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Composition and Preparation of Plant Tissue Culture Medium

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Abstract

Plant tissues and organs are grown *in vitro* on artificial media, which supply the nutrients necessary for growth. A culture medium is a complete mixture of nutrients and growth regulators .The basic nutrient requirements of cultured plant cells are very similar to those of whole plants. The clue for developing a basic culture medium seems to have initially come from the nutritional requirements of plants growing in soil, and later from nutrient solutions used for whole plant culture . When mineral salts are dissolved in water, they undergo dissociation and ionization. The active factor in the medium is the ions of different types rather than the compounds. One type of ion may be contributed by more than one salt in the medium. Therefore, a meaningful comparison between two media can be made on the basis of total concentrations of different types of ions in them. The most commonly used medium is the formulation of Murashige and Skoog (1962). The Murashige and Skoog (1962) (MS) or Linsmaier and Skoog (1965) (LS) are the most widely used salt compositions, especially in procedures where plant regeneration is the objective.

Introduction

The growth, development and morphogenic response of an explant in culture depends on its genetic make-up, surrounding environment and composition of the culture medium. The success of a plant tissue culture experiment largely depends on the selection of right culture medium. A culture medium is a complete mixture of nutrients and growth regulators. The clue for developing a basic culture medium seems to have initially come from the nutritional requirements of plants growing in soil, and later from nutrient solutions used for whole plant culture. Nutritional requirements for optimal growth of a tissue *in vitro* may vary with the species. Even tissues from different parts of a plant may have different requirements for satisfactory growth (Murashige and Skoog, 1962) [4]. As such, no single medium can be suggested as being entirely satisfactory for all types of plant tissues and organs. When starting with a new system, it is essential to work out a medium that will fulfill the specific requirements of that tissue. During the past 25 years, the need to culture diverse tissues and organs has led to the development of several recipes. Some of the earliest plant tissue culture media, e.g. root culture medium of White (1943) and callus culture medium of Gautheret (1939), were developed from nutrient solutions previously used for whole plant culture. White evolved the medium from Uspenski and Uspenskaia's medium (1925) for algae, and Gautheret's medium is based on Knop's (1865) salt solution. All subsequent media formulations are based on White's and Gautheret's media.

Constituents Media (amount in mg 1-)							
	White's	**Heller's[3]**	**MS**	**ER**	**B5**	**Nitsch's[5]**	**NT**
Inorganic							
NH_4NO_3	-	-	1650	1200	-	720	825
KNO_3	80	-	1900	1900	2528	950	950
$CaCl_2.2H_20$	-	75	440	440	150	-	220
$CaCl_2$	-	-	-	-	-	166	-
$MgSO_4$. 7 H_20	750	250	370	370	247	185	1233
KH_2PO_4	-	-	170	340	-	68	680
$(NH_4)2SO_4$	-	-	-	-	134	-	-
$Ca(NO_3)_2.4H_20$	300	-	-	-	-	-	-
$NaNO_3$	-	600	-	-	-	-	-
Na_2SO_4	200	-	-	-	-	-	-
NaH_2 PO_2 .H_20	19	125	-	-	150	-	-
KCl	65	750	-	-	-	-	-
KI	0.75	0.01	0.83	-	0.75	-	0.83
H_3BO_3	1.5	1	6.2	0.63	3	10	6.2
$MnSO_4$. 4 H_20	5	0.1	22.3	2.23	-	25	22.3
$MnSO_4$. H_20	-	-	-	-	10	-	-
$ZnSO_4$. $7H_20$	3	1	8.6	-	2	10	-
$ZnSO_4.4H$							
$ZnNa_2$.EDTA	-	-	-	15	-	-	-
Na_2Mo $O_4.2H_2$							
MoO_3	0.001	-	-	-	-	-	-
$CuSO_4$. $5H_20$	0.01	0.03	0.03	0	0.03	0.025	0.025
$COC1_2.6H_20$	-	-	0.03	0	0.03	-	-
$COSO_4.7H_20$	-	-	-	-	-	-	0.03
$A1CI_3$	-	0.03	-	-	-	-	-
$NiC1_2.6H_20$	-	0.03	-	-	-	-	-
$FeC1_3.6H_20$	-	1	-	-	-	-	-
$Fe_2(SO_4)$ $_3$	2.5	-	-	-	-	-	-
$FeSO_4$. $7H_20$	-	-	27.8	27.8	-	27.8	27.8
Na_2 EDTA. $2H_20$	-	-	37.3	37.3	-	37.3	37.3
Sequestrene 330Fe	-	-	-	-	28	-	-

