Essentials Nutrients

For Plant Tissue Culture

SC30253 Biotechnology

22 Jan. 2019

Essentials Nutrients of Plants

An element can be considered to be essential for plant growth if

- A plant fails to complete its life cycle without it
- Its action is specific and cannot be replaced completely by any other element
- Its effect on the organism is direct, not indirect on the environment
- It is a constituent of a molecule that is known to be essential.

Essentials Nutrients of Plants

Element (Form Primarily Absorbed by Plants)	% Mass in Dry Tissue	Major Functions	Early Visual Symptoms of Nutrient Deficiencies
Macronutrients			
Carbon (CO_2)	45%	Major component of plant's organic compounds	Poor growth
Oxygen (CO_2)	45%	Major component of plant's organic compounds	Poor growth
Hydrogen (H_2O)	6%	Major component of plant's organic compounds	Wilting, poor growth
Nitrogen (NO_3^-, NH_4^+)	1.5%	Component of nucleic acids, proteins, and chlorophyll	Chlorosis at tips of older leaves (common in heavily cultivated soils or soils low in organic material)
Potassium (K^+)	1.0%	Cofactor of many enzymes; major solute functioning in water balance; operation of stomata	Mottling of older leaves, with drying of leaf edges; weak stems; roots poorly developed (common in acidic or sandy soils)
Calcium (Ca^{2+})	0.5%	Important component of middle lamella and cell walls; maintains membrane function; signal transduction	Crinkling of young leaves; death of terminal buds (common in acidic or sandy soils)
Magnesium (Mg^{2+})	0.2%	Component of chlorophyll; cofactor of many enzymes	Chlorosis between veins, found in older leaves (common in acidic or sandy soils)
Phosphorus ($H_2PO_4^-$, HPO_4^{2-})	0.2%	Component of nucleic acids, phospholipids, ATP	Healthy appearance but very slow development; thin stems; purpling of veins; poor flowering and fruiting (common in acidic, wet, or cold soils)
Sulfur (SO_4^{2-})	0.1%	Component of proteins	General chlorosis in young leaves (common in sandy or very wet soils)

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Essentials Nutrients of Plants

Element (Form Primarily Absorbed by Plants)	% Mass in Dry Tissue	Major Functions	Early Visual Symptoms of Nutrient Deficiencies
Micronutrients			
Chlorine (Cl^-)	0.01%	Photosynthesis (water-splitting); functions in water balance	Wilting; stubby roots; leaf mottling (uncommon)
Iron (Fe^{3+}, Fe^{2+})	0.01%	Respiration; photosynthesis: chlorophyll synthesis; N_2 fixation	Chlorosis between veins, found in young leaves (common in basic soils)
Manganese (Mn^{2+})	0.005%	Active in formation of amino acids; activates some enzymes; required for water-splitting step of photosynthesis	Chlorosis between veins, found in young leaves (common in basic soils rich in humus)
Boron ($H_2BO_3^-$)	0.002%	Cofactor in chlorophyll synthesis; role in cell wall function; pollen tube growth	Death of meristems; thick, leathery, and discolored leaves (occurs in any soil; most common micronutrient deficiency)
Zinc (Zn^{2+})	0.002%	Active in formation of chlorophyll; cofactor of some enzymes; needed for DNA transcription	Reduced internode length; crinkled leaves (common in some geographic regions)
Copper (Cu^+, Cu^{2+})	0.001%	Component of many redox and lignin-biosynthetic enzymes	Light green color throughout young leaves, with drying of leaf tips; roots stunted and excessively branched (common in some geographic regions)
Nickel (Ni^{2+})	0.001%	Nitrogen metabolism	General chlorosis in all leaves; death of leaf tips (common in acidic or sandy soils)
Molybdenum (MoO_4^{2-})	0.0001%	Nitrogen metabolism	Death of root and shoot tips; chlorosis in older leaves (common in acidic soils in some geographic areas)

