Chapter 2

General Laboratory Supplies and Practices

2.1 Selection and preparation of plant material

In order to establish rose (*Rosa hybrida* L.) plants in controlled conditions, we collected explants, also called nodal segments, from the Rajkot, Gujarat, India. These were initially grown in the neighbouring local farming region.

To prepare the rose nodal segments for culture establishment, they were initially washed thoroughly with running tap water to eliminate dirt and debris. After cleaning, the segments were trimmed, and 5 cm long shoots were selected as explants. These explants were then soaked in Bavistin for 30 minutes and subsequently rinsed with distilled water. Next, the explants underwent several treatments: first, they were exposed to sodium hypochlorite and a few drops of Tween-20 for 5 to 20 minutes, followed by surface disinfection with sodium hypochlorite for 3 to 5 minutes. After each chemical treatment, the explants were rinsed 3 to 4 times with autoclaved double-distilled water. The treated explants were then placed on MS medium (comprising MS salts, 3.0% sucrose, 0.8% agar, and 3 mg L^{-1} BAP) in a laminar airflow cabinet under sterile conditions. This equipment was supplied by Yorkco. For further multiplication, the developing shoots were transferred to fresh media of the same composition after the initial proliferation stage. The precise composition of the MS medium is detailed in Table 2.1.

Chemicals

The tests utilized a variety of chemicals, including analytical reagents (AR), laboratory reagents (LR), guaranteed reagents (GR), and sometimes locally produced chemicals, tailored to the specific needs of the study. The chemical brands employed in the investigation included HI-Media Laboratories, Fine chemicals, and Sigma chemicals company from the USA.

Glasswares and plasticwares

For tissue culture studies, various types of culture vessels were utilized, including 100 ml narrow-mouth and 100 ml and 150 ml wide-mouth Erlenmeyer

flasks made by Borosil, as well as 400 ml neutral glass culture bottles from Hindustan National Glass Industries Ltd. (Bahadurgarh, Haryana). Measurement cylinders with capacities of 10, 25, 100, and 1000 ml from Borosil and Laboplast (India) were also employed, in addition to phyta jars with capacities of 100, 2000, and 5000 ml, and beakers with capacities of 100, 250, 1000, and 2000 ml from Laboplast. For precise tasks, variable volume transfer pipettes from Merck and Eppendorf (Germany) were used, alongside glass pipettes from Borosil (India). These pipettes were particularly useful for biochemical examinations and the accurate administration of plant growth regulators.

A variety of equipment and materials were employed throughout the studies. This included 15 x 50 mm test tubes, 70 cm diameter funnels, and a neutral glass pestle-mortar, all manufactured by Borosil (India). Microtips were supplied by Tarsons (India), and polypropylene centrifuge tubes were provided by Eppendorf (India). Locally constructed 7500 cm^3 acrylic chambers were also utilized. For histology studies, slides were prepared using microslides and both round and rectangular micro-cover glass from Bluestar (India).

To sterilize glassware before reuse, it was soaked in chromic acid overnight. Subsequently, it underwent a thorough cleaning process with tap water, followed by washing with liquid detergent and another rinse with tap water to ensure all detergent residues were removed. The glassware was then rinsed with distilled water and dried in a hot air oven at 120°C for an entire day. For plasticware, including tubes and containers, the cleaning process started with liquid soap and tap water, followed by a careful rinse with distilled water. After cleaning, the plastic items were air-dried in a dust-free environment.

2.2 Methods of plant tissue culture

2.2.1 Culture media

The plant tissue culture medium used in all the studies had a consistent composition, comprising vitamins, amino acids, inorganic nutrients, carbon sources, plant growth regulators, and a gelling agent when needed. The foundational medium was based on Murashige and Skoog (1962). To prepare the medium, lukewarm double-distilled water was combined with carbon sources, gelling agents, and

inorganic nutrients, all of which were stored as stock solutions. This mixture was heated until fully dissolved. The medium's pH was adjusted to 5.8±0.2 using 1N HCl or 1N NaOH, with a pre-standardized pH meter from Systronics (India) ensuring accuracy before sterilization. Depending on the specific requirements of each experiment, the prepared medium was manually dispensed into various vessels. Culture bottles were sealed with polypropylene caps, either vented or unvented, while other containers were stoppered with non-absorbent cotton plugs. The culture medium was sterilized by autoclaving for 15 to 17 minutes at 121°C and 15 psi (1.06 kg/cm²). Post-autoclaving, all culture vessels containing the medium were stored in a contamination-free environment.

Inoculation and maintenance of cultures

All aseptic inoculation activities were conducted using a Yorco (India) Laminar flow clean air bench. Initially, the bench surface was thoroughly cleaned with cotton soaked in 70% isopropanol. The following items were then arranged on the bench: a spirit lamp filled with 90% alcohol, filter papers, mercuric chloride, a Coplin jar, culture jars containing media, and double-distilled water. Additionally, a glass bead sterilizer (Dent-eq, Bangalore, India) was placed on the bench to heat-sterilize forceps and scalpels. After setting up, the bench's shutter was closed, and the entire chamber's contents were sterilized using ultraviolet radiation from the UV tube inside the confined chamber.

Sterilized forceps were used to place the precisely cut explants onto the media after autoclaving and placement on filter paper. Following inoculation, the culture containers were kept in a culture room for growth and monitored regularly. To ensure the cultures remained healthy, a routine subculturing schedule was followed, involving transferring cultures to fresh media at specific intervals. If necessary, shoot cultures were divided into smaller groups for subculturing. Any unwanted callus or necrotic tissues were carefully removed with a scalpel before placing the healthy propagules onto new media. Detailed descriptions of the frequency and duration of these subculturing techniques can be found in the respective chapters for further study.