Organic							
Inositol	-	-	100	-	100	100	100
Nicotinic acid	0.05	-	0.5	0.5	1	5	-
Pyridoxine.HCl	0.01	-	0.5	0.5	1	0.5	-
Thiamine-HCl	0.01	-	0.1	0.5	10	0.5	1
Glycine	3	-	2	2	-	2	-
Folic acid	-	-	-	-	-	0.5	-
Biotin	-	-	-	-	-	0.05	-
Sucrose	2%	-	3%	4%	2%	2%	1%
D-Mannitol	-	-	-	-	-	-	12.70%

Table 1: Composition of Some Plant Tissue Culture Media.

The Plant Tissue Culture Medium components

One of the most important factors governing the growth and morphogenesis of the plant tissues in culture is the composition of the culture medium. The basic nutrient requirements of cultured plant cells are very similar to those of whole plants. The basic requirements of mineral elements required for the growth of plant tissues are fulfilled by providing their common salts in the medium. When mineral salts are dissolved in water, they undergo dissociation and ionization. The active factor in the medium is the ions of different types rather than the compounds. One type of ion may be contributed by more than one salt in the medium. Therefore, a meaningful comparison between two media can be made on the basis of total concentrations of different types of ions in them. Plant tissue culture media provide not only these inorganic nutrients, but usually a carbohydrate (sucrose is most common) to replace the carbon which the plant normally fixes from the atmosphere by photosynthesis. To improve growth, many media also include trace amounts of certain organic compounds, notably vitamins, and plant growth regulators.

Plant tissue culture media are generally made up from solutions of the following components:

- Macronutrients
- Micronutrients
- Vitamins
- Amino acids or other nitrogen supplements
- Carbohydrates or sugars
- Solidifying agents or supporting systems and
- Growth regulators (plant hormones)

Macronutrients/Microelements

Macronutrients are the components which the plants need in major or high quantities. They provide the six major elements; nitrogen, phosphurus, potassium, calcium, magnesium and sulfur in addition to oxygen, carbon and hydrogen. The optimum concentration of each nutrient for achieving maximum growth rates varies considerably among species. According to the recommendations of the international association for plant physiologist the elements needed by plants in quantities greater than 0.5 g/l is classified as **macronutrients.**

Constituent	Forms of availability	Role
Potassium	K^+	Necessary for normal cell division, and synthesis of proteins and chlorophyll
Magnesium	Mg_2^+	Component of chlorophyll molecule
Calcium	Ca_2^+	Constituent of cell wall; involved in the regulation of hormone responses and could have a pre-emptive role in morphogenesis; deficiency may cause shoot tip necrosis
Nitrogen	NO^{3-} NH_4^+ Organic Nitrogen (vitamins/ amino acids	Important constituent of amino acids, vitamins, nucleic acids and proteins; indirectly affects growth by its influence on pH of the medium; NH4+ is necessary for somatic embryogenesis in cell and callus cultures
Phosphorus	PO_4^{3-}	Vital for cell division; storage and transfer of energy (part of AMP, ADP and ATP)
Sulphur	SO_4^{2-}	Present in some amino acids (cysteine, cystine and methionine) and proteins

Table 2: Role of macronutrients plant tissue culture media

Micronutrients/Microelements/Minor elements

Micronutrients are elements required by the plants in small quantities, which usually does not surpass a few milligrams. The essential micronutrients for plant cell and tissue growth include iron, manganese, zinc, boron, copper, cobalt and molybdenum. According to the recommendations of the International Association for Plant physiologist the elements needed by plants in quantities less than 0.5 g/l will be considered as micronutrients.

