Chapter 2

General Laboratory Supplies and Practices

2.1 Selection and preparation of plant material

In order to initiate the growth of banana plants in a controlled environment, we obtained explants (referred to as Banana Suckers) from the Sokhada village in the Baroda district, which were originally being cultivated in the nearby local farming region.

For the establishment of cultures, the banana suckers were first thoroughly washed under running tap water to remove soil and other debris. Following the washing, the banana suckers were cut, and shoots measuring 10 cm in length were isolated to serve as explants. Subsequently, these explants underwent a 30-minute treatment with Bavistin, followed by a rinse with distilled water. The explants then underwent a series of treatments. They were initially subjected to sodium hypochlorite along with a few drops of Tween-20 for a duration of 5 to 20 minutes. This was followed by a surface disinfection using sodium hypochlorite for 3 to 5 minutes. After each chemical treatment, the explants were thoroughly rinsed with autoclaved double-distilled water, repeating this process 3 to 4 times. The treated explants were then placed onto MS media (consisting of MS salts, 3.0% sucrose, 0.8% agar, and 5 mg l^{-1} BAP) under aseptic conditions within a laminar airflow cabinet (Yorco) was the supplier of this equipment. Following the initial proliferation phase, the developed shoots were transferred to fresh medium of the same composition for further multiplication. The detailed composition of the MS medium can be found in Table 2.1.

Chemicals

Based on the specific needs of the study, a range of chemicals including AR, LR, GR, and occasionally locally sourced chemicals were employed for the experiments. Throughout the investigation, various brands of chemicals were utilized, including HI-MEDIA Laboratories, Fine Chemicals, and Sigma Chemicals Company from the USA.

Glassware and Plasticware

Borosil manufactured Erlenmeyer flasks of different capacities (100 ml narrow mouth, 100 ml wide mouth, 150 ml), along with neutral glass culture bottles with a capacity of 400 ml from Hindustan National Glass Industries Ltd. in Bahadurgarh, Haryana, were employed as culture vessels for tissue culture investigations. Phytajars (100 ml, 2000 ml, 5000 ml capacity) and beakers (100 ml, 250 ml, 1000 ml, 2000 ml capacity) from Laboplast

in India, as well as measuring cylinders of 10-, 25-, 100-, and 1000-ml capacity sourced from either Borosil or Laboplast in India, were used. Glass pipettes from Borosil in India were utilized, and for precise tasks, variable volume transfer pipettes from Merck and Eppendorf in Germany were employed. These pipettes were particularly valuable during the precise administration of plant growth regulators and for tasks involving biochemical work.

For the diverse studies conducted, a variety of equipment and materials were employed. Pestle–mortar made from Neutral Glass, test tubes sized at 15 x 50 mm, and funnels with a diameter of 70 cm, all of which were manufactured by Borosil in India, were utilized. Polypropylene centrifuge tubes were sourced from Eppendorf in India, while microtips from Tarsons in India were also used. Acrylic chambers with a volume of 7500 cm^3 were locally fabricated and employed for the experiments. When it came to histological investigations, microslides and micro–cover glass (both round and rectangular) from Bluestar in India were used for slide preparation.

Glassware, upon reuse, underwent a chromic acid treatment by being immersed in the acid overnight. Subsequently, a comprehensive washing procedure was performed using tap water. This was followed by washing with liquid detergent and then again with tap water to ensure complete removal of the detergent residue. After this thorough washing with distilled water, the glassware were subjected to drying in a hot air oven set at 120°C for a duration of 24 hours. In the case of plasticware, such as plastic containers, tubes, and other items, they were initially washed using liquid detergent, followed by a rinse with tap water. To finalize the cleaning process, the plasticware were thoroughly rinsed with distilled water. Once cleaned, these plastic items were placed in a dust-free environment for the purpose of drying.

2.2 Methods of plant tissue culture

2.2.1 Culture media

All the plant tissue culture media shared a common composition, encompassing inorganic nutrients, vitamins, amino acids, plant growth regulator(s) carbon source(s), a gelling agent, and potentially organic supplements as required. The fundamental medium employed in all experiments was Murashige and Skoog's medium (1962).