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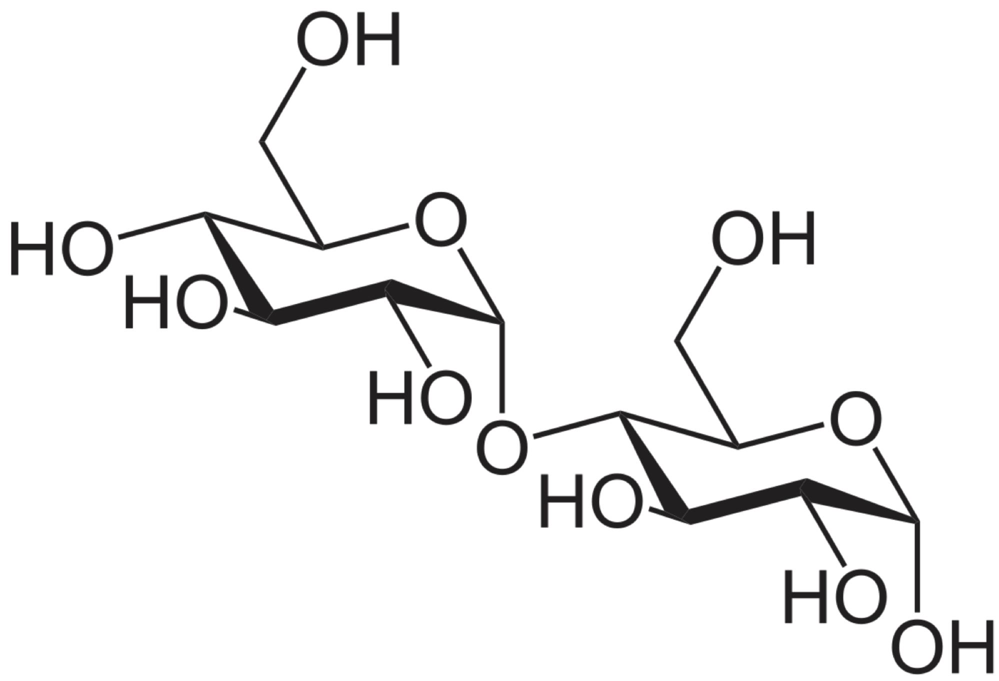
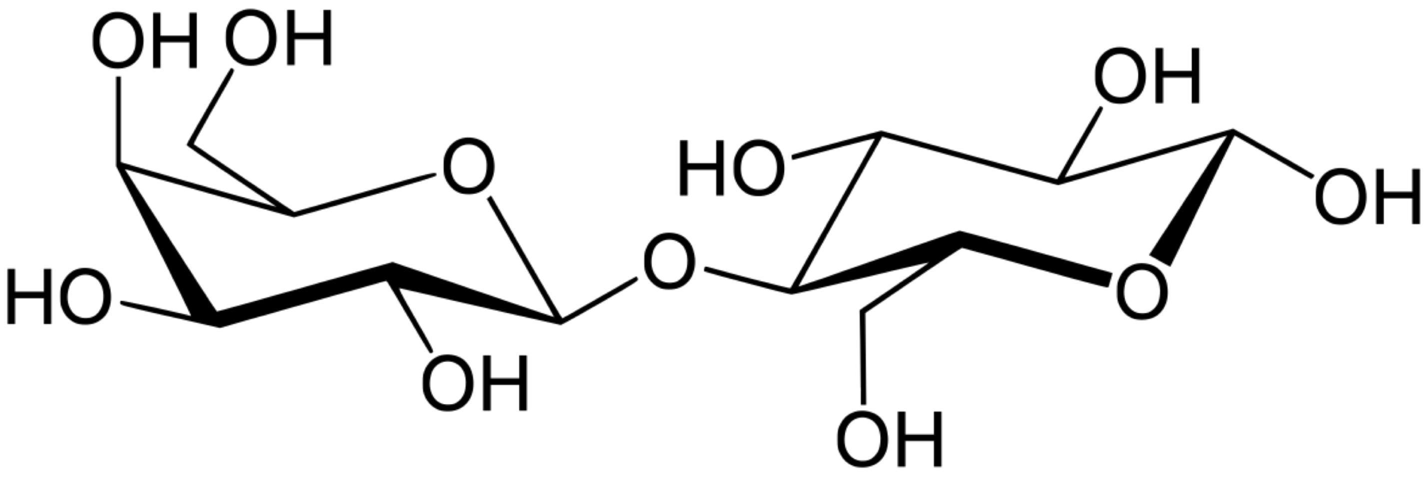
Macronutrients : Carbon

- The carbon source of PTC is commonly found in form of carbohydrate and carbon dioxide.