Constituent	Forms of availability	Role
Iron	Fe^{2+}	Part of certain enzymes; functions as respiratory electron carrier through such compounds as cytochrome and oxidative enzymes, peroxidases and catalase
Manganese	Mn^{2+}	It is known to be required for the activity of several enzymes, which include decarboxylases, dehydrogenases, kinases and oxidases and superoxide dismutase enzymes. Manganese is necessary for the maintenance of chloroplast ultra-structure. Because manganese plays an important role in redox reactions.
Zinc	Zn^{2+}	Zinc is a component of stable metallo-enzymes. Zinc deficient plants suffers from reduced enzyme activities and a consequent diminution in protein, nucleic acid and chlorophyll synthesis. Plants deprived of zinc often have short internodes and small leaves.
Boron	$H_2BO_3^-$	Boron is thought to promote the destruction of natural auxin and increase its translocation.
Copper	$Cu^{2+.}$	Part of certain oxidative enzymes such as cytochrome oxidases, tyrosinase and ascorbic oxidase which serve to oxidize phenolic substances.
Cobalt	β-$^+$	It is able to inhibit oxidative reactions catalyzed by ions of copper and iron The Co_2^+ ion can inhibit ethylene synthesis
Molybdenum	MoO_4^{2-}	Component of some plant enzymes, such as nitrate reductase, and therefore, essential for nitrogen metabolism n

Table 3: Role of micronutrients in plant tissue culture media.

Vitamins

Plants can produce their requirements of vitamins. However, plant cell cultures need to be supplemented with certain vitamins. Vitamins that act as coenzymes are required to be added to the medium for healthy growth of tissue cultures. The most widely used vitamins are those of B group, viz., thiamine (vitamin B1), nicotinic acid (also known as niacin or vitamin B3), pyridoxine (vitamin B6) and myo-inositol (sometimes referred to as meso-inositol). Certain other vitamins which find specific uses in cell cultures are pantothenic acid, vitamin C, vitamin D and vitamin E. Myo-inositol, a sugar alcohol, is added in a relatively larger quantity (100 mg L-1).Thiamine, nicotinic acid and pyridoxine are used in the Hydrochloride (HCl) form. Of all the vitamins used in plant tissue culture, only thiamine and myo-inositol (considered a B vitamin) are considered essential ingredients of plant tissue culture media. Cultured plant cells and tissues can however become deficient in some factors; growth and survival is then improved by their addition to the culture medium. Ascorbic acid Vitamin C an antioxidant, prevents blackening during explant isolation.

Amino acids

The amino acids serve as the organic source of reduced nitrogen. The presence of inorganic nitrogen in the medium (NH_4^+ and NO_3^-) is generally sufficient to ensure protection against any possible nitrogen deficiency, and supplementation with amino acids may not be required. However, an organic source of nitrogen is preferred only when an inorganic source is lacking or exhausted. Glycine, the simplest amino acid, is a common constituent of plant tissue culture media. Cysteine has been included in media as an antioxidant to control the oxidation of phenolics and prevent blackening of tissue.

Carbon and energy source

Most plant tissue cultures are unable to photosynthesize because of the absence of chlorophyll or poorly developed chloroplasts, limited CO_2 in the culture vessel due to poor gaseous exchange and absence of optimum light intensity. It is, therefore, obligatory to add to the culture medium an utilizable source of carbon necessary for various metabolic activities. The most commonly used carbon source is sucrose at a concentration of 2–5 % (w/v). Generally, sucrose autoclaved along with the medium supports better growth of tissues than filter-sterilized sucrose. Autoclaving causes hydrolysis of sucrose into more efficiently utilizable sugars, such as glucose and fructose. Sucrose not only is an energy source but is also the major osmotic component of the medium. Nutrient salts contribute approximately 20–50 % to the osmotic potential of the medium, and the rest is taken care by sucrose. Some other forms of carbon that plant tissues are known to utilize include maltose, galactose, mannose and lactose. Maltose has especially been found superior to sucrose in promoting somatic embryogenesis in soybean, alfalfa and rubber.

