Standard Operating Procedure (SOP) for banana tissue culture media preparation

Authors & Contributors

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1. Introduction

Plant tissue culture involves growing plant cells, tissues, or organs on a sterile growth medium *in vitro* under controlled conditions of temperature, light, and humidity. An ideal plant tissue culture medium should have all the required minerals, nutrients, and vitamins which are sometimes supplemented with growth regulators and solidifying agents. Plant tissue culture is an integral part of banana breeding where it is used for embryo germination and micropropagation through shoot tip culture, requiring Murashige and Skoog |(MS) basal medium supplemented with various growth regulators. Morel and Wetmore vitamins (MWV) is used in place of MS vitamins for embryo germination.

2. Purpose

The purpose of this SOP is to provide guidance to tissue culture laboratory staff on culture media components and preparation.

3. Scope

This SOP describes tissue culture media preparation procedures implemented by the IITA Musa breeding program. The SOP covers preparation of culture media for embryo culture, shoot proliferation, and rooting of plants.

4. Definition of terms

- **Culture medium:** It is a source through which plants receive nutrients for growth, it is in either solid or liquid form.
- **Stock solution:** Stock solutions are concentrated solutions of groups of media components that are prepared ahead of time and are used to make several batches of culture media.
- **Hormones:** These are growth regulators influencing plant growth and development.

5. Roles and Responsibilities

Tissue culture expert: Responsible for managing and overseeing all tissue culture laboratory activities conducted by the banana breeding programme in IITA and collaborations with public and private sector labs in Africa.

Research associate / supervisor, Tissue culture laboratory: Responsible for managing and overseeing all laboratory activities, quality control, report writing, stock taking, training and demonstration/exhibition.

Laboratory research technician: Perform laboratory tasks such as embryo and shoor tip explant preparation, aseptic transfers and culture management, proper labelling including use of BTracT and data entry.

Laboratory attendant/assistant: Responsible for cleaning of the instruments and the laboratory

6. Procedure/Protocols

6.1 Required tools

- Analytical weighing balance

- Weighing boats
- Measuring cylinders
- Spatulas
- Droppers
- Magnetic stirring bars
- Heaters and stirrer
- pH Meter
- Dispenser
- Glass/plastic jars/vials, tubes, falcon tubes
- Culture tube racks
- Autoclave and autoclave baskets
- Refrigerators

7.0 **MS Basal Medium with vitamins**

MS Basal medium containing macro elements, microelements and vitamins is commercially available as a dehydrated powder. If using MS Basal medium powder, follow the instructions stated by the manufacturer on the pack.

Alternatively, MS basal medium (with vitamins) can be prepared from MS stock solutions (I, II, III, and IV) as described below. Note that MS stock solutions are not required if MS basal medium powder is used.

7.1 MS I – Macronutrients

Start with about 500ml of sterile water in a beaker. While stirring, weigh and add each chemical as listed below until completely dissolved before adding the next. Dissolve

$MgSO_4.7H_20$ and KH_2PO_4 separately before adding to others. Top up to 1000 ml with sterile distilled water, label appropriately with date and stock name and store stock in the refrigerator (4°C) in case it is not used instantly.

Table 1: Macronutrients: medium composition and stock components

Component	Final medium Concentration g/L	Stock concentration (10x in 1L)	Stock quantity to dispense per L of medium
KNO_3	1.9000	19.0 g	100 ml
NH_4NO_3	1.6500	16.50 g	
$CaCl_2.2H_20$	0.4400	4.40 g	
$MgSO_4.7H_20$	0.370	3.70 g	
KH_2P0_4	0.1700	1.70 g	

7:2 MS II- Iron-EDTA solution

Dissolve each component listed below separately in about 70 ml of SD water and heat until completely dissolved. Allow both solutions to cool slightly, mix and top up to 200 mL with SD water. Stock solution in amber bottle or clear bottle wrapped with aluminum foil, label appropriately and store in the refrigerator for maximum of one month.

Table 2: Iron-EDTA: medium composition and stock components

Component	Final medium Concentration mg/L	Stock concentration: 50x in 500ml	Stock quantity to dispense per L of medium
$FeSO_4.7H20$	27.8	1.3925 g	10 ml
$Na_2EDTA.2H_20$	37.3	1.8625 g	

7.3 MS III Micronutrients

Start with about 200 ml of sterile water in a beaker. While stirring weigh and add each component listed below until completely dissolved before adding the next. Top up with sterile water to 250 ml, label appropriately and store in refrigerator.

Table 3: Micronutrients: medium composition and stock components

Component	Final medium Concentration mg/L	Stock concentration: 50x in 250ml	Stock quantity to dispense per L of medium
$MnSO_4.H_20$**	16.9	0.8450 g	5 ml

H_3BO_3	6.2	0.3100 g	
$ZnSO_4.7H_20$	8.6	0.4300 g	
KI	0.83	0.0415 g	
$Na_2MoO.2H_20$	0.25	0.0125 g	
$CuSO_4.5H_20$	0.025	0.0013 g	
$C_OCl_2.6H_20$	0.025	0.0013 g	

**If using $MnSO_4.7H_20$, note that final medium concentration is 22.3 mg/L so weigh 1.1150 g for stock

7.4 MS IV – Vitamins

Start with 400 mL of SD water in a beaker. While stirring, weigh and add each component listed below until completely dissolved before adding the next. Top up with SD water to 500 ml. Dispense in falcon tubes and store in freezer.