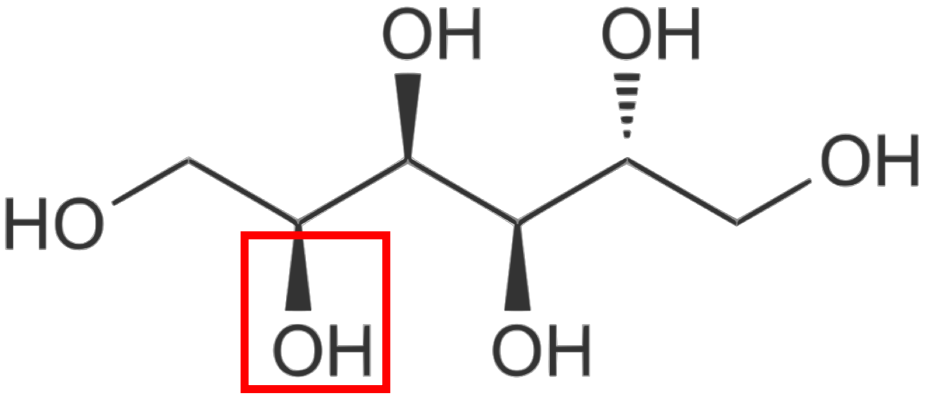


Maltose

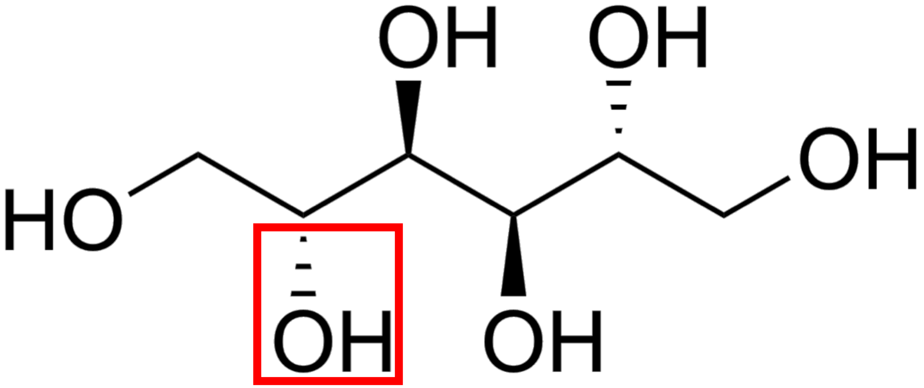


Lactose

Macronutrients : Carbon



Sorbitol



Mannitol

African Journal of Biotechnology Vol. 8 (23), pp. 6529-6535, 1 December, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effect of sorbitol in callus induction and plant regeneration in wheat

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Accepted 27 July, 2009

Six wheat genotypes were evaluated for their response to callus induction and regeneration on MS medium modified with different concentrations of sorbitol, that is, 0, 10, 20, 30 gL^{-1} along with optimum (3 mgL^{-1}) concentration of 2,4-D. Variability was observed among different genotypes for callus induction. Highest callus induction frequency was shown by Wafaq- 2001, which was about 85.62% followed by Inqalab-91 which showed 71.94% callus induction. While minimum callus induction frequency was shown by Saleem-2000 which was about 51.21%. Regarding sorbitol concentration highest average callus induction frequency (79.20%) was obtained at 20 gL^{-1} and lowest average callus induction frequency (59.20%) was observed at 30 gL^{-1}. In Wafaq-2001 and Inqalab-91 plant regeneration increased gradually by increasing the sorbitol concentration from 0 to 20 gL^{-1} but then it decreased. Similarly Auqab-2002 had no regeneration al all on non-sorbitol medium but showed regeneration on addition of sorbitol. Similarly time duration required for plant regeneration also decreased by increasing the concentration of sorbitol. It was also observed that sorbitol has given more strength to regenerated plant.