Solidifying agents or supporting systems/gelling agents

In static liquid cultures, the tissue would get submerged and die of anaerobic conditions. To circumvent this problem, the medium is solidified with a suitable gelling agent. The most desirable properties of a gelling agent are that it should: be inert, withstand sterilization by autoclaving and be liquid when hot so that the medium could be dispensed in culture vessels in desired quantities. Some of the gelling agents used in plant tissue cultures are agar, agarose and gellan gum (phytagel, Gel rite) [6].

Agar

This is the most commonly used gelling agent obtained from red algae, especially *Gelidium amansii.* Firmness of the gel produced by a given concentration of agar varies according to the brand and the pH during autoclaving. Agar [6] is partly hydrolyzed if it is autoclaved in an acidic medium. Agar is used at varying concentrations from 0.8 to 1%.

Agarose

Agarose consists of β-D (1-3) galactopyranose and 3,6-anhydro-α-L (1-4) galatopyranose linked into polymer chains of 20-160 monosaccharide units. Agarose is obtained by purifying agar to remove agaropectins with its sulphate side groups. It is only used where high gel strength is required, such as in single cell or protoplast cultures. Agarose is adequate at 0.4%.

Gel rite

Gelrite or Phytagel, a gellan gum, is a linear polysaccharide produced by the bacterium *Pseudomonas elodea.* It comprises of linked K-glucuronate, rhamnose and cellobiose molecules. Unlike agar [6]., which requires heating, gel rite can be readily prepared in cold solution. To prevent clumping it should be added to rapidly stirring culture medium at room temperature. Gel rite is a good alternative to agar because of: Its low cost per liter of medium (0.1-0.2% is sufficient), It sets as a clear gel which assists easy observation of cultures and their possible contamination, Unlike agar, the gel strength of gel rite is unaffected over a wide range of PH. However, certain plants show hyperhydricity on gel rite, apparently due to more freely available water.

Growth regulators/ Growth hormones

Growth regulators, or hormones, are not nutrients, but they influence growth and development. They are generally produced naturally in plants. Cultures; however, usually do not manufacture sufficient quantities of growth regulators, so they must be added selectively to culture media. The growth regulators are required in very minute quantities (μmol^{1-} values). It often requires testing of various types, concentrations and mixtures of growth substances during the development of a tissue culture protocol for a new plant species.

There are different groups of PGRs commonly used in the media. They are auxins, cytokinins, gibberellins, ethylene and Abscisic acid.

Auxins

In tissue cultures auxins have been used for cell division and root differentiation. The auxins commonly used in tissue culture are: Indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), Naphthoxyacetic acid (NOA), Para-chlorophenoxyacetic acid (p-CPA), Dichlorophenoxyacetic acid (2,4-D), and Trichlorophenoxyacetic acid (2, 4, 5-T). Of these: IBA and IAA are widely used for rooting and, in interaction with a cytokinin, for shoot proliferation. 2, 4-D and 2, 4, 5-T are very effective for the induction and growth of callus. 2, 4-D is also an important factor for the induction of somatic embryogenesis.

Auxins are usually dissolved in either ethanol or dilute NaOH.

Cytokinins

In tissue culture media, cytokinins are incorporated mainly for cell division and differentiation of adventitious shoots from callus and organs. These compounds are also used for shoot proliferation by the release of axillary buds from apical dominance. More commonly used cytokinins are: Benzyl Amino Purine (BAP), Isopentenyl-adenine (2-ip), Furfurylamino Purine (kinetin), Thidiazuron (TDZ) and zeatin. Compared to the other cytokinins, thidiazuron are generally used at very low concentrations (0.1-5 $\mu g\ 1^{-1}$). Cytokinins are generally dissolved in dilute HC1 or NaOH. For thidiazuron, DMSO may be used as the solvent.

