchid flower, Reproduction process

Introduction

An orchid flower can be in the form of a small or tree-like liana - the smallest of them reaches only a few millimeters, and the largest orchid can grow up to 35 meters. Among orchids, there are long-lived species that can grow for 70 years or more. The famous scientist Charles Darwin did research on orchid pollination and he admired the process of fertilization in orchids and gave many examples and details about these flowers. In London, the Kew Royal Botanic Gardens has an extensive collection of orchids, some of which have been preserved since the beginning of the last century. No wonder they are 100 years old [1]. By 2000, about 250000 artificial and natural varieties of orchids had been identified and produced by enthusiasts and biologists around the world. A number of species of the orchid family are widely used not only in decorative floriculture, but also in the food, perfume industry, and medicine. In particular, the substance that gives the familiar vanilla aroma is obtained from the fruit of the orchid family liana. In addition, cut orchid flowers keep better in a vase than other flowers. Paphiopedilum sanderianum, the world's largest scientifically named orchid leaf, reaches 120 centimeters. The most expensive orchid in the world, Golden Kinabalu, blooms for the first time only at the age of 15. Because of their uniqueness and originality, these orchid buds can fetch up to $5000 [2]. This flower grows in Malaysia. The orchid family can adapt to any environment - it can grow in swamps and slopes, underground, in the desert, tundra and other places with a dry climate. Species and cultivars of orchids have different odors, such as their appearance - they can be distinguished by their scent of honey and vanilla, depending on the species, or they can spread the smell of rotten flesh. The richest collection of orchids in the world can be seen in the garden created for these flowers in Singapore, where about 60,000 different varieties of orchids grow. In eastern countries, the Salep drink, made from orchid roots, is known throughout the world for its ability to remove a number of substances toxic to the body, such as oxytocin, from the body.

The name orchid comes from the ancient Greek word órcus, which means a metamorphic root with a deformed rhizome. The root and flower forms give the shape of a human or a mammal. The classification of orchids is based mainly on the structure of their style and the nature of the location of the anther and stigma [3]. A new classification of orchids was developed by the American scientist R. L. Dressler [4]. He divided the Orchid family into 5 small families, 22 genera and 70 species. Today, orchids are found on every continent except Antarctica. Most species are found in the tropics. Areas with a short dry season and high rainfall have been found to have the most favorable conditions for orchid growth. The peculiarity of the flora of orchids on different continents lies in the originality of their distribution. Moderate width orchids are perennial weeds with underground rhizomes or tubers, and epiphytic orchids are most common in the tropics [5]. In the temperate zone, the orchid flora is much poorer than in the tropics. In the temperate latitudes of the Northern Hemisphere, there are only 75 genera (10% of the total) and 900 species (4.5%). Even fewer - 40 generations and 500 species - are located in the southern temperate zone.

According to the latest research from around the world, it has the following classification:

Domain: eukaryotes

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Kingdom: Plants
Section: Flowering
Class: single-stage
Order: Asparagus
Family: orchids
Apostasioideae: HORAN 1847 2 series and 16 species, Southeast Asia
Cypripedioideae: KOSTEL., 1831. 5 generations and 130 species
Vanilloideae: (LINDL.) SZLACH., 1995 15 generations and 180 species
Epidendroideae: LINDL., 1821 More than 500 genera and about 20.000 species
Orchidoideae: EATON, 1836 208 generations and 3630 species

In nature, orchid seeds germinate only after infection with mycorrhizal fungi, which provide the developing embryo with water, carbohydrates, minerals and vitamins. Orchid seeds first germinated in the wild in the case of potted orchids, but germination was unreliable and seedling mortality was high. In vitro germination techniques developed in the early 1900s resulted in more reliable growth and reproduction of many orchid taxa. The oldest methods of in vitro fertilization of orchid seeds used naturally occurring mycorrhizal fungi to stimulate seedling germination and growth. In 1922, Lewis Knudson germinated orchid seeds in vitro by sowing them in a sterile nutrient medium modified with sucrose. This method is called asymbiotic seed germination because the fungal mycobion is not used for germination, but takes into account many conditions such as photoperiod, temperature and mineral nutrition, so that the growth of symbiotic fungi and asymbiotic orchid seeds is efficient. Another important factor in symbiotic fertilization is fungal compatibility. Recent years have also introduced restrictions on the seed dormancy period for orchid seed germination. This chapter discusses methods and techniques for growing asymbiotic and symbiotic orchid seeds depending on photoperiod, temperature, nutrition, seed dormancy, and fungal microsymbiosis.