Key words: Callus indu

Scientific Research Publishing

J. Biomedical Science and Engineering, 2016, 9, 58-64
http://www.scirp.org/journal/jbise
ISSN Online: 1937-688X
ISSN Print: 1937-6871

Mannitol and Sorbitol Improve Uniformity of Adventitious Shoots Regeneration in *Echinacea purpurea* L. Moench

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How to cite this paper: Chen, X.L., Li, D.L., Yang, Y.S. and Wu, H. (2016) Mannitol and Sorbitol Improve Uniformity of Adventitious Shoots Regeneration in *Echinacea purpurea* L. Moench. *J. Biomedical Science and Engineering*, **9**, 58-64.
http://dx.doi.org/10.4236/jbise.2016.910B008

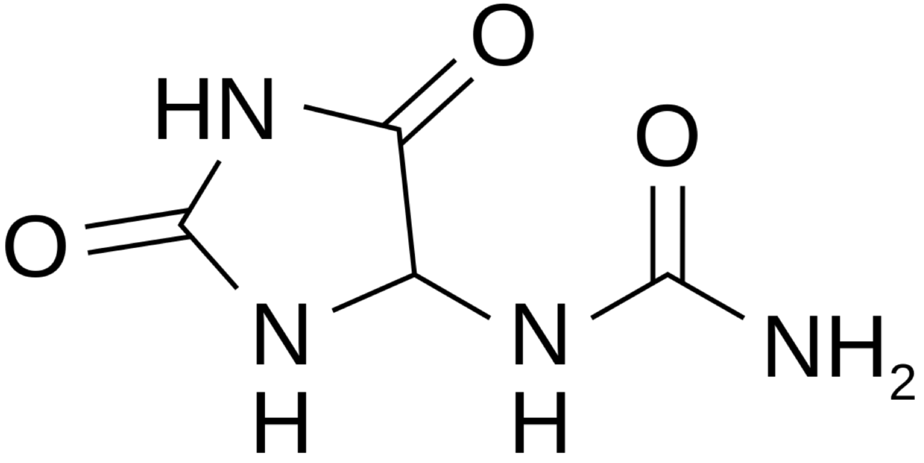
Received: June 20, 2016
Accepted: September 20, 2016
Published: September 23, 2016

Abstract

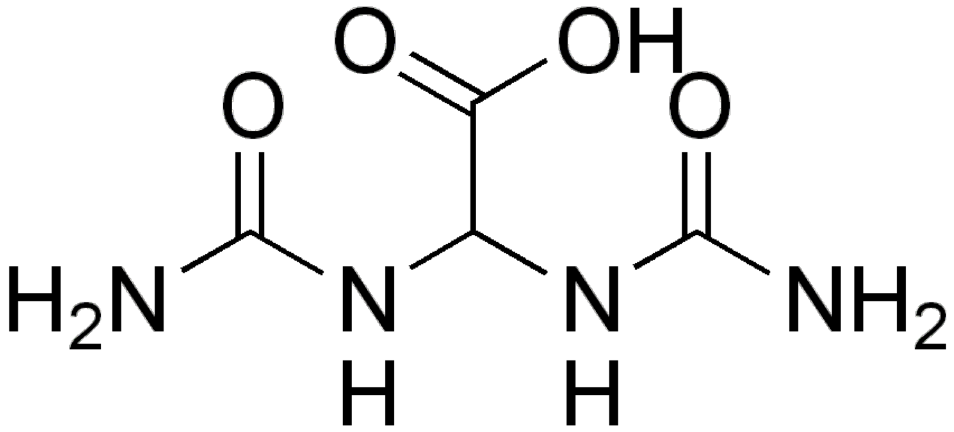
Mannitol or sorbitol was added into the Murashige and Skoog (MS) medium containing certain concentrations of 6-Benzyladenine (BA) which was used to induce adventitious buds of *Echinacea purpurea* L. Results showed that the induced adventitious buds growing from medium added with 15 $g{\cdot}L^{-1}$ mannitol or sorbitol of the same concentration were more consistent in height. The regeneration rates in MS medium containing 0.2 $mg{\cdot}L^{-1}$ BA and 15 $g{\cdot}L^{-1}$ mannitol were increased, while in MS medium containing 0.2 and 0.5 $mg{\cdot}L^{-1}$ BA, and 15 $g{\cdot}L^{-1}$ sorbitol, the regeneration rates were suppressed. On the other hand, genotype of explants and the concentration of BA influenced the incidence of hyperhydricity, and the hyperhydricity of regenerated buds was more severe when the petiole explants were inoculated on medium with 15 $g{\cdot}L^{-1}$ mannitol or 15 $g{\cdot}L^{-1}$ sorbitol. The present study offers new possibility to the production of uniform plantlets for commercial cultivation in this important medicinal plant.

