

Practical write up- C14PPlant Biotechnology1(a)

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MS MEDIA PREPARATION FOR PLANT TISSUE CULTURE

OBJECTIVE:

Media preparation is one of the primary and most essential steps in tissue culture. Media is prepared based on the type of tissue being cultured. Most media differentiate from each other based on the requirements of the growth of the specimen it supports.

Things needed-

- - Sterile Beakers and test tubes
- -Spatula
- -Volumetric flask
- -Autoclave
- -Electronic balance
- -magnetic stirrer
- -PH meter
- Chemicals
- Distilled water

First we will make five different stocks of higher concentrations and then mix them to prepare the media.

We will weigh the following components on a digital weighing balance and make these five stocks according to the given composition in distilled water. We will use sterile tubes and flasks and spatula the whole time. We will use magnetic stirrer if needed to mix the components.

Stock A(1000ml) strength 20X

NH₄NO₃- 33g

KNO₃-38g

CaCl₂.2H₂O-8.8g

MgSO₄.7H₂O-7.4g

KH₂PO₄-3.4g

Stock B(100ml) strength 500 X

H₃BO₃-0.310g

KI-0.0415g

NaMoO₂.2H₂O-0.0125g

CoCl₂.6H₂O-0.00125g

MnSO₄.4H₂O-1.115g

ZnSO₄.7H₂O-0.430g

CuSO₄.5H₂O-0.00125g

Stock C(200ml) strength 500X

FeNa₂EDTA-3.67g

Stock D(100ml) strength 500X

Thiamine HCl- 5mg

Pyridoxine HCl-25mg

Niacine-5mg

Glycine-100mg

Stock F(100ml) strength 500X

Myoinositol-5g

After making the stocks, we will take the following amounts(written below) from the respective stocks and mix them, then we will add the following amount of sucrose(written below) and make up the volume upto 500 ml with distilled water. We will then add the following amount(written below)of Agar.

Stock A-25ml

Stock B-1ml

Stock C-1ml

Stock D-1ml

Stock F-1ml

Sucrose-15gm

Agar-4gm(0.8%)

After mixing everything we will check the pH, the pH should be between 5.6-5.8. If the pH is less or more we will have to adjust it by adding base or acid accordingly.

We will pour the media in a autoclavable flask and cover its mouth properly with cotton plug and aluminium foil and autoclave the media.

Note- We will add required hormones and antibiotics depending upon the requirement of the specific experiment.