Probably no plant family is as interesting and complex as the Orchidoseae. Orchidoseae is the largest plant family with 17.000–35.000 species (Dressler 1993). Representatives of this family are found on all continents except Antarctica, with the greatest diversity in the tropics of Southeast Asia, South America and Central America. Seventy percent of all orchid species are epiphytes, but terrestrial, aquatic, and lithophytic species also occur (Dressler 1993).

A distinctive feature of orchids is their seeds, which are adapted to the wind. Orchid seeds are extremely small and contain undifferentiated embryos that lack the enzymes for polysaccharide metabolism (Manning and van Staden 1987; Molvray and Kores 1995). Orchid seed testicles (seed coat) are often hard but thin (Molvray and Kores 1995). Sugars are present in orchid embryos in the form of sucrose, fructose, maltose, rhamnose, and glucose, but these sugars are either fully utilized prior to fertilization or absent in sufficient amounts to support and maintain it (Manning and van Staden 1987). Although seeds use lipids and proteins as their main source of nutrients, embryos do not have the enzymes to convert lipids into soluble sugars (Manning and van Staden 1987). Since orchid seeds are unable to metabolize polysaccharides and lipids, they use mycorrhizal interactions with the respective fungi (microsymbiosis) during germination and early development (Rasmussen and others 1990). Once the embryo has entered, microsymbiosis provides it with water, carbohydrates, minerals and vitamins (Rasmussen 1992; YOder et al. 2000). Microsymbiosis plays a key role in the production of glucose and enzymes, which is important for initiating seed germination by mobilizing reserves and maintaining nutrients after fertilization (Manning and van Staden, 1987).

Over the past 15 years, great strides have been made in improving the efficiency of in vitro orchid seed production. In this chapter, we present a brief history of orchid seed germination and then discuss current issues and methods for asymbiotic and symbiotic germination. Although several important old articles have been discussed, most of them have been published in the last 15 years.

Materials and methods

Reagents used for growth media

The following reagents were used: MS-murosigu skugo nutrient medium, ribofilavin (vitamin B2), vitamin B12, kinetin, folic acid (vitamin B9), thiamine hydrochloride (vitamin B), inositol, calcium pantosinate (vitamin B5), nicotinic acid, adinine, D-biotin, gebirylic acid, 6-BAP, D-glucose, sucrose, agar,

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NaCl (HIMEDIA) from India. All reagents used are of purity qualification for chemical analysis in the experiment.

Experiment 1 was prepared on a simple basis, mainly for the production of plant flower seeds.
- A mixture of mineral salts Murashege skoog 1l / 4.4 gr
- Carbohydrates (sucrose) 1 l/25g
- Inositol 1l/0.01gr
- Amino acid protein (casein) 1l/0.01g
- Agar agar 1l/6gr
- NaOH, HCl for neutralization
- Medium solution 5.6-5.8 PH

Experiment 2 was prepared on a simple basis, mainly for the cultivation of the peduncle of the plant.
- A mixture of mineral salts Murashege skoog 1l / 4.4 gr
- Carbohydrates (sucrose) 1 l/30g
- Inositol 1l/0.02g
- Amino acid protein (Casein) 1l/0.02g
- BAP 1l/0.01mg
- Agar agar 1l/6gr
- NaOH, HCl for neutralization
- Medium solution 5.6-5.8 PH

Experiment 3 was prepared on a simple basis, mainly for the production of flower seeds of the plant.
- A mixture of mineral salts Murashege skoog 1l / 4.4 gr
- Carbohydrates (sucrose) 1 l/25g
- Inositol 1l/0.01gr
- Amino acid protein (casein) 1l/0.01g
- Agar agar 1l/6gr
- NaOH, HCl for neutralization
- antibiotics (tetracycline, benzylpenicillin) 100 mg/l
- Adsorbent-activated carbon in the amount of 0.5-1%
- Medium solution 5.6-5.8 PH

Experiment 4 was prepared, on a simple basis, mainly for the production of flower seeds of the plant.
- A mixture of mineral salts Murashege skoog 1l / 4.4 gr
- Carbohydrates (sucrose) 1 l/25g
- Inositol 1l/0.01gr
- Amino acid protein (casein) 1l/0.01g
- Agar agar 1l/6gr
- NaOH, HCl for neutralization
- antibiotics (tetracycline, benzylpenicillin) 100 mg/l
- Adsorbent-activated carbon in the amount of 0.5-1%
- Trichoderma veride-1l/1.5g
- Medium solution 5.6-5.8 PH