Macronutrients : Nitrogen

- Nitrogen is generally supplied in the form of NH_4^+ along with NO_3^-
- also amine or amide compounds such as urea.

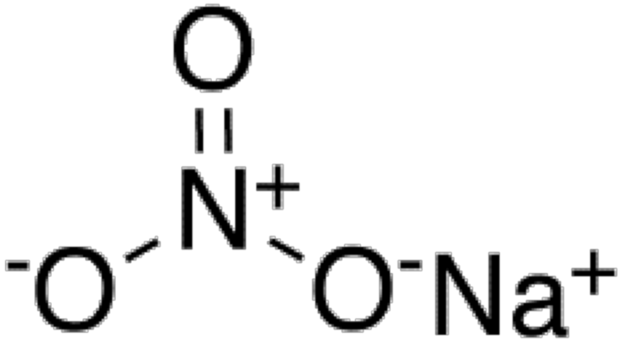


Allantoin

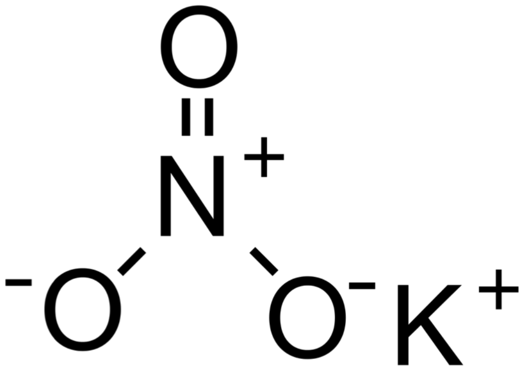


Allantoic acid

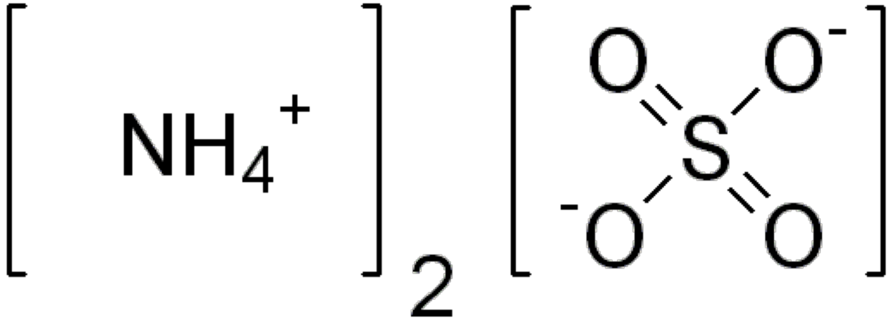
