Tissue Culture Application of *Maranta arundinacea*

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Abstract The reproductive methods of ornamental arrowroot (*Maranta arundinacea*) mainly include plant division, cutting propagation, truncation and sprout promotion propagation, and tissue culture. Large-scale seedling production mainly relies on tissue culture. In the process of tissue culture, the culture conditions can be artificially controlled, and the plant materials used for tissue culture grow completely in artificially-provided culture media and microclimate environment, free from the adverse effects of catastrophic climate, seasonal and diurnal changes. Because the conditions are relatively uniform, tissue culture is very beneficial for plant growth and more convenient for stable long-term cultivation and production. In the process of tissue culture, the inoculation process is more important. How can we achieve standardized operation and reduce pollution during the inoculation process is worth exploring.

Key words *Maranta arundinacea*; Tissue cultivation; Inoculation

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Arrowroot (*Maranta arundinacea*) is a perennial evergreen foliage plant of Marantaceae. It is often used for indoor potted plant listing and hall layout, with good ornamental effect. In recent years, arrowroot has been introduced and cultivated in a relatively small amount in China, but it still cannot meet market demand. The reason is that the speed of propagation mainly by natural branching or cuttings is slow. Meanwhile, due to viruses and other reasons, the phenomena of evolution, degradation and gradual death of arrowroot in the planting process are more serious, so that many imported rare varieties are gradually reduced. Therefore, plant tissue culture is a rapid reproduction method in this situation.

Research Background

Arrowroot is a foliage plant of Marantaceae. It is common in southern China and is native to tropical regions of the Americas. It is widely cultivated in various tropical regions and blooms in summer and autumn. Arrowroot prefers a warm, humid, and semi-shaded environment, with a suitable temperature of 18 – 28 ℃[1]. It is afraid of dryness and should not be exposed to strong light. It is very sensitive to moisture. Arrowroot prefers to grow in semi-shaded environments or low-light conditions, and its leaves are prone to ambustion under exposure to strong light. The propagation of arrowroot is ideal at the temperature of about 20 ℃. As long as the temperature reaches 20 – 28 ℃ and the humidity exceeds 80%, it can be planted throughout the year.

In China, arrowroot is often used as an ornamental plant. The branches and leaves of arrowroot grow densely and softly, and the whole plant looks plump in shape. Different varieties of arrowroot have different colors on their leaf surfaces. *Calathea sanderiana* has dark green and bright leaves, and the veins show clear double-line white, forming a sharp contrast. The whole leaves of Hongruyi have a color of red mixed with rose, so it is very suitable to purchase Hongruyi as a Chinese New Year flower during the Spring Festival. When used to decorate bedrooms, living rooms, offices, and other places, arrowroot appears quiet and dignified, and can be appreciated for a long time. When arranged on both sides of corridors and indoor flower beds in public places, different varieties have different display effects, but the same is the extremely pleasing ornamental effect. Therefore, the growth cycle of conventional branching and cutting is too long to meet a large number of people's needs. Meanwhile, arrowroot is vulnerable to red spiders, scale insects, leaf spot disease, brown spot disease and black spot disease. Due to the existence of diseases and pests, the phenomena of arrowroot degradation and gradual death in the planting process are more serious, which will lead to the gradual reduction of many introduced precious varieties. Most of the ornamental plants of Marantaceae are foliage plants, and vegetative reproduction is usually adopted for foliage plants. However, if plant culture technology is used to propagate tissue culture plantlets, the objectives of rapid propagation, large quantity, neat growth and stable quality can be achieved, while yielding twice the result with half the effort[2].

Research Significance

Ornamental arrowroot rarely bears seeds during cultivation in China and cannot be propagated by seed sowing. In China, the reproductive methods of ornamental arrowroot mainly include division propagation, truncation and sprout promotion propagation, and tissue culture propagation, and large-scale plantlet production mainly relies on tissue culture[3]. Under certain environmental conditions, providing fixed conditions such as temperature, light, moisture, nutrients and hormones manually is conducive to achieving automatic production control of arrowroot. On an inoculation table, over 1 000 plants can be cut per day, and greenhouses only need to be used for adult plantlet cultivation. Compared with division and cuttage, it avoids a lot of complicated work such as weeding, watering and fertilization, and pest control, and greatly saves manpower, material resources and land required for field

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planting.

