

A REVIEW OF INSTRUMENTS USED IN PLANT TISSUE CULTURE-PLANT BIOTECHNOLOGY

Abstract

Plant tissue culture biotechnology plays a vital role in plant propagation, genetic engineering, and conservation efforts. The success of tissue culture techniques heavily relies on the efficient utilization of various instruments throughout the process. This review provides a comprehensive overview of the instrumentation used in plant tissue culture biotechnology, highlighting recent advances and their applications. The review begins by discussing the fundamental equipment required for establishing a tissue culture laboratory, including laminar flow hoods, autoclaves, and incubators. It explores the significance of these instruments in maintaining aseptic conditions, sterilizing culture media and equipment, and providing optimal growth conditions for plant tissues. In addition to culture vessel-related instruments, the review highlights the crucial role of microscopes and imaging systems in plant tissue culture. It discusses the application of compound microscopes in observing tissue cultures, monitoring cell division, and assessing morphological changes during in vitro plant regeneration. Overall, this review provides a comprehensive understanding of the instrumentation used in plant tissue culture biotechnology and their role in achieving successful tissue culture outcomes. By examining recent advances and applications, this review aims to contribute to the advancement of plant biotechnology research and its practical applications in agriculture, horticulture, and conservation.

Keywords: Plant tissue culture, Instruments, biotechnology and application.

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I. INTRODUCTION

Plant biotechnology is a branch of biotechnology deals with the cultivation, modification and processing of food and medicinal plants. Plant biotechnology contribute in three major ways they are, growth and development of plants, production of food and pharmaceutical crude plant source and protection of plants against the exotic stress (1). The increase in food and medicinal plant demand throughout the world, as well as the unequal distribution of wealth, has increased the need for new technology that allows for greater yield and better quality of food products (2). A survey conducted by China on plant biotechnology shows that China is developing the largest plant biotechnology capacity outside of North America. The increase in government regulation and rising costs are the major factors that diverted the farmers in China to adopt genetically modified crops in their agriculture (3). Plant tissue culture (PTC) is a developing of plant tissue in nutrient media, *in vitro* growth of plant cells was first conducted or introduced by Haberlandt, Australian botanist. In the world, 80% of the population depends on traditional herbal medicine to meet their health needs. Plant tissue culture also refers to the sterile culture or *in vitro* culture of commercial plants. Plant tissue culture is an *in vitro* culture of cells, tissues, and their components in artificial physical and chemical conditions. It is the growth of isolated plants or their tissues in an artificial medium (4, 5).

The plant tissue culture is a laboratory experience or exciting process conducted on plant cells; tissue using various instruments, PTC needs the basic requirements for developing an *in vitro* growth of tissue in a aseptic aspect. Equipment used in the tissue and cell culture is Petri dish, Beaker, Laboratory flask, Erlenmeyer flask, Graduated cylinders and Laboratory glassware. The plant growth rooms will provide suitable parameters such as temperature and lighting regime (6). Yong Xu, Hanbin Wang, Walter Nsengiyumva in their paper concluded that the use of three dimensional optimal cultivation area in plant growth chamber produce aideal uniformity of light in the growth area (7). The temperature is the major factor that involves in the growth of the plant tissue in a growth chamber, study conducted by Song Woo Jeong and his team members found that cultivation of the lettuce leaves for 20days in 12°C and 20°C temperature produce a polyphenols rich- leaves(8). Plant tissue culture have evolved the development of the callus, anther, protoplasts etc., in the culture medium made of agar or liquid suspension .The most commonly used nutrient media's are Murashige and Skoog(MS), Modified MS, Gambotg's B5 medium and its modification, Woody Plant medium (WPM) and Driver and Kuniyuki Woody Plant medium (DKW). Petri dishes is a transparent lidded dish which is used by the biological in the culture of cells of bacteria, fungi etc (9). The goal of the paper is to explain the instruments used in plant tissue culture laboratories, their purpose, and the methodology. Instruments used in the plant tissue culture laboratories are Autoclave, Centrifuge, Hot air oven, laminar air flow, Microscope, CO2 incubator, pH meter, Chromatography, Spectrophotometer and Colorimeter. This paper reviews an overview of the instruments role in the modern plant tissue culture technology.