Macronutrients : Nitrogen



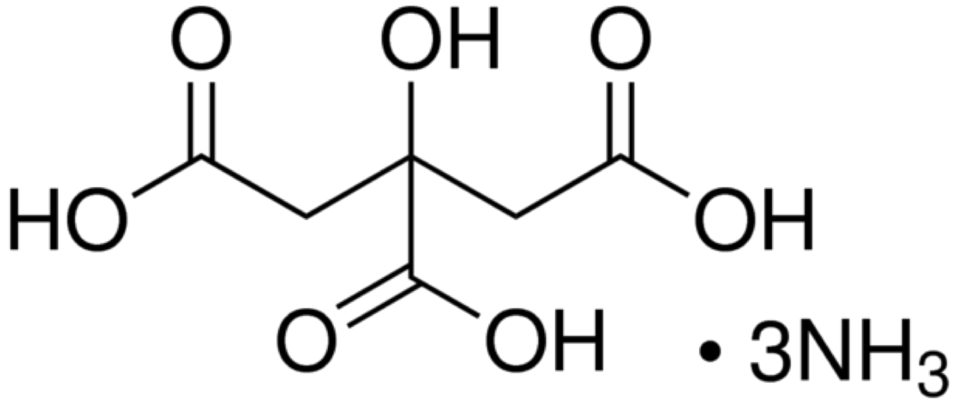
Sodium Nitrate



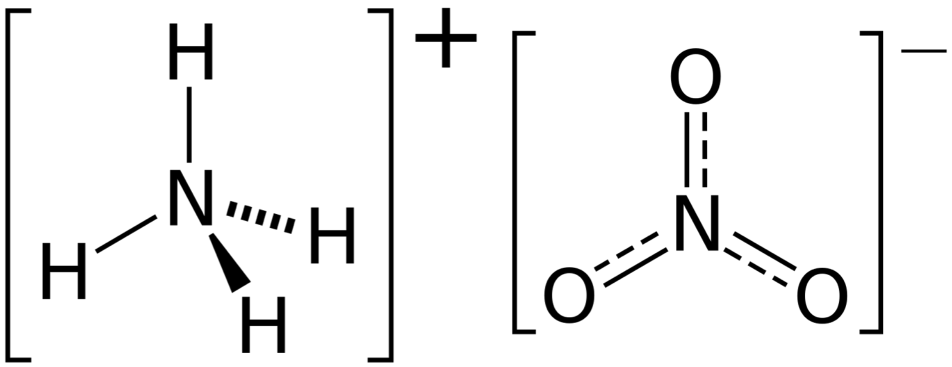
Potassium Nitrate



Ammonium Sulfate



Ammonium Citrate



Ammonium Nitrate

Macronutrients : Nitrogen

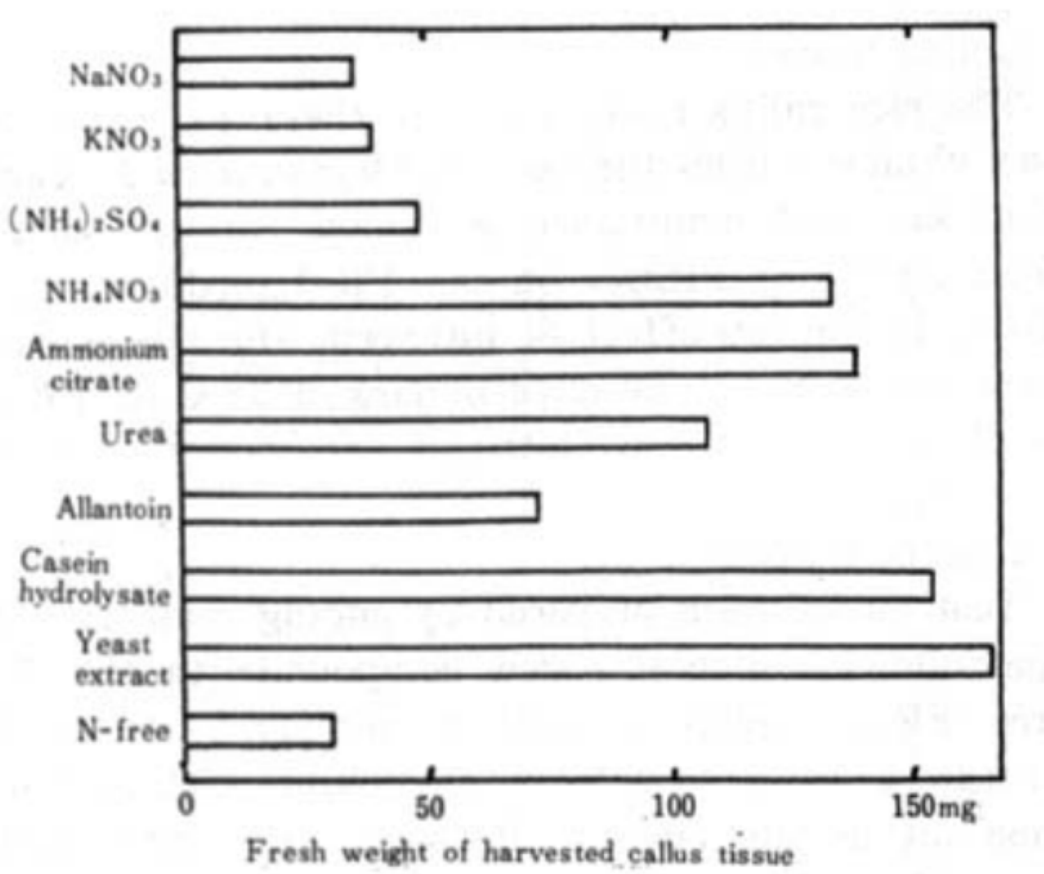
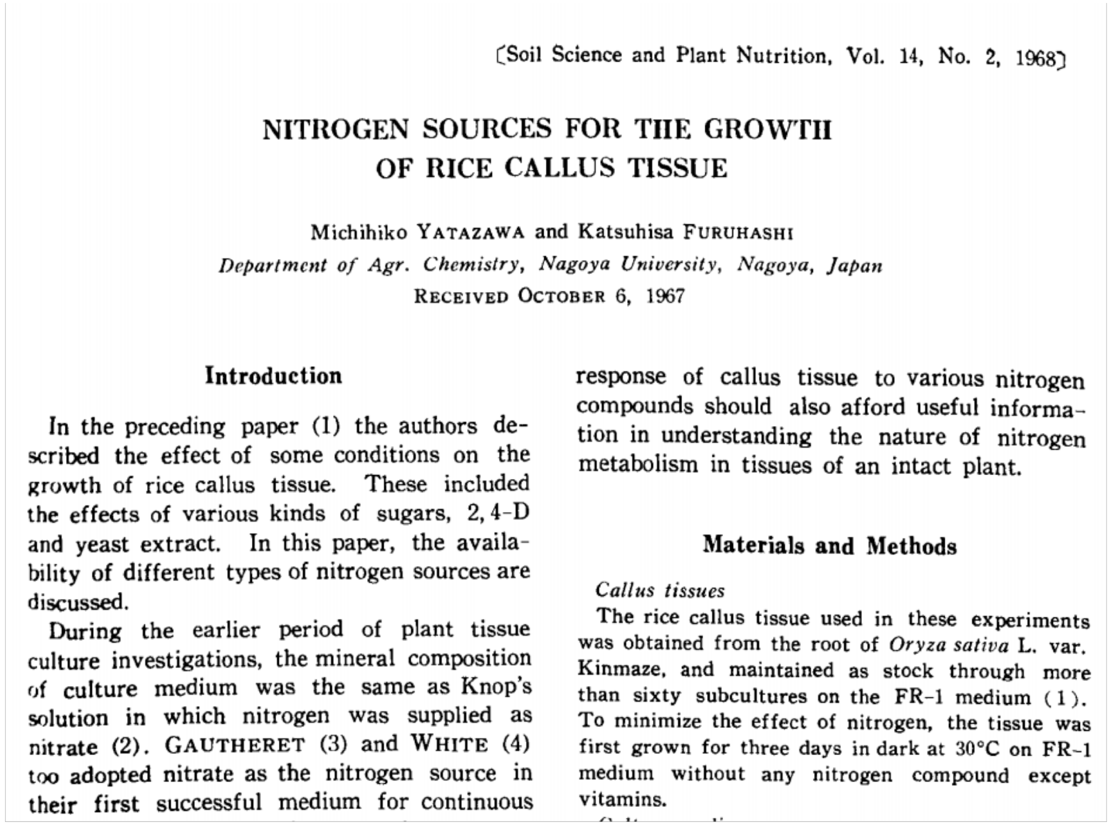