The cultivation environment for bud germination and plantlet growth is entirely controlled manually, and different cultivation conditions can be provided according to the different requirements of different parts, so the growth of arrowroot will be faster. Moreover, arrowroot plantlets are also relatively small, and a cycle is often 30 – 40 d. Therefore, although tissue culture requires certain equipment and energy consumption, plant materials can be generated through geometric reproduction, resulting in lower overall costs and timely provision of high-quality or virus-free plantlets. It greatly reduces the damage and death of plantlets caused by external environmental influences, and different cultivation conditions can be used to control their development process.

Due to the controllability of the environment, high-quality parents can be selected in the laboratory stage, without any variation. All genetic characteristics of original parents can be maintained, and continuous breeding and reproduction can be carried out to ensure the uniformity of subculture quality. Meanwhile, scholars can also try to cross different varieties to cultivate and obtain new varieties.

Tissue Culture of Arrowroot

Inoculation equipment

During the inoculation process, inoculation equipment is very important. Without equipment, any process cannot proceed. Inoculation equipment includes clean benches, tweezers, scalpels, inoculation plates, high-temperature inoculation sterilizers, alcohol, etc. We introduced various types of inoculation equipment from the purpose of equipment.

Clean bench: A clean bench, also known as purification workbench, is designed to meet the cleaning needs of local working areas in scientific experiments. The air filtered by the fan is then filtered into a high-efficiency filter through a static pressure chamber. The air will be filtered in a vertical or horizontal flow state to achieve a purity level of 100 in the operating area, ensuring the environmental sterility requirements of arrowroot during cutting and inoculation. Clean benches can only protect arrowroot operated inside them from pollution, but cannot protect the workers. Therefore, workers also need to spray alcohol to wipe the workbenches before inoculation, in order to reduce the amount of pollution during the arrowroot cutting process.

Tweezers, scalpels and inoculation plates: Tweezers, scalpels and inoculation plates are the main inoculation tools, which are used together in the process of plant cutting. Tweezers are employed to fix arrowroot plants, and scalpels are used to cut the effective part of arrowroot, and inoculation plates are used to carry arrowroot plantlets that need to be inoculated. Each bottle of arrowroot is fixed with only one set of tools. The inoculation plates needs to be cleaned and sterilized before reuse. Tweezers and scalpels need to be placed in a high-temperature inoculation sterilizer for disinfection and sterilization for 3 – 5 min before reuse. The blades of scalpels need to be changed every day to reduce unnecessary pollution caused by breakage of blades due to excessive use during the inoculation process.

High-temperature inoculation sterilizer: Before inoculation, sterilizers need to be turned on 20 min in advance to reach a temperature of 300 ℃ before normal use. If the temperature is too low, the sterilization effect cannot be achieved. If the temperature is too high, the blades will become brittle and easily break when used. Before each use, the exterior of the sterilizer used needs to be sprayed with alcohol and wiped to reduce unnecessary contamination caused by the splashing of culture media during the inoculation process.

Alcohol: 70% alcohol is used in the laboratory. It is usually used to wipe and disinfect inoculation benches and arrowroot plantlet bottles before opening, and sterilize inoculation workers' hands, but it cannot directly contact the original plants, tweezers and scalpels, because it is not pure alcohol and contains a small amount of water. If it contacts the original plants, tweezers and scalpels, it may cause the original plants to be polluted and wasted.