II. INSTRUMENTS USED IN PTC

- 1. Autoclave:** Autoclave is an instrument used for sterilization of the glassware used in the preparation of plants tissue, cells or organs by killing the bacteria, fungi, viruses in the glass ware before the culture process (10). The apparatus such as petridishes, beaker, flask, pipette, graduated cylinders and Erlenmeyer flask, the glass ware are mainly made

of Pyrex or Borosilicate glass. The autoclave is also used in sterilizing of nutrient media. The autoclaving temperature of the plant Growth nutrient media is 121°C and 15-20 psi. The Northern Arizona University in the college of Engineering, Forestry and Natural Science had developed a solar autoclave for rural areas at 2012 by Dr.Nelson – professor at CEFNS (11).

- **Principle:** Autoclave operates on the principle of moist heat sterilization, in which the inside material is sterilized under high pressure. The main principle of autoclave is steam sterilization; the other major principles of steam sterilization include Temperature, Time, Moisture, Drying, Air removal and direct steam contact (12).
 - **Methodology:** The nutrient media is sterilized before the culture, the pH of the media before the sterilization is 5.2 to 5.8 and after sterilization is maintained at greater of 5.7. There two types of sterilization mechanism include Physical and Chemical. Autoclave is based on the physical mechanism, the physical methods carried out by heat, radiation and filtration. Heat is used in autoclave for sterilization, it is transferred for a period Of 20 minutes which increases the pressure inside the chamber. The cycle of the sterilization may also vary for different laboratory procedures; the common cycle is between 1-1.5 hours (13).
 - **Purpose:** The Autoclave is used to prevent the growth of bacteria, fungi and other microbes during the cultivation process; the Autoclave is used to sterilize the culture vessels, Petri dishes, filter units, baby food jar, flask, stirring bar, beaker and pipette. Eg: The study conducted by the Joana Alves (microbiologist), he found that the single use plastics in the laboratory can be reused by chemical decontamination and Autoclaving. This process after the 7 weeks of study had reduced the plastic waste in the laboratory (14).
 - **Risk factor:**
 - Contamination due to moist heat mechanism.
 - The major risk factor is violent ejection of:
 - ❖ Pieces of apparatus;
 - ❖ Pressuring medium;
 - ❖ The vessel constants.
 - **Precautions:**
 - Proper orientation program for the person's going to use the autoclave or work on the plant tissue culture project.
 - Maintenance of temperature at 121°C or greater.
 - No person is allowed to use autoclave until the autoclave is in good condition.
 - Only professionals are allowed to use the instruments (15)
2. **Hot Air Oven:** Hot air oven is an electronic instrument used in the drying, sterilizing of surgical tools and glass ware used in Plant tissue culture. Moist heat used by the Autoclave can cause contamination in the sterilizing glass wares, so the alternative way for sterilizing the glass ware is dry heat mechanism of Hot air oven. But the hot air oven

takes long time for the sterilization process as compared to the Autoclave sterilization (16).

- **Principle:** The Hot air oven depends on the principle of dry heat sterilization; it doesn't need any moisture for the sterilization. There are four categories under which the equipment required to supply dry heat for sterilisation can be categorised. First, there is the hot air oven, then there is the conveyor oven, then there is transmitted heat, and finally there is dry heat in a vacuum. It is suggested to have a closer look at each of these approaches (17).
 - **Methodology:** Dry heat sterilization timing for sterilizing the materials is 170°C for 30 minutes, 160°C for 60 minutes and 150°C for 150 minutes (18).
 - **Purpose:** It is used to sterilize the oil containing materials, powders and glass & metal apparatus sterilization. It is smaller than the Autoclave and it is easy to use. Eg: The solar hot air oven manufactured using the simple material can able to generate temperature above 180°C and it changed the colour of the Browne's type 3 and 5.
 - **Risk factor:**
 - It does not kill some kind of microorganisms for every time
 - Burning of materials at high temperature oven.
 - Damage is the sharp ended equipment (19).
 - **Precaution:** Make careful to put on heat-resistant gloves, eye/face protection, and a lab coat before operating the device to prevent burns. You might also wish to put on a rubber apron, rubber sleeve protectors, and a face protection (20).
- 3. Plant Growth Rooms:** In experimental plant biology research, controlled settings created by plant growth chambers are essential for obtaining repeatable results. Commercial plant growth chambers can offer accurate controls of environmental factors including temperature, humidity, and light cycle, as well as the capacity to alter these factors through intricate programming. The injected culture vials is moved to a growth environment with regulated lighting and temperature for incubation. The upkeep of cleanliness in this area is essential. Positive air pressure in the "clean area" or an overhead air curtain at the entrance can be used to get rid of surface dust (21).
- **Principle:** The plant growth chamber or rooms has three major principles they are
 - **Temperature:** The most necessary and significant factor that influences the host is temperature. Temperature can be measured reliably, easily controlled, and is simple to measure. Typically, a non-calibrated thermometer is used, so the reading can change. Because it reads ambient air but not tissue temperature, the data that are recorded are erroneous. Using a mercury thermometer or an RMO couple, we can accurately measure ambient temperature, which almost constantly monitors the body temperature of plants. A fine thermocouple, a needle inserted into the plant tissue, or infrared remote sensors can all be used to measure the temperature of the tissue (22, 23).

