Inquiry Teaching Design of Plant Tissue Culture Experiments in Secondary Vocational Education

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Abstract. Based on the experiment of "establishing plant tissue regeneration culture system", this paper briefly discusses the general steps of inquiry teaching design of plant tissue culture experiments in secondary vocational education. Inquiry experiment teaching begins with posing questions, which is the premise that makes students think. Ask questions to inspire students' desire and curiosity for inquiry and in-depth learning, guide students to design the experiment procedure, and conduct students to implement laboratory activities. Not only can inquiry experiment teaching deepen students' understanding of the knowledge they have learned, but also improve students' experimental operational level. More importantly, it can cultivate students' exploration consciousness and enhance teaching effect.

Keywords: inquiry teaching · plant tissue culture · secondary vocational education

1 Introduction

In the experiment of "establishing plant tissue regeneration culture system", the aseptic operation method is used to culture the organs, tissues or cells of plants in vitro, so that they can grow into complete regenerated plants on the synthetic medium through cell growth, proliferation, and differentiation [1]. This experiment is selected from the plant tissue culture courses of agriculture and forestry majors in secondary vocational schools. Plant tissue culture is a practical applied course developed on the basis of botany, plant physiology and other courses, and the corresponding plant tissue culture technology has penetrated into not only agriculture and forestry, but also industry and other fields [2]. Actually, "establishing plant tissue regeneration culture system" is a necessary basic skill for graduates of this major. Mastering this skill will help to enhance students' competitiveness in the workplace and be more competent for relevant positions, which is the fundamental reason for setting up this experimental course in secondary vocational schools.

Inquiry-based experimental teaching refers to the process of asking questions, designing experiments, and obtaining scientific conclusions through teachers' organization and guidance under the educational theory of "students as the subject and teachers as the lead", when students know little about the characters, regulations and interrelations

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of the research object [3]. This experiment has the characteristics of continuity, integrity and long period, and it is difficult to realize the repeated practice of each student, so we must pay attention to the standardization and rigor of the experimental operation, which requires high proficiency in the operation. On the other side, it also poses a challenge to how to adopt the inquiry teaching method. The inquiry experiment teaching of plant tissue culture is problem-oriented and puts forward high requirements for teachers and students [4]. First of all, teachers should have higher professional knowledge and experimental operational skills of plant tissue culture course, be able to control the progress of the inquiry experiment classroom and deal with some unexpected events in the process of inquiry, and so on. Students should have rich theoretical knowledge related to plant tissue culture, open thinking in the face of science and the courage to discover [5]. Inquiry-based experimental teaching is conducive to changing students' learning style, from passive learning to active learning, which is exactly what we advocate. With the advancement of curriculum reform, more and more attention has been paid to the inquiry experiment of plant tissue culture, which can guide students to carry out inquiry learning and cultivate students' innovative spirit and practical ability [6].

2 Make the Students Clear About the Purpose of the Experiment

This experiment "establishing plant tissue regeneration culture system" has the characteristics of continuity, integrity and long period, which includes four projects, namely "preparation of the mother solution and medium", "aseptic operation and callus induction", "subculture and differentiation culture of callus" and "rooting culture of test-tube plantlets". We need to set the purpose of the experiment according to the curriculum standards and the reality of the students. It is worth noting that teachers should not only make themselves clear the purpose of the experiment, but also make the students clear about it. That is to say, we need to put forward the experimental purpose of each project from the beginning, so that the students can have a clear understanding of it. The experimental purpose of each project is as in Table 1.

Table 1. The experimental purpose of each project

Project	Experimental purpose
Preparation of the mother solution and medium.	Comprehend what kinds of nutrients and phytohormones are required that for in vitro culture.
	Master the preparation method of mother solution.
	Master the preparation method of the medium.
	Prepare the medium for follow-up experiments.
	Master how to autoclave.

(*continued*)

Table 1. (*continued*)

Project	Experimental purpose
Aseptic operation and inducing callus formation.	Proficient in disinfection and inoculation of explants.
	Understand the dedifferentiation process of explants, and design different concentrations combinations of exogenous phytohormones.
	Comprehend the environmental conditions of callus induction.
Subculture and differentiation culture of callus.	Comprehend the environmental conditions of callus culture in vitro.
	Master how to subculture and differentiation culture of callus.
	Understand the internal relations and differences between the two essential stages of subculture and differentiation culture of callus.
Rooting culture of callus.	Comprehend the environmental conditions of rooting culture in vitro.
	Understand the internal relations and differences between the two essential stages of differentiation culture and rooting culture.