Sterilization of orchid tissue and seeds and utensils

One of the most important conditions for the growth of isolated organs, tissues, cells, and protoplasts is the importance of sterility. The importance of sterility lies in the fact that microorganisms also thrive in artificial nutrient media designed to grow isolated organs, tissues, cells, and protoplasts. Microbial growth poses a double threat to growing cells and tissues. First, during the life cycle of microorganisms, the composition of the nutrient medium changes significantly, stopping cell growth under certain stable conditions. Secondly, plant tissue, cells, and especially protoplasts are easily damaged by microorganisms. Therefore, experiments with isolated organs, tissues, cells, and protoplasts are carried out in sterile chambers, boxes, or laminar boxes. Boxes, utensils, utensils, plants, culture media, cotton swabs and other work items are sterilized.

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Laminar-box sterilization. The inner surface of the laminar, where work is performed, is wiped with 70% alcohol. Then the laminar is filled with alcohol, matches, a glass of 96% alcohol, sterilized containers, utensils and a sterilized water tube. A binocular loupe is also placed on the laminar to separate the meristems. 2 hours before work, the laminar box is irradiated with a bactericidal ultraviolet lamp. Two hours before work, the inner surface of the laminar is once again wiped with 70% alcohol.

Before starting work, hands should be thoroughly washed with soap and water, wiped with alcohol, put on a sterile gown and a sterile mask.

Sterilizing containers. Containers are sterilized in ovens in an autoclave with dry heat or wet steam. Containers should be thoroughly washed and dried before sterilization. Various detergents and chromium (a solution of potassium bichromate in sulfuric acid) are used to wash containers. Washed containers are rinsed in distilled water and dried in an oven. Before sterilization, the test tubes are closed with cotton swabs and wrapped in paper to prevent airborne infection. Then the containers are placed in drying cabinets and heated at a temperature of 160 ° C for 2 hours. Such heating kills not only bacteria, but also their spores. The temperature in the drying oven must not be raised above 1750°C, as cotton plugs turn yellow and parchment paper becomes brittle. Even better sterilization can be achieved under pressure in an autoclave, because when heated to moist heat, microorganisms and their spores die even better. Various beakers, Petri dishes, pipettes, flasks with distilled water are autoclaved. The dishes are wrapped in foil or wrapping paper and autoclaved at 2 atmospheres for 25-30 minutes. When autoclaving pipettes, cotton wool is placed on top of them and wrapped separately.

Sterilization of equipment. Tools, scalpels, tweezers, needles, etc. are sterilized by boiling in an oven at 1400 C with dry heat or water for 12 hours. Iron instruments are not autoclaved because they corrode and do not pass under the influence of wet steam. Before and during the operation, the instruments are placed in porcelain cups, sterilized in 96% ethanol and heated in an alcohol flame. Lancets, tweezers and microbiological loops are heated in an alcohol flame and stored between sterile papers. Sterilized instruments are used once, when reused, they are sterilized again in alcohol and heated in a flame. Needles and packaging are sterilized by adding alcohol.

Sterilization of materials. Cotton wool, gauze, cotton swabs, filter paper, gowns and handkerchiefs used in the experiment are sterilized in an autoclave at 2 atmospheres for 25-30 minutes.

Sterilization of plant materials. From various sterilizing solutions for sterilizing seeds, upper meristems, tissue fragments of different parts of the plant: Use aqueous solutions of 15%, 10% and 5% calcium hypochlorite.

Parts of the plant are thoroughly washed in running water with soap and rubbed, rinsed in distilled water and soaked for a few seconds in absolute alcohol. After sterilization, plant objects should be washed several times in distilled water to remove sterilizers.

The nutrient medium is sterilized with steam under pressure (in an autoclave). A test tube with nutrient media is closed with cotton stoppers, wrapped in wrapping paper and autoclaved at a pressure of 1200 C 1 atmosphere for 20 minutes.