Fig. 1. Effect of nitrogen compounds (100mg N/l) on the growth of rice callus tissue; grown in dark at 30°C for 20 days.



[Soil Science and Plant Nutrition, Vol. 14, No. 2, 1968]

NITROGEN SOURCES FOR THE GROWTH OF RICE CALLUS TISSUE

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RECEIVED OCTOBER 6, 1967

Introduction

In the preceding paper (1) the authors described the effect of some conditions on the growth of rice callus tissue. These included the effects of various kinds of sugars, 2, 4-D and yeast extract. In this paper, the availability of different types of nitrogen sources are discussed.

During the earlier period of plant tissue culture investigations, the mineral composition of culture medium was the same as Knop's solution in which nitrogen was supplied as nitrate (2). GAUTHERET (3) and WHITE (4) too adopted nitrate as the nitrogen source in their first successful medium for continuous response of callus tissue to various nitrogen compounds should also afford useful information in understanding the nature of nitrogen metabolism in tissues of an intact plant.

Materials and Methods

Callus tissues

The rice callus tissue used in these experiments was obtained from the root of *Oryza sativa* L. var. Kinmaze, and maintained as stock through more than sixty subcultures on the FR-1 medium (1). To minimize the effect of nitrogen, the tissue was first grown for three days in dark at 30°C on FR-1 medium without any nitrogen compound except vitamins.

Michihiko Yatazawa & Katsuhisa Furuhashi (1968) Nitrogen sources for the growth of rice callus tissue, Soil Science and Plant