Table 4: MS Vitamins: medium composition and stock components

Component	Final medium concentration mg/L	Stock concentration: 100x in 500 ml	Stock quantity to dispense per L of medium
Glycine	2	0.2 g	5 ml

Thiamine	0.4	0.04 g	
Pyridoxine	0.5	0.05 g	
Nicotinic Acid	0.5	0.05 g	

Table 5: Morel & Wetmore vitamin (MWV) solution: medium composition and stock components used for embryo culture.

	Final medium concentration mg/L	Stock concentration: 50x in 100 ml	Stock quantity to dispense per L of medium
Myo-inositol	100.00	5000mg	2 mL
Nicotinic acid	1.00	50	
Pyridoxine-HCl	1.00	50	
Thiamine-HCl	1.00	50	
Calcium Panthothenate	1.00	50	
Biotin*	0.01	0.5	

*Prepare Biotin stock, weigh 5 mg and dissolve in 10 mls ethanol 95%. Add I ml to other components stirring in water and bring up to volume. Keep stock in freezer.

7:5. Ascorbic acid

Start with about 200 ml of sterile water in a beaker. While stirring, weigh and add ascorbic acid until completely dissolved. Pour in a bottle, label appropriately and store in a refrigerator.

Table 6: Ascorbic acid: medium concentration and stock quantity

	Final medium	Stock	Stock quantity

Component	Concentration mg/L	50x in 250 ml	To dispense /L
Ascorbic acid	20	1	5 ml

7:6 Hormones

7:6:1 BAP (for shoot proliferation)

Weigh 0.1 g BAP and dissolve in 1-5 ml of NaOH(1M). Top up to 100 ml with sterile water and dispense 5 ml/L of medium. Label stock appropriately and store in a refrigerator if not used instantly or dispense in falcon tubes and store in freezer if not used regularly. BAP is the cytokinin of choice for bud/shoot proliferation in vitro and is required at a concentration of about 5 mg/L. Note that BAP concentrations can be adjusted depending on the proliferation rates and stages of genotypes.

7.6. 2NAA (for rooting)

Weigh 0.00372 g NAA and dissolve in few drops of absolute ethanol. Top up with sterile water to 100 ml and dispense 5 ml/L of medium. The final concentration in media is 5mg/L. Store in a refrigerator if not used instantly or dispense in falcon tubes and store in freezer if not used regularly.

8.0 MEDIA PREPARATION PROTOCOLS

The following general steps apply for all media preparation using the underlisted component and stock amounts for various culture media types (Table 7 and 8).

1. Decide on the type and quantity of media to prepare and write down components (media preparation checklist).
2. Begin with about ½ the final volume of distilled water in a beaker or flask.
3. While stirring, weigh and add sucrose and allow dissolving.
4. Add appropriate quantities of the various MS basal medium (with vitamins) or various stock solutions according to the list of components, one at a time, waiting till each dissolve before adding the next and check it off on the list as it is added.
5. Measure and add the hormones stock depending on the type of media (proliferation, rooting or embryo culture) and ascorbic acid.
6. Top up the volume with distilled water to the final volume leaving off few mls for pH adjustments later.
7. Measure the pH and adjust to the required pH 5.8 using 1M HCL or 1M NaOH.
8. Weigh and add the gelling agent (phytagel) and heat up media for until gelling agent is completely dissolved (boiling).
9. Dispense the medium in the desired culture vessels (baby jars, 30 ml; test tubes, 10 ml; magenta vessels 40 ml) and autoclave at 121^{0}C for 15mins.
10. Leave in sterile area to cool and harden before use or seal properly and store in refrigerator for use later.

8.1 Shoot tip culture proliferation and rooting media

Table 7: Components for shoot tip proliferation and rooting media preparation

Component	**Amount to weigh or dispense for 1L of medium**	
	Proliferation	**Rooting**
Sucrose	30 g	30 g

MS basal medium (with vitamins) powder	4.33 g	4.33 g
Or MS stock solutions		
MS I Macro elements stock	100 ml	100 ml
MS II Fe-EDTA stock	10 ml	10 ml
MS III Micro elements stock	5 ml	5 ml
MS IV Vitamins stock	5 ml	5 ml
Ascorbic acid stock	5 ml	5 ml
BAP (1 mg/ml)	5 ml or 2ml	0
NAA (1mg/ml)	0	5 ml
pH	5.8 ± 0.1	5.8± 0.1
Phytagel	2.35g	2.35g

8.2 Embryo culture medium

The steps followed for media preparation are the same as above but following the components in table 8.

Table 8: Components for embryo culture medium (Bakry, 2008).

Component	Amount to weigh or dispense for 1L of medium
MS I	100 ml
MS II	10 ml
MS III	5 ml
Casein hydrolysate	500 mg
MWV	2 ml
Sucrose	25 g
BAP 1mg/ml	1 ml
IAA 0.1mg/ml	4 ml
Gelrite	1.5 g
pH	5.8±0.1

10. REFERENCES

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Title & Version History

Crop: One of the mandate crops of IITA breeding focus

Function: A specific module or task under a breeding program

SOP Owner: Function lead responsible for implementing and updating the SOP

SOP #: Record the SOP version number

Revision #: Record the SOP new version number

Implementation Date: Record effective date of the SOP

Last Reviewed/Update Date: Record the last reviewed date of the SOP

Approval Date: Record the date the SOP was approved for implementation