Preparation of nutrient media for growing cells and tissues of an orchid flower

The nutrient medium in which cells and tissues isolated from an orchid flower are grown must contain all the macronutrients necessary for plants: nitrogen, phosphorus, potassium, calcium, sulfur, magnesium, iron and trace elements: boron, zinc, copper, cobalt, manganese, iodine, molybdenum , as well as vitamins, carbohydrates, carbonic water, phytohormones. Some culture media should contain casein hydrolyzate and some amino acids. In addition, EDTA (ethylenediamine-tetrazircanoic acid) or its sodium salt should be included in the nutrient medium to meet the needs of cells in iron at various pH values. Carbohydrates are the main component of the nutrient medium in which isolated cells and tissues are cultivated, since cells and tissues do not have the ability to autotrophic nutrition. Most often, a solution of sucrose or glucose, 20-40 g / l, is used as a source of carbohydrates. Polysaccharides are not used as a source of carbohydrate nutrition, since some tissues, mainly tumors, contain active hydrolytic enzymes (amylase, etc.) and can grow on nutrient media containing starch. Growth regulators are essential for cell differentiation and induction of cell tissue. Therefore, when obtaining callus tissue, it is necessary to include auxin (cells causing cell differentiation) and cytokines (inducing division of dedifferentiated cells) into the culture medium. Induction of stem morphogenesis can result in low or no auxin in the culture medium. The autonomy of both hormones or one

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of them depends on the ability of these cells to produce hormones. 2,4-dichlorophenoxyacetic acid (2,4-D) 1–10 mg/ml on nutrient media as a source of auxin; indolisilicic acid (ISA) -1-30 mg/l, α-naphthylacetic acid (NSA) -0.1-2 mg/l, etc. 2.4-D is often used. ISK is 30 times less active than 2.4-D. For the development of callus tissue, an increased content of auxin is often used; with subsequent tissue transplantation, tissue growth continues, even if the amount of auxin is several times less. In artificial nutrient media, kinetin, 6-benzylaminopurine (6-BAP) and zeatin (0.001-10 mg/l) are used as sources of cytokinin. In tissue growth and induction of organogenesis, BAP 6 is more active than kinetin. Some nutrient media contain adenine.

In the composition of some nutrient media, in addition to auxin and cytokinins, gibberal acid (HA) is added. Although the presence of HA in the nutrient medium is not necessary, in some cases it accelerates the growth of isolated tissues. To accelerate the primary induction of callus and its growth activity, plant extracts or juices are added to the nutrient medium. Coconut milk - the liquid endosperm of the coconut has the property of increasing the growth rate. In the preparation of yogurt nutrient medium, a polysaccharide, agar-agar, is used, which is obtained from seaweed. Usually, when preparing a solid nutrient medium, 5-7% agar is used. For better use of time, solutions of macro- and micro-vitamins and vitamins can be prepared without a large amount of stock solution and can be used by multiple dilutions. Concentrated solutions are stored in a refrigerator, vitamin solutions are stored at sub-zero temperatures. When cultivating cells, tissues and organs of plants belonging to different species, nutrient media of various compositions are used. The most commonly used food media are Murashige-Skoog, White, Gamborg. Murasige-Skuga nutrient media can be used for growing apical meristems with various modifications and for plant microclimate.

Results and discussions

When breeding an orchid flower, we initially selected several varieties most suitable for our local conditions (Orchis purpurea, Orchis pallens, Orchis purpurea, tridentate and oprys opifera, Orchidea coriophora). These plants stand out for their beautiful and long flowering and the high price of tanning in the world market. In our project, we used the most modern method of propagation of this plant - microclonaria, in which we selected stems and germinated seeds, on which various parts of the plant were presented. Orchids face many challenges in naturally propagating plants. The first of these problems is that the orchid that has formed in nature weakens the seeds and does not germinate for a long time. the size of orchid flower seeds will be 0.25-1.2 mm long and 0.09-0.27 mm wide, and weight 0.3-1.4 mg. These seeds have a powdery texture and even one seed is difficult to distinguish by eye. Related to this is the difficulty of propagating this plant. These seeds do not have endosperm, in other words, they are nutritious tissue. Contains "embryo" only in the seed coat. In addition to the appropriate environmental conditions that seeds require in order for these seeds to germinate in nature, there is a need to partner with "Mushroom" in nature. By working with orchid mycorrhiza, the energy needed to germinate your embryos comes from carbohydrates such as glucose. In mycorrhiza, the fungus first infects the seeds of the orchid as a parasite; after a short time, the fungus is terminated by germ cells, assimilated, and a balance is established. The small, nail-like structure that occurs when a seed germinates is called a mycorrhiza or protocorm. The fungus converts starch and similar compounds produced by the decomposition of organic humus in the environment into water-soluble sugars and sends them to the young orchid plant. The young plant has a supply of nutrients that allows it to germinate. The growth of mycorrhiza is very slow, as the orchid tubers from Salep produce one seed tuber per year. As a result, large-scale production is reduced due to the low productivity of many plants. Difficult to grow and slow growing, this plant can still be found today despite being unknowingly dismantled for human consumption. In order for the seeds to be very small and germinate without lack of nutritional value and defects, it needs the cooperation of the mycorrhiza fungus; even after germination, it takes many years before it becomes a mature plant; reasons such as the limitation or lack of opportunities for propagating an orchid flower in a vegetative way; limits orchid production by quantity. At the same time, obtaining asymbiotic sprouts and plants using the "tissue culture" method makes it possible to eliminate the risk of extinction of orchids and to produce them in large quantities.