Disinfection of operators and inoculation benches

All exposed objects and objects in contact with water sources have bacteria on their surfaces. Accordingly, untreated sterile rooms, clean bench, unsterilized culture media, container surfaces, inoculation knives, body surfaces, and respiratory tract are all contaminated with bacteria[4]. Before daily work, the inoculation operator needs to change into laboratory specific shoes and work clothes in a partition, and finally enter a wind shower room. The wind shower room will blow away sand, stones, and dust attached to the human body and carried items. Meanwhile, it also acts as an air brake to prevent impure air from entering clean areas. The sterilization operation on a workbench also requires two steps. One is to use a UV light for 20 min of irradiation before operation, which can be carried out at night of the previous day. The prerequisite is to ensure that no one enters after sterilization. The other is to turn on the machine for air blowing. Before formal inoculation, the workbench is wiped carefully with 75% alcohol[5]. After arriving at the laboratory, the operator need to spray the inoculation table with alcohol and wipe it with a high-temperature sterilized cloth before starting the inoculation operation.

Arrowroot bottle plantlets

The subculture growth cycle of arrowroot plantlets in bottles is 30 – 40 d. For daily inoculation of bottle plantlets, bottle plantlets that are not contaminated and grow and develop well for 30 to 40 d are selected for subculture. Every time the bottle plantlets selected for cutting are subcultured for one generation, they will not be subcultured again after the number of subculture generations reaches 8. Although plantlets over 8 generations can still be used, when cutting plantlets, it can be felt that the tubers are relatively hard, and the operation is relatively difficult. Compared with plantlets with fewer generations, plantlets subcultured for more generations will have darker and older colors, and their leaves will be drier and harder. Therefore, bottle plantlets subcultured for more than 8 generations will no longer be subcultured.

Culture media (bagged, bottled)

Through practice, it was found that the more important aspect of the inoculation process is culture media. The culture media put into production need to be divided into bottled culture media and bagged culture media, both of which adopt MS culture medium, which is then added with different kinds and concentrations of plant hormones for different cultures[6]. Sterilized inoculation bottles cannot be left in the air for too long. It is generally best to perform inoculation within 1 – 2 d after taking the bottles out of a sterilizer to reduce contamination caused by microorganisms on the surface of culture bottles[7]. Bottled culture media are used to hold tubers and newly sprouted ultra-small plantlets, in order to provide sufficient lateral and vertical growth space for the tubers. Bagged culture media are used to hold plantlets with a length of about 6 cm or more, which are placed in bagged culture media and allowed to grow in a seedling room at a fixed temperature for another 15 d. Stronger plantlets are selected and weak plantlets are removed before being sent to a greenhouse and transferred to soil culture.

Primary bud burst

The materials used for tissue culture, namely explants, come from a wide range of sources. Roots, stems, leaves, flowers, fruits and seeds of plants can all be used as culture materials[8], while the explants of arrowroot are usually stems.

For each complete round of subculture, a batch of high-quality plantts is required as the original plants. Plants that have a longer growth time and relatively stable traits of adult flowers should be selected as the original plants. Firstly, the external leaves are peeled off, and only the middle and front segments are left, while all the rear segments connecting the roots and stems are removed. At this moment, the stems can be brought into the laboratory and wiped with 70% alcohol and cotton to clean the dust, pests, and waste leaves carried on the outside (Fig. 1). Next, a blade can be used to thinly cut off the circular outer leaf bark of each bud point, exposing the bud point to the outside, making it easier for subsequent sprouting. Next, cotton dipped in alcohol is used to wipe along each bud point to perform disinfection for the second time. Next, a glass bottle is added with alcohol to half its level, and about 10 stems are added and soaked for 10 – 15 min to perform the final disinfection and sterilization. Finally, all stems are taken out, and cut into segments, with each two ring buds forming one segment. The segments are put separately into bagged culture media, which are sealed and placed in a fixed-temperature seedling room (Fig. 2). After two weeks of cultivation and growth, pollution-free sprouts will burst smoothly. The stem segments with buds can be transferred to bottled culture media to wait for growth and development again and obtain the firstgeneration of plantlets.

The first generation of plantlets is relatively young and tender. At this stage, it can be judged from the touch that the stems are soft when cutting the plantlets, and the plantlets are relatively pink, and bud burst occur at a high rate.

Fig. 1 Plantlets obtained from primary subculture

Fig. 2 Treated original plants

Inoculation

Subculture The main purpose of arrowroot subculture is to allow the tubers to sprout and grow. Some can be grown into plantlets and transferred to soil culture, while others can be kept for further subculture. Different methods of cutting will have different results, and the main methods include topping and vertical cutting, and transition.