3 Create an Active Classroom Teaching Situation to Stimulate Students' Interest in Independent Inquiry

If we directly adopt the conventional method of explanation, in fact, it will not stimulate students' interest in learning effectively, and of course it will not achieve a better teaching effect. Creating situations is one of the teaching methods to stimulate students' interest in learning effectively, including problem situations, intuitive situations, life situations, story situations and game situations. Among them, problem situations are more commonly used in the real process of classroom teaching. Make flexible use of the problem situations, guide students to carry out inquiry activities independently, and think throughout the process. The problem is a starting point of learning, and is also the basis for choosing knowledge. Problem-orientated learning is to take problems as a link with learning, let students study with problems, analyse problems through independent thinking and cooperative exploration, and find solutions to solve problems.

The main purpose of designing questions is to guide the inquiry process, and we must realize that the students are the subject of inquiry. Therefore, it is necessary to arouse the students' initiative and enthusiasm in learning as much as possible. Designing questions are completely crucial and should be operable in practice. On the one hand, the problem should not be too simple; otherwise the students will have no desire to explore. On the other hand, the problem should not be too difficult; otherwise the students will lose their confidence in inquiry. What matters is that designing questions should be close to the

students' life experience and existing knowledge background. Only in this way can we guide the students to actively participate in the whole teaching process.

For example, in the first part of "preparation of the mother solution and medium". According to the students' existing knowledge background, some questions are designed as follows: (1) What kinds of medium are commonly used? What are the characteristics of each? (2) What are the components of the medium? What is the function of each? (3) Why should the mother solution be prepared first? How to prepare mother solutions?

4 Divide Students into Some Groups Reasonably, and Instruct Students to Cooperate in Inquiry

4.1 Put Forward the Theme of Inquiry

At present, it is generally believed that the factors affecting callus induction include the following: (1) the callus induction ability of different plants and different explants of the same plant; (2) the effect of type of medium; (3) the effect of different concentrations combinations of exogenous phytohormones; (4) the effect of culture conditions, namely humidity, temperature and light. In fact, the above factors are also the influencing factors of differentiation culture and rooting culture.

4.1.1 The Callus Induction Ability of Different Plants and Different Explants of the Same Plant

Whether the experimental material is suitable or not will play a decisive role in the experimental results. Some parts are difficult to dedifferentiation, or the rate of redifferentiation is very low, so the rate of induction is related not only to different species of plants, but also to different organs of the same plant. In general, woody plants, with high tannin or pigment content, are not suitable for experimental material than herbaceous plants, which because synthetic precursors of tannin or pigment, phenolic glycosides, are more prone to browning than herbaceous plants in the process of callus induction and redifferentiation. Therefore, it is more difficult for woody plants to induce callus compared with herbaceous plants. As far as herbs are concerned, generally, annual herbs are easier to induce callus than perennial herbs.

Explants are usually disinfected by soaking in 75% alcohol solution for 30 s, and then soaking in 0.1% liter mercury solution for 5–12 min. Its disinfection effect is great, but it also has shortcomings, such as high toxicity of liter mercury solution. Liter mercury solution is easy to be inhaled, ingested and absorbed through the skin, so everyone should be extra careful in the use process. In addition, it is difficult to remove the residual liquid, and the recovery treatment of waste liquid after use is troublesome and costly. If not properly treated, it will also bring considerable pollution to the environment. It is worth noting that some studies have shown that sodium hypochlorite solution can replace the liter mercury solution, but the specific concentration and duration of use are unknown, which can be used as a topic for us to inquiry.

To sum up, we can come to two topic as follows: (1) evaluate the effect of different organs of the same plant on the rate of callus induction; (2) how to substitute liter mercury with sodium hypochlorite solution in the process of disinfecting the explants.