Dark conditions are generally suitable for the germination of orchid seeds; but some species of orchids require both light and photoperiodic conditions, some have shown that this species can germinate equally in both light and darkness. Accordingly, for the first time, orchid seeds are considered symbiotic with mycorrhizal fungi by Noel Bernard, who in 1899 investigated germination in the laboratory. Also note his

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success in germinating the seeds of the Knudson orchid; In the 1950s, a new era began in the field with the growth of assimilation sprouts in a simple vanilla-type mineral + sugar blend. The role of endophytic fungi in the development of germination was achieved despite slight differences between the two species of orchids when they added sucrose, organic nitrogen and vitamins literally asymmetrically to the basic nutrient medium containing ammonium nitrate. Vitamin tests on Orchis lagiflora have shown that thiamine is essential. Mycorrhizal fungus has a positive effect on germination, except in vitro conditions.

Propagation of an orchid flower under in vitro conditions has the following advantages:

1. Because orchid seeds are very small and there is little endosperm at the top of the seed, in vitro propagation in the laboratory is more successful than natural propagation.

2. In the natural case, the seed has a positive effect on bud germination and symbiotic life, which is maintained with the fungus in later stages of development. However, with the help of cell culture, the need of the fungus is completely eliminated, and symbiotic germination is obtained.

3. Some special purpose hybrids may produce a limited number of seeds obtained on a suitable growth medium. Yield can increase seedling growth rate

4. Sowing in vitro on a nutrient medium ensures seed germination. This reduces the time it takes for the plant to reproduce and produces genetically identical plants that are free from exogenous influences.

5. In the In Vitro environment, seeding and growth are faster because the conditions are completely controlled and there is no need to survive in the race with fungi and bacteria.

Germination of orchid seeds in the in vitro medium proceeds as follows: first, in the nutrient medium of styrene, the embryo is immersed from the seed coat into the nutrient medium and absorbs water, which swells. After cell division, the embryo separates from the seed coat.

Orchid flower propagation methods by in vitro technology basically we can propagate and grow by planting the seeds of various plant tissues or germinated plant flowers into a special nutrient. The main goal of this technology is to obtain micro-clones of plants that are difficult or impossible to propagate vegetatively in the external environment, and to grow these clones in large quantities. when propagating the “orchid” flower using in vitro technology, the morphological origin of the plant is mainly used to breed it, creating an artificial nutrient medium. This nutrient medium mainly consists of micro-macronutrients and carbohydrate-based phytohormones. In March 2021, on the basis of the primary material and technical base, based on domestic and foreign experience, the composition of an artificial nutrient medium for the reproduction of this plant was formed and the first experiments were started.

1 experimental sample was prepared on a basically simple basis for seed germination of a plant flower.

- Murashege scoog mix of mineral salts 1l/ 4.4 gr
- Carbohydrate (sucrose) 1 l/25g
- Inositol 1l/0.01 g
- Amino acid proteins (casein) 1l/0.01 gr
- Agar agar 1l / 6gr
- NaOH, HCl for neutralization
- Solution medium 5.6-5.8 PH

2 experimental sample was prepared on a basically simple basis for growing the flower spike of a plant.

- Murashege scoog mix of mineral salts 1l/ 4.4 gr
- Carbohydrate (sucrose) 1 l/30g
- Inositol 1l/0.02 g
- Amino acid proteins (casein) 1l/0.02 gr
- BAP 1l/0.01 mg
- Agar agar 1l / 6gr
- NaOH, HCl for neutralization
- Solution medium 5.6-5.8 PH

3 experimental sample basically prepared on a simple basis for the germination of plant flower seeds.