Gibberellins

Gibberellins are less commonly used in plant tissue culture. There are over 20 known gibberellins, of which GA3 is used most often. They are reported to stimulate elongation of internodes, meristem growth for some species and more importantly to attain normal development of plantlets from *in vitro* formed adventive embryos. GA3 is readily soluble in cold water (up to 1000 mg L-1). Being heat sensitive (90 % of the biological activity is lost after autoclaving), GA3 is filter sterilized and added to autoclaved medium after it has cooled.

Ethylene (ethane, C_2H_4)

Ethylene is an unusual, gaseous plant hormone. It is produced by ageing and stressed tissues. In plant tissue cultures, ethylene is also produced by the organic constituents of the medium on exposure to heat, oxidation, sunlight or ionizing radiation. Ethylene appears to influence various morphogenic processes, such as embryogenesis and organogenesis, but its effects are not clear cut. It generally inhibits growth and differentiation, but in some cases, it promoted somatic embryogenesis.

Abscisic acid

Abscisic acid is most often required for normal growth and development of somatic embryos and only in its presence do they closely resemble zygotic embryos.

Stock solutions

Stock solutions are concentrated solutions of groups of media chemicals that are prepared ahead of time and used to make several batches of media. They are prepared in 10 or 100 fold concentrations. The stocks can consist of groups of ingredients or nearly complete media. The use of stock solutions reduces the number of repetitive operations involved in media preparation and, hence, the chance of human or experimental error. Moreover, direct weighing of media components (e.g., micronutrients and hormones) that are required only in milligram or microgram quantities in the final formulation cannot be performed with sufficient accuracy for tissue culture work.

Preparing Stock Solution

The kinds of stock solutions routinely made vary widely. Each operation selects a system which is in line with needs and convenience. The salts should always be dissolved by adding one compound at a time. Some of the ingredients will form precipitate (insoluble compounds) if mixed together in concentrated form, so each group is made up of chemicals that usually will not precipitate at the concentration of the stocks. Precipitation is usually avoided by dissolving the inorganic nitrogen sources first. Dissolving the inorganic nitrogen sources of the major salts first will avoid precipitation between phosphate and calcium sources when added subsequently, which can occur when the PH approaches 6.0. Dissolving the calcium salt separately before adding it will also help to avoid precipitation.

For these components, preparation of concentrated stock solutions and subsequent dilution into the final media is standard procedure.

General Steps in the Preparation of Stock Solutions (1 liter)

- Pour about ¾ of final distilled water in to a 1-liter flask
- Slide the magnetic stirrer bar into the flask
- Place the flask on the magnetic stirrer and turn on the stirrer
- Refer to the check list of chemical composition of the medium and place a weighing paper (or a piece of aluminum foil) on the balance, weigh and add one compound at a time.
- Stir until dissolve

- Turn off the stirrer and remove the stir bar with long forceps, or pour the stock solution into a clean flask, saving out the stir bar.
- Add distilled water to bring the level of the solution up to 1000ml (1liter) after mixing well, top up with double distilled water in an accurate graduate vessel
- After the stock ingredients are dissolved, the solution is distributed in plastic bags with zip per seals.
- ***NOTE: Important points to be considered while preparing stock solution:***
- For the preparation of stock solutions, the chemicals are dissolved in distilled or high- purity demineralized water.
- Before adding any chemicals in making the stock solutions, there must be water in the flask, otherwise precipitates are likely to form.
- Adding one compound at a time will usually avoid precipitation.
- All the bottles containing stock solution should be properly labeled indicating the type of stock, date of preparation and name of the investigator.
- All the stock solutions are stored in proper plastic or glass bottles under refrigeration.
- The stock solution of iron must be stored in amber coloured bottle.
- Do not pipette directly from the stock bottles, and do not return any unused stock solutions to the stock bottles.
- If you spill some chemicals or remove more than you need, it is bad practice to return the excess to the bottle from which it came because it may have mixed with other substances.
- It is also important that you be sure to clean the spoon or spatula between weighing different chemicals.
- When mixing stock solutions or media, a running checklist should be followed to ensure that the right chemicals and amounts are used.
- If the items are checked off the moment they are added to the mixture, there should be no problem if the technician is interrupted.