4.1.2 The Effect of Type of Medium

As a carbon and energy source, sucrose can better maintain the hypotonic circumstance in the medium than glucose. Such as the preparation of the same mass fraction of medium, the osmotic pressure of sucrose is significantly lower than that of glucose. If glucose is used as carbon source, it is easy to make plant cells dehydration and poor growth, and the rate of sucrose absorption by plant cells is much slower than that of glucose, so the osmotic pressure formation of sucrose can be relatively stable for a long time. Additionally, in terms of energy supply, sucrose provides more energy than glucose at the same concentration. In the process of plant tissue culture, special attention should be paid to prevent the medium from being contaminated by microorganisms. It is known that glucose is the most suitable carbon source for microbial growth, and sucrose is less used, so using sucrose as the carbon source of medium can reduce microbial contamination to a certain extent.

The mass purity and dosage of agar not only affect the hardness of the medium, but also affect the absorption of nutrients by the explants, and then affect the experimental results. It is stated in the experimental textbook that the amount of medium agar is generally 0.3–1.0%, but the quality, the purity and the degree of gel are not marked.

To sum up, we can come to a topic as follows: analysis the effect of different agar dosage on the experimental effect.

4.1.3 The Effect of Different Concentrations Combinations of Exogenous Phytohormones

A different concentrations combination of exogenous phytohormones is suitable as the scheme of inquiry because it contribute to understand the internal relations and differences between the three essential stages of subculture, differentiation culture and rooting culture. Exogenous phytohormones mainly include auxin and cytokinin, which are necessary to induce callus formation, especially when the combination of both can stimulate callus formation more rapidly and strongly. The auxin common used are 2,4-D, IAA and NAA, in the range of 0.01–10 mg/L. The cytokinin are kinetin, zeatin and 6-BA, in the range of 0.1–10 mg/L. According to the actual situation, NAA and 6-BA are selected for this experiment.

To sum up, we can come to a topic as follows: how to induce callus formation, callus budding and callus rooting by adjusting the concentration of NAA and 6-BAA.

4.2 Let Each Group Choose Their Own Materials

Due to long training period or the limitation of region and season of plant materials used in many textbooks and research papers, it is not suitable for teaching experiment as experimental materials. This not only causes a lot of inconvenience of arrangement of experimental contents, but also affects the cultivation of students' scientific inquiry ability. Therefore, we let each group choose their own materials according to the experimental purpose and the scheme of inquiry. Groups can choose any feasible plant materials that can be easily obtained in the campus, the city and the surrounding areas according to local conditions, and collect information related to the materials they choose.

4.3 Instruct Each Group to Design the Experiment Scheme

Eventually, each group write an experimental plan and submit it to the teacher for review, and the adopted plan will carry out experimental inquiry under the guidance of the teacher. Only in this way can we change the role of students from "passive receiver" to "active learner". While the role of teachers is a guide, assistant and participant, this greatly stimulates students' enthusiasm for experimental inquiry. In the meanwhile, it urges students to combine theoretical knowledge with practice so close that students have a deep understanding of theoretical knowledge learned by them, which is the fundamental reason for carrying out experimental teaching in secondary vocational schools.

4.4 Instruct Each Group to Implement the Experimental Scheme

Teachers play the role of guiding, enlightening, answering questions and making demonstration, while students are the main body of the experiment, and the experiment process should give full play to the initiative of students. In the course of doing the experiment, the students' irregular behaviour was corrected in time, and the relevant problems were explained in detail. Focus on summarizing the harvest and shortcomings of each experiment after the class. Through interactive teaching, the multi-level communication between teachers and students and between students is formed, and the learning effect is optimized, so that students can gradually master the method of analysing problems, and master the thinking and methods of solving problems.

5 Conclusion

By reforming the teaching method of plant tissue culture experiment, the experiment is used to transform students' passive learning into active learning. In the process of designing the experimental scheme, the students' understanding of the theoretical knowledge of plant tissue culture was further deepened. In the process of implementing the experimental scheme in group, the students exercised their operational skills. Secondary vocational schools need to train practical and innovative graduates, and inquiry teaching can achieve this. But, there are few cases of applying inquiry teaching in secondary vocational professional courses. Therefore, it is also very meaningful to study how to introduce inquiry teaching into the experimental teaching of other courses.

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