- Murashege scoog mix of mineral salts 1l/ 4.4 gr
- Carbohydrate (sucrose) 1 l/25g

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- Inositol 1l/0.01 g
- Amino acid proteins (casein) 1l/0.01 gr
- Agar agar 1l / 6gr
- NaOH, HCl for neutralization
- antibiotic (tetracycline, benzylpenicillin) 100 mg/l
- Adsorbent in the amount of 0.5 -1% - activated carbon
- Solution medium 5.6-5.8 PH

4 experimental sample prepared on a simple basis, mainly for the germination of plant flower seeds.

- Murashege scoog mix of mineral salts 1l/ 4.4 gr
- Carbohydrate (sucrose) 1 l/25g
- Inositol 1l/0.01 g
- Amino acid proteins (casein) 1l/0.01 gr
- Agar agar 1l / 6gr
- NaOH, HCl for neutralization
- antibiotic (tetracycline, benzylpenicillin) 100 mg/l
- Adsorbent in the amount of 0.5 -1% - activated carbon
- Trichoderma veride-1l/ 1.5 gr
- Solution medium 5.6-5.8 PH

Growing orchid seeds in a hormone-free environment.

Seeds were collected and prepared for sterilization. During sterilization, 40%, 30% and 15% calcium hypochloride solutions and 96% alcohol were used. Seed separation was carried out in laminar boxes sterilized with bactericidal lamps. Before starting work, workplaces, tables, jars with a nutrient are wiped with alcohol. Instruments used during planting (tweezers, scalpels, needles) are sterilized, for which the instruments are soaked in alcohol and set on fire with an alcohol flame.

Seeds are laid out on the surface of the nutrient medium in jars. The neck of the jar was sterilized in the flame of an alcohol lamp and the development of sprouts from seeds was observed.

1 experimental sample was prepared on a basically simple basis for seed germination of a plant flower.

- Murashege scoog mix of mineral salts 1l/ 4.4 gr
- Carbohydrate (sucrose) 1 l/25g
- Inositol 1l/0.01 g
- Amino acid proteins (casein) 1l/0.01 gr
- Agar agar 1l / 6gr
- NaOH, HCl for neutralization
- Solution medium 5.6-5.8 PH

The sown seeds were stored in the dark at 23-24C. After a week, seed germination was not observed. At intervals of 3-4 weeks, microorganisms developed in the nutrient medium, but the development of flower seeds was not observed.

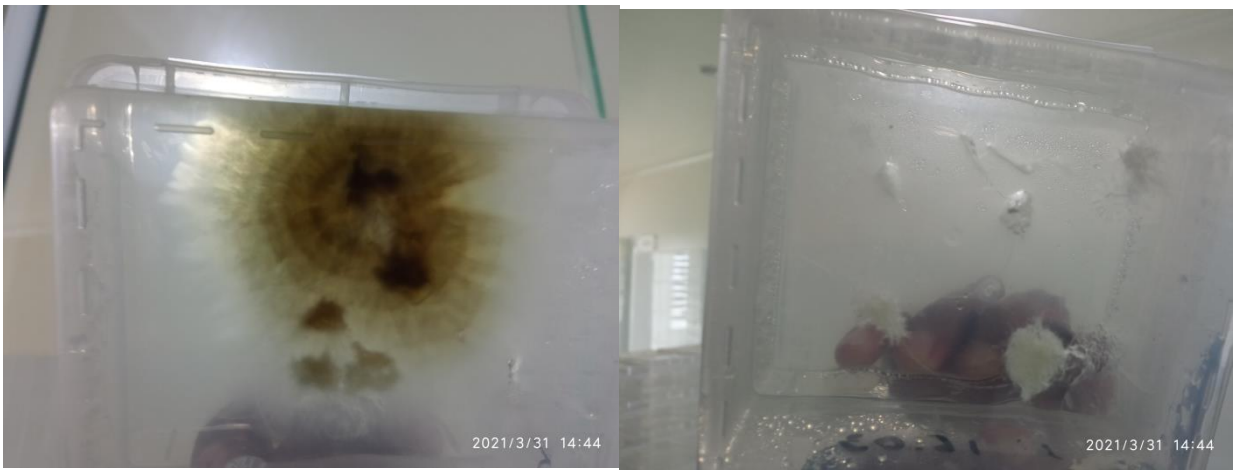

Figure 1 Fungal microbes in the process of growing orchid seeds on a hormone-free medium