Topping and vertical cutting refers to the method including steps of leaving one centimeter of stem while cutting off other parts, and cutting from the middle of the left stem with a scalpel through the center to obtain divided plantlets, which is a way to achieve good doubled growth of plantlets.

In the transition method, the plantlets are left without topping, but old and hard stems are removed, and only tender tubers with bud points are added in bottled culture media for transitional growth. Soon, the bud points of these high-quality tubers will begin to burst and grow.

Plantlet culture In production, the main modes of profit are flower sales and plantlet sales. Plantlets refer to individual plantlets that are about 7 cm long during the subculture stage. And plantlets from different generations have different effects.

Arrowroot plantlets should be left with a portion of their stems and roots about 1 cm long when loading culture media. These

stems and roots can promote the absorption of nutrients in the culture media, but bud points should not be left on the plantlets. Leaving bud points on the plantlets will cause them to compete for nutrients and affect the growth of the plantlets.

When the number of subculture generations is low, bud tips are mostly chosen for subculture. At this time, the bud tips grow relatively tender and are only suitable for subculture, while plantlets that grow more robust can be grown or sold as finished plantlets.

Plantlets that have been subcultured for 3 – 6 times can be used for bud burst and proliferation by way of truncation and vertical cutting, and leaving bud points and tender tubers to realize doubling of the number of plantlets. Of course, not all plantlets at this stage are used for subculture, and high-quality parts will also be selected for planting or sale.

Plantlets that have been subcultured 6 times will no longer be used for subculture, as the tubers are relatively hard and the number of sprouts is low. They not only have little subculture value, but also consume more manpower. Therefore, in the later stage, only finished plantlets are cut off for planting or sale.

Transplantation after multiple generations

After a certain number of subculture generations, all arrowroot plantlets will no longer be subcultured. At this time, the arrowroot plantlets can be removed from cultivation bottles for the last time from laboratory cultivation to greenhouse planting without strict disinfection. Instead, the plantlets need to be taken out of cultivation bottles, and too many rhizomes are cut off, leaving a small amount of rhizomes. Then, the plantlets are soaked with copper hydroxide for 5 min for disinfection, and then transferred to greenhouses for soil culture. During the soil cultivation process, attention should be paid to watering, shading, and heat insulation, and a survival rate of over 95% could be reached. After transplanting for about 40 d, plants can be cultivated in pots.

Waste plantlet treatment

The pollution of plantlets is mainly divided into internal and external pollution. Internal pollution, also known as endogenous pollution, refers to pollution caused by the presence of microbes (endophytic microbes) on the surface or inside explants. External pollution is mainly caused by environmental pollution and improper operation, and refers to the pollution caused by microbial invasion during inoculation or cultivation[10]. After cutting, all plantlets will be uniformly sealed and placed in a seedling room at a fixed temperature for cultivation and growth. During this stage, it is necessary to regularly select contaminated, moldy and dry plantlets from the seedling room and dispose them uniformly.

Moldy plantlets: Moldy plantlets will present hyphae interwoven into fluff, floc or mesh, and the mycelia are usually white. They often appear in a block-like form within seedling bags.

Contaminated plantlets: Contaminated plantlets are often caused by bacterial and fungal infections. Bacterial infections often manifest after 1 to 2 d of inoculation, and mucoid bacterial plaques often appear on the surfaces of culture media. Fungal infections usually manifest within 3 d after inoculation, with the main symptom being the appearance of villous hyphae that form spore layers of red, orange, pink, yellow, and other colors. Generally, they appear in the form of spots within seedling bags.

Dry and withered plantlets: As the name suggests, the plantlets themselves are relatively thin or of poor quality, and often turn yellow and dry within 10 d of inoculation. Such plantlets are also useless and can only be discarded.

Conclusions

This paper analyzed the process of tissue culture and inoculation of arrowroot, including different reasons and objectives at different stages of the inoculation process. It will greatly reduce seedling damage caused by external environmental influences and provide reference for the cultivation of new varieties.

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