2.2.2 Culture conditions

After inoculation, the cultures were placed on specialized racks equipped with fluorescent tubes, providing 2000–3000 lux (approximately 30-45 μmol $m^{-2}s$-) of light. To mimic natural day-night cycles, the cultures were exposed to 16 hours of light and 8 hours of darkness, controlled by timers. The culture room temperature was maintained at a consistent 26 ± 2°C using Mitsubishi Heavy Industries air conditioners with temperature control units and Olympus hot air convectors (India) to ensure optimal conditions.

Greenhouse environment and nursery maintenance

The plantlets were transferred to the greenhouse facility at Atmiya University's Department of Biotechnology in Rajkot for hardening and acclimatization. In this greenhouse, a Fan–Pad evaporative cooling system was used to gradually reduce humidity from 70% to 50%. The temperature was consistently maintained at 28±2°C.

Statistical analyses

The data collected from multiple studies was analysed using established statistical methods. To ensure valid comparisons across studies, an ample number of replicates and control groups were incorporated. A completely randomized design (CRD) was employed for the studies. Standard deviation calculations were conducted using Duncan's Multiple Range Test (DMRT) and software packages like XLSTAT, while standard statistical techniques were applied to analyse the collected data.

Table 2.1 List of inorganic/organic salts, carbon source, vitamins, and solidifying agents used in generalized plant tissue culture media.

Sr. No.	Components	Elements	Inorganic/Organic salts
1	Macroelements	Nitrogen (N)	NH_4NO_3
			$(NH_4)_2SO_4$
		Potassium (K)	KNO_3
			KCl
			KH_2PO_4
			K_2SO_4
		Calcium (Ca)	$Ca(NO_3)_2.4H_2O$
			$CaCl_2.2H_2O$
		Magnesium (Mg)	$MgSO_4.7H_2O$
		Sodium (Na)	Na_2- EDTA
			$NaH_2PO_4.2H_2O$
			Na_2SO_4
		Ferrous (Fe)	$FeSO_4.7H_2O$
2	Microelements	Potassium (K)	KI
		Boron (B)	H_3BO_3
		Ferrous (Fe)	$Fe_2(SO_4)_3$
		Manganese (Mn)	$MnSO_4.4H_2O$
		Zinc (Zn)	$ZnSO_4.7H_2O$
		Sodium (Na)	$Na_2MoO_4.2H_2O$
		Copper (Cu)	$CuSO_4.5H_2O$
		Cobalt (Co)	$CoCl_2.6H_2O$
3	Vitamins	Calcium pantothenate	
		Thiamine HCl	
		Inositol	
		Nicotinic acid	
		Pyridoxine HCl	
4	Amino acids	Glycine	
		Cysteine HCl	
		Glutamine	
5	Carbon source	Sucrose	
6	Solidifying agents	Agar	

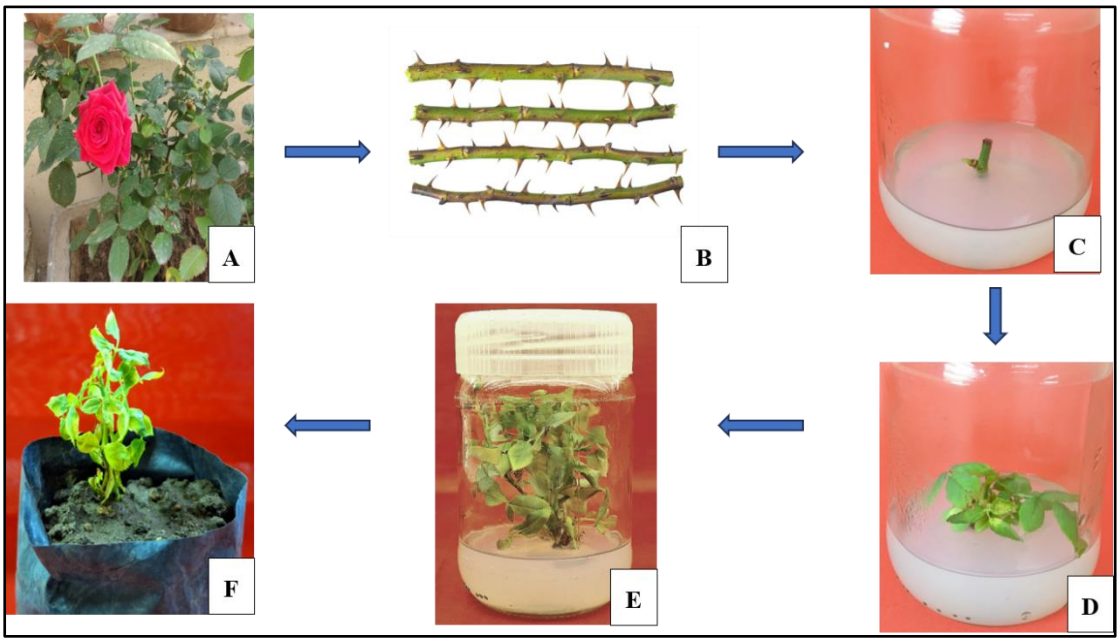

Figure 2.1 Establishment of rose tissue culture in the plant biotechnology laboratory, Department of Biotechnology, Atmiya University for Ph.D. work (A) Mother plant of rose (*Rosa hybrida* cv.), (B) Nodal segments collected from mother plant, (C) Explant inoculation on MS medium, (D) Shoot formation in explant, (E) *In vitro* multiplication stage and (F) Hardening of plantlets produced under greenhouse conditions (after rooting formation)