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After that, we continued our research by adding an antibiotic and activated charcoal to the composition of the nutrient medium (as indicated in the literature), the seeds were selected and prepared for sterilization. During sterilization, 40%, 30% and 15% solutions of calcium hypochloride and 96% alcohol were used. Separation work was carried out in laminar boxes sterilized with bactericidal lamps. Before starting work, workplaces, tables, nutrient jars are wiped with alcohol. The instruments used for sowing (tweezers, scalpels, needles) are sterilized, for this; the instruments are immersed in alcohol and kept on the flame of an alcohol lamp.

Seeds are laid out on the surface of the nutrient medium in jars. The neck of the jar was sterilized in the flame of an alcohol lamp and the development of sprouts from seeds was observed.

- the experimental sample was prepared on a basically simple basis for the germination of seeds of a plant flower.
- Murashege scoog mix of mineral salts 1l/ 4.4 gr
- Carbohydrate (sucrose) 1 l/25g
- Inositol 1l/0.01 g
- Amino acid proteins (casein) 1l/0.01 gr
- Agar agar 1l / 6gr
- NaOH, HCl for neutralization
- antibiotic (tetracycline, benzylpenicillin) 100 mg/l
- Adsorbent in the amount of 0.5 -1% - activated carbon
- Solution medium 5.6-5.8 PH

Посеянные семена хранили в темноте при 23-24С. Через неделю всхожести семян не наблюдалось. С интервалом в 3-4 недели в питательной среде наблюдались признаки прорастания 10-15% семян цветка.

Figure 2 Growing process

We continued to add antibiotic and activated charcoal and Trichoderma veridesa fungus to the composition of the culture medium during our studies (listed in the literature), the seeds were selected and prepared for sterilization. During sterilization, 40%, 30% and 15% calcium hypochloride solutions and 96% alcohol were used. Work on the separation of seeds was carried out in sterilized laminar boxes with bactericidal lamps. Before starting work, workplaces, tables, nutrient jars are wiped with alcohol. The instruments used for sowing (tweezers, scalpels, needles) are sterilized, for this, the instruments are immersed in alcohol and kept on the flame of an alcohol lamp.

Seeds are laid out on the surface of the nutrient medium in jars. The neck of the jar was sterilized in the flame of an alcohol lamp and the development of sprouts from seeds was observed.

- Murashege scoog mix of mineral salts 1l/ 4.4 gr
- Carbohydrate (sucrose) 1 l/25g
- Inositol 1l/0.01 g
- Amino acid proteins (casein) 1l/0.01 gr
- Agar agar 1l / 6gr
- NaOH, HCl for neutralization

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- antibiotic (tetracycline, benzylpenicillin) 100 mg/l
- Adsorbent in the amount of 0.5 -1% - activated carbon
- Trichoderma veride-1l/ 1.5 gr
- Solution medium 5.6-5.8 PH

The sown seeds were stored in the dark at 23-24 C. After a week, no seed germination was observed. With an interval of 3-4 weeks, signs of germination of flower seeds in a nutrient medium were observed.

Conclusions

Separation and cultivation of orchid seeds

Work progress. Work on the separation of seeds is carried out in sterilized laminar boxes with bactericidal lamps. Before starting work, workplaces, a table, binocular magnifiers and stands with test tubes are wiped with alcohol. Tools used for separation into parts (tweezers, scalpels, needles) are sterilized after each separation, for this, the tools are immersed in alcohol and kept on the flame of an alcohol lamp.

Seeds are placed on the surface of the nutrient medium in a test tube. The mouth of the test tube and the cotton swab are sterilized in the flame of an alcohol lamp, closed and packed in a tripod. After filling the rack with test tubes, the food is covered with a plastic lid so that the medium does not dry out, and the development of sprouts from seeds is monitored.

There are several studies on the germination and temperature of orchid seeds. Like many other species, orchid seeds germinate within a temperature range, but maximum germination is only achieved within a narrow range. Seeds of Dactylorhiza majalis germinate between 10 and 30 °C, but the optimal temperature range is between 23 and 24.5 °C. The percentage of germination has decreased below 15 °C and above 27 °C (Rasmussen et al. 1990b; Rasmussen and Rasmussen 1991).

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