I. Macronutrient stock solution(s):

Usually, the stock solution of macronutrients is prepared as 10x. Dissolve all the macronutrients one by one except ($CaCl_2$ for macronutrient stock solution). A separate stock solution for calcium salts may be required to prevent precipitation.

II. Micronutrient stock solution.

Micronutrient stock solutions are generally made up at 100 times their final strength.

III. Iron-EDTA/iron stock solution:

For making stock solution of iron, the required quantities of $FeSO_4.7H_2O$ and $NA_2EDTA.2H_2O$ are weighed and dissolved separately in 450 ml of distilled water by heating and constant stirring and then the two are mixed. The final stock solution should be deep golden yellow. Then it should be stored in an amber bottle or a bottle covered with an aluminum foil.

IV. Vitamins stock solution:

Vitamins are prepared as 100x or 1000x stock solutions and stored in a freezer (-20° C) until used. Vitamin stock solutions should be made up each time media is prepared if a refrigerator.

V. Growth Regulators stock solution

Depending on the levels of growth regulators used, their stock solutions may be prepared at strength of 1 or 10 mM. The growth regulators are dissolved in a minimum quantity of the solvent, and the final volume made up with distilled water.

Plant Tissue culture Media Preparation

Preparation of culture media is a critical step in tissue culture work wherein great precision is required on the part of the investigator. Whenever a medium is to be prepared, the required amounts are drawn from the stock solutions and mixed.

Steps in the preparation of the MS medium (1-liter volume)

A standard protocol for media preparation is described below, along with recipes for preparation of MS and BS media. This generalized approach can be adapted to any medium.

- Add approximately 750 ml of the final volume of double distilled water to a 1-liter beaker
- Add appropriate quantities of the various stock solutions according to the list of ingredients and *check* it off on the list as it is added.
- Slide the magnetic stir bar into the flask
- Place the flask on the hot plate/stirrer and turn on the stirrer
- Add each component according to the list of ingredients and check it off on the list as it is added.
- Weigh the required quantities of *myo*-inositol and sucrose and add to the flask
- After all the ingredients have been added and dissolved, with the exception of the gelling agent and growth regulators, the final volume of the medium is made up with double distilled water by using a graduated cylinder.

- Add the required amount of growth regulator and adjust the PH of the medium to the required value, by adding drop wise, while stirring using 1 N NaOH OR 1 N HCl with separate Pasteur pipettes
- Turn on the hot plate/stirrer heat. Continue to heat and stir until the medium boils vigorously, but do not allow it to boil over.
- When the medium boils vigorously, turn off the hot plate/stirrer heat, Weigh and add 7g of agar while stirring
- After adding agar, turn on the hot plate/stirrer heat and continue to heat until the medium boils vigorously, but do not allow it to boil over.
- Turn off the hot plate/stirrer and remove the flask
- Dispense the medium in to the desired culture vessels using a pitcher or automatic pipette
- Cap the culture vessels
- The culture vessels containing medium are transferred to appropriate wire baskets/racks, and sterilized by autoclaving at 121˚C at 105 kPa for 15 min (time of sterilization varies with volume).
- When the autoclave pressure is back to zero and temperature not more than 50 C˚, remove the racks/ wire baskets from the autoclave
- The medium is allowed to cool at room temperature and is stored at 4C˚ in clean place

STOCK SOLUTIONS FOR MURASHIGE AND SKOOG (MS) MEDIUM FOR BANANA MICRPROPAGATION

List of Chemical Compositions Murashige T and Skoog F (1962) [4]

Macronutrient		
Components	Chemical formula	Weight (mg/l)
Ammonium Nitrate	NH_4NO_3	1650
Potassium Nitrate	KNO_3	1900
Calcium Chloride Anhydrate	$Cacl_2.2H_2O$	440
Magnesium Sulphate Anhydrate	$MgSO_4.7H_2O$	370
Potassium Dibasic phosphate	KH_2PO_4	170
Micronutrient		
Boric Acid	H_3BO_3	6.2
Potassium Iodide	KI	0.83
Manganese Sulphate	$MnSO_4.4H_2O$	22.3
Zinc Sulphate	$ZnSO_4.7H_2O$	8.6
Sodium Molbidate	$Na_2MoO_4.2H_2O$	0.25
Copper Sulphate	$CuSO_4.5H_2O$	0.025
Cobalt Chloride	$CoCl_2.6H_2O$	0.025
Iron / Chelated form		
Sodium Ethylene Diamine Tetra acetate	$Na_2EDTA.2H_20$	37.3
Ferrous Sulphate.7H_2O	$FeSO_4.7H_20$	27.8
Vitamins		
Thiamine HCl		0.1