The medium preparation process involved the addition of inorganic nutrients, gelling agents, and carbon sources (all stored as stock solutions) to lukewarm double

distilled water. The mixture was then heated until full dissolution was achieved. Following this heating process, the pH of the medium was adjusted to a value of 5.8±0.02 using 1N NaOH and/or 1N HCl. This pH adjustment was performed prior to sterilization, and the pH meter used was pre-standardized from Systronics in India.

The prepared medium was manually dispensed into various vessels, including culture tubes, conical flasks, and culture bottles, according to the specific needs of each experiment. Culture bottles were sealed with either vented or unvented polypropylene caps, while the remaining vessels were stoppered using non-absorbent cotton plugs.

To ensure sterility, the culture media were autoclaved at 15 psi (1.06 kg cm^{-2}) and a temperature of 121°C for a duration of 15-17 minutes. Following autoclaving, all culture vessels containing the media were stored in an environment free from dust.

Inoculations and maintenance of cultures

All aseptic inoculation procedures were conducted within a Yorco Laminar Flow Clean Air Bench located in India. The process initiated with thorough swabbing of the bench's surface using cotton soaked in a 70% isopropanol. Subsequently, various items such as culture vessels containing media, filter papers, double distilled water, mercuric chloride, a Coplin jar, and a spirit lamp filled with 90% alcohol were positioned on the bench floor. Additionally, a glass bead sterilizer (Dent–eq, Bangalore, India) was placed on the bench for heat sterilization of forceps and scalpels. The Laminar Flow Bench's shutter was then closed, and the contained chamber, along with all the items inside, was subjected to sterilization using ultraviolet radiations emitted by the UV tube within the chamber.

The explants that had been sterilized were carefully cut on autoclaved filter paper and subsequently placed onto the medium with the aid of sterilized forceps. Once inoculated, the culture vessels were placed within a culture room, where they were allowed to grow and observations were recorded.

To ensure the well-being of the cultures, a consistent subculturing routine was maintained, involving the transfer of cultures to fresh medium at predefined intervals. During subculturing of shoot cultures, they were divided into smaller groups if necessary. Any undesirable callus or necrotic tissues were meticulously removed using a scalpel, and the healthy propagules were then introduced onto fresh medium. The specific frequency

and duration of these subculturing procedures have been comprehensively outlined in the corresponding chapters for further reference.

2.2.2 Culture conditions

Following the inoculation process, the cultures were placed in specialized culture racks equipped with fluorescent tubes, ensuring a light intensity of 2000-3000 lux (approximately 30-45 μmol $m^{-2}s^{-1}$). To simulate natural day-night cycles, the cultures were subjected to 16 hours of light and 8 hours of darkness, regulated by timers. To ensure optimal conditions, the temperature within the culture room was upheld at a constant 28 ± 2°C, achieved through the use of Hot Air Convectors (Olympus, India) and Air Conditioners (Amtrex and Carrier Aircon) equipped with Temperature Controlling Units.

Greenhouse environment and nursery maintenance

For the process of hardening and acclimatization, the plantlets were transferred to the greenhouse facility within the Department of Biotechnology, Atmiya University in Rajkot. In this greenhouse environment, a controlled reduction in humidity levels was achieved, transitioning from 70% to 50%, through the implementation of a Fan–Pad evaporative cooling system. The temperature was maintained at 28±2°C.

Statistical analyses

The data collected for various studies underwent analysis using established statistical methods. Adequate replicates and control groups were included as needed for proper comparisons in different investigations. The experiments followed a completely randomized design (CRD), and standard deviation calculations were performed on the recorded data using standard statistical procedures, and use DMRT employing software like XLSTAT.

Table 2.1 Composition of Murashige and Skoog's (MS) and Schenk and Hildebrandt's (SH) Nutrient Medium.