- **Humidity:** Humidity is one of the critical elements that directly influence plant growth through transpiration, gas exchange, and altered energy balances. The primary factor contributing to the humidity in the room was the discharge of water content from plant surfaces. Vapour saturation difference is an important piece of information that can be used to directly calculate evaporation and stomata activity in plants.
 - **Light:** The main energy source for plant growth chambers and fields alike is radiation. Numerous research study opportunities, including those in photosynthesis, bioenergetics, and morphogenetic ets , are generated by the radiation action on plants. The radiation travels over space like a wave, but it actually consists of energy packets called photons (24).
 - **Problem:** Climates are defined as long-term changes in environmental variability, such as changes in temperature, light, humidity, and air movement. Take place during the growth season. Simple, low-cost equipment is unable to create circumstances with variations of less than 0.5 °F or 1% relative humidity. The complexity of constantly changing environments as they exist in the field cannot be accurately replicated with current technology (25).
 - **Guidelines:** It might be on all the time, 365 days a year, 24 hours a day. A growth chamber's maintenance costs should be taken into account when making the purchase. The upkeep and energy expenses of Cornell University's growth chambers were examined in the early 1980s (Langhans and Redder, 1981). \$30 for maintenance per square foot of chamber space (26).
4. **Microscope:** An instrument used to magnify small objects called a microscope. Even at the cellular level, some microscopes can be used to study an object, enabling researchers to view a cell's form as well as its nucleus, mitochondria, and other organelles (27). There are two major type of microscope used they are,
- **Scanning electron microscope (SEM) (28):** A focussed stream of electrons is projected and scanned over a surface by a scanning electron microscope (SEM) to produce a picture. The interaction between the beam's electrons and the sample results in a variety of signals that can be used to learn more about the surface's topography and composition.

The following parts make up the SEM machine:

- An electron gun is a device that produces highly energetic electrons.
- The electrons are transported through two or more electromagnetic lenses in column
- The scan coils that make up the deflection system.
- A secondary and backscattered electron detector.
- A sample-holding chamber.

The keyboard is used to control the electron beam in the computer system, and the viewing screen is used to display the scanned images.

- **Transmission Electron Microscope (TEM) (29):** Three key systems make up a transmission electron microscope: (1) an electron cannon that creates the electron beam and a condenser system that directs the beam onto the subject; (2) an image-producing system made up of the image-recording system, which transforms the electron image into a form perceptible to the human eye. The objective lens, movable specimen stage, objective, intermediate, and projector lenses, which focus the electrons passing through the specimen to form a real, highly magnified image. The image-recording system typically comprises of a digital CCD camera for long-term records and a fluorescent screen for viewing and focussing the image. In addition, power supplies, a vacuum system made up of pumps and the gauges and valves that go with them, are needed.
 - **Purpose:** To encourage a sterile environment and support the preservation of cell health, tissue culture microscopes are frequently installed inside bio safety cabinets. Therefore, a portable microscope may be useful for such activities. Pathologists can use microscopes to see anomalies that they would not be able to perceive with the unaided eye, such as minute structures, minute variations in colour, or the presence or absence of specific cell types in a specimen. Additionally, a number of contrast techniques can reveal even more details (30).
5. **Centrifuge:** A centrifuge is a machine that uses a rotor to utilise to separate particles from a solution. The elements in biology are typically, particles are referred to as cells, subcellular organelles, or big molecules (31).
- **There are two different kinds of Centrifuge Procedures:** the preparatory one, whose goal is to isolate particular particles, and the analytical one, whose task is to measure the physical characteristics of the Sedimenting particles. Each particle in the sample experiences a centrifugal force as the rotor of the centrifuge rotates; the force is proportional to the sedimentation rate of the particle. Globally, geotechnical centrifuges are used to examine constructions whose behaviours are highly reliant on the physical characteristics of soil (31).
 - **Methodology:** The method for the separation of the plant DNA using the centrifuge, in a food dehydrator, tissue is dried for 12 to 24 hours before being ground into a powder for DNA extraction. Dicot tissue can be mass-powdered using glass beads and a commercial paint mixer in centrifuge tubes. When using the paint mixer, tissue never comes into contact with common surfaces that could cause cross contamination, which could be advantageous if the DNA is going to be used in PCR experiments. The DNA is of a calibre comparable to that derived from either lyophilized or fresh frozen tissue, which is frequently utilised in labs. The presented method has the advantages of being quick, not requiring expensive equipment, and being able to be utilised in circumstances when a large number of samples need to be extracted (32).
6. **CO₂incubator:** Numerous benefits of tissue culture include the quick growth of plants, the bulk production of seedlings free of viruses and disease, and the protection of plant species resources. However, generally used tissue culture techniques are typically based on mediums with added sugar, which is in favour of bacteria's quick proliferation and

expansion in the culture media, which eventually contaminates the habitats where plants grow (33).

- **Purpose:** Later, scientists carried out numerous studies on sugar-free tissue culture that included enhancing CO₂ concentration, enhancing illumination, introducing large-scale culture containers with gas-permeable membrane, and formulating sugar-free cultural media. There were numerous varieties of growth channels. Constructed and created [9–11] in which CO₂ was indirectly enhanced. In order to indirectly raise the CO₂ content in the tissue cultural containers in the chambers, gas-permeable sheet covering tissue culture containers was placed within large-scale growth chambers. It is conceivable that a substantially higher CO₂ concentration than that required in the containers would need to exist in the chambers where the containers are placed in order to enhance the CO₂ concentration in the containers (34, 35).

7. **Laminar Air Flow Bench:** In the US, laminar airflow systems were initially used in operating rooms in 1964. In 1972, Charnley introduced the concept of an operating room atmosphere that was “ultra-clean” due to worries about contamination in the surgical field. He demonstrated a drop in PJI from 7% to 0.5% during a ten-year period without the use of perioperative antibiotics. Other studies have revealed that this clean air technology can reduce infection rates by as much as 92% (36).

Advantages

- The contamination inside the chamber is comparatively lower than the outside.
 - Air flow at one direction
 - UV radiation protection (37)
8. **Colorimeter:** The study of the phenomenon of light absorption by molecules in solution is called photometry. In quantitative measurements, the ability of a chemical to specifically absorb light at a given wavelength is helpful. A certain quantity of light is absorbed by a solution when a beam of light of a certain wavelength is passed through it; as a result, the intensity of the light that emerges from the solution is reduced (38). When light is absorbed by a solution, Beer-Lambert’s law is followed. According to Beer’s law, the amount of light that is transmitted diminishes exponentially as the concentration of the absorbing substance increases. According to Lambert’s law, the amount of light that is transmitted diminishes exponentially as the thickness of the absorbing substance increases (39).
- **Purpose:** A crucial part of the plant life cycle is played by nitrogen (N). It is the primary mineral nutrient for plants that is required for the synthesis of chlorophyll and other components of plant cells. Plant N condition affects crop output. As a result of optimisation, the nitrogen fertilisation has such a positive influence on the environment and the economy; it has become the focus of significant research. It also finds the concentration of nitrate, ammonia and phosphorus in the soil (40).

III. CONCLUSION

In conclusion, the instrument used in plant tissue culture biotechnology plays a crucial role in the success of laboratory experiments and the overall progress of plant biotechnology research. These instruments are specifically designed to provide a controlled environment for the growth and development of plant tissues, ensuring optimal conditions for cellular regeneration and plant propagation. The instrumental tools used in plant tissue culture biotechnology are indispensable in providing a controlled and sterile environment for the propagation and manipulation of plant tissues. Their contributions have greatly facilitated advancements in plant biotechnology research, leading to improved crop production, disease resistance, and the development of novel plant varieties. As technology continues to advance, we can expect further innovations in instrument design and functionality, propelling the field of plant tissue culture biotechnology to new heights.

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