Nicotinic Acid		0.5
Pyridoxine		0.5
Inositol		100
Glycine		2
Sucrose		3%

STOCK SOLUTIONS FOR MURASHIGE AND SKOOG (MS) [4] MEDIUM FOR BANANA MICRPROPAGATION

A. Stock solution of Macronutrient (40X)

Chemical Reagents	Chemical formulas	g/250ml	g/500ml
Ammonium Nitrate	NH_4NO_3	**16.5**	**33**
Potassium nitrate	KNO_3	**19**	**38**
Magnesium sulphate	$MgSO_4.7H_2O$	**3.7**	**7.4**
Potassium, dibasic phosphate	KH_2PO	**1.7**	**3.4**

B. Stock solution of Calcium Chloride (40X)

Chemical Reagent	Chemical formula	g/250ml	g/500ml	g/1000ml
Calcium Chloride	$Cacl_2.2H_2O$	4.4	8.8	17.6

C. Stock Solution of Micronutrient (1000X)

Chemical reagents	Chemical formulas	g/250ml	g/500ml	g/1000ml
Boric Acid	H_3BO_3	1.55	3.1	6.2
Manganese Sulphate	$MnSO_4.4H_2O$	5.575	11.15	22.3
Zinc Sulphate	$ZnSO_4.7H_2O$	2.15	4.3	8.6
Sodium Molbdate	$Na_2MoO_4.2H_2O$	0.0625	0.125	0.25
Copper Sulphate	$CuSO_4.5H_2O$	0.00625	0.0125	0.025
Cobalt Chloride	$CoCl_2.6H_2O$	0.00625	0.0125	0.025

D. POTASSIUM IODIDE KI (1000X)

Chemical Reagent	Chemical formula	g/250ml	g/500ml	g/1000ml
Potassium Iodide	KI	0.2075	0.415	0.83

E. Stock Solution of Fe-EDTA (100x)

For making stock solution of iron, the required quantities of $FeSO_4.7H_2O$ and NA2EDTA.$2H_2O$ are weighed and dissolved separately in 450 ml of distilled water by heating and constant stirring and then the two are mixed. The final stock solution should be deep golden yellow.

Chemical Reagents	Chemical formulas	g/250ml	g/500ml	g/1000ml
Sodium Ethylene Diamine Tetra acetate	$Na_2EDTA.2H_20$	0.9325	1.865	3.73
Ferrous Sulphate	$FeSO_4.7H_20$	0.695	1.39	2.78

Calculation of Concentration of Stock Solution

For example, for NH_4NO_3:

For 1L working solution (1x), 1650 mg /l NH_4NO_3 needed=1.65 g/l (1x)

1x=1.65 g/l,» 40x1.65 g/l=66 g/l

40x=?

- ✍ For **1L** stock solution at 40x, 66 g/l of NH_4NO_3 is needed
- ✍ So, to prepare **500ml** of stock solution at 40x, we divide by 2=66 g/2=**33g** NH_4NO_3
- ✍ To prepare **250m**l of stock solution at 40x, we divide by 4=66 g/4=**16.5g NH_4NO_3**

Working Solution

From stock solution, we need to dilute the stock solution to prepare working solution.

To prepare working solution, we use the dilution formula:

M1V1=M2V2; where 1. indicates the stock solution

2. indicates the working solution

(40x) v1= (1x) (1000ml);

V1= (1x) (1000ml)/ (40x) =25ml

❖ Therefore, in order to prepare 1L of media, 25ml of each stock solution are needed to be added in to the beaker.

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