Nutrients	MS	SH
Macronutrients		
NH_4NO_3	1650.0	–
KNO_3	1900.0	2500.0
$CaCl_2.2H_2O$	440.0	200.0
$MgSO_4.7H_2O$	370.0	400.0
KH_2PO_4	170.0	–
Na_2–EDTA	37.35	20.0
$FeSO_4 7H_2O$	27.85	15.0
$NaH_2PO_4.2H_2O$	–	300.0
Micronutrients		
KI	0.83	1.0
H_3BO_3	6.2	5.0
$MnSO_4.4H_2O$	22.3	10.0
$ZnSO_4.7H_2O$	8.6	1.0
$Na_2MoO_4.2H_2O$	0.25	0.10
$CuSO_4.5H_2O$	0.025	0.20
$CoCl_2.6H_2O$	0.025	0.10
Vitamins		
Nicotinic acid	0.5	0.5
Pyridoxine HCl	0.5	0.5
Thiamine HCl	0.1	5.0
Inositol	100.0	1000.0
Others		
Glycine	2.0	2.0
Agar	0.8%	0.8%
Sucrose	3.0%	3.0%
pH	5.8	5.8

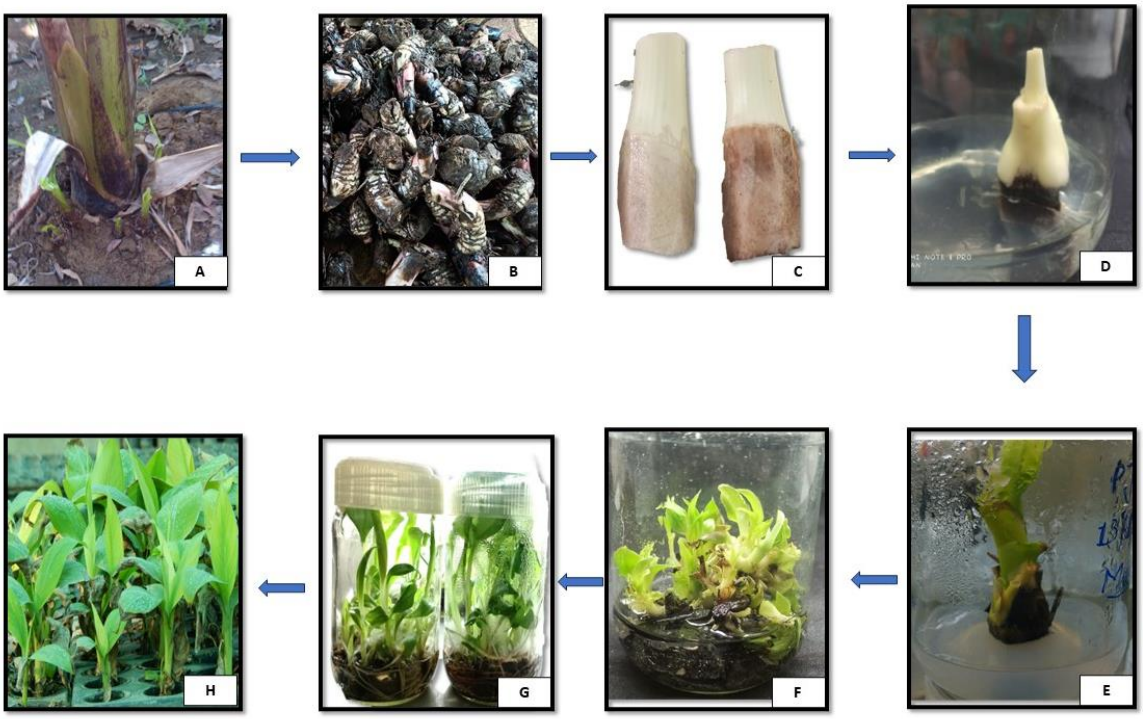

Figure 2.1 Establishment of banana tissue culture in the Plant Biotechnology Laboratory, Department of Biotechnology, Atmiya University for Ph.D. dissertation. (A) Mother plant of banana, (B) Sucker collected from mother plant, (C) Prepare explant for inoculation, (D) Explant inoculation on MS medium, (E) Shoot formation in explant, (F) *In vitro* multiplication stage, (F) *In vitro* rooting stage, (G) Hardening of plantlets produced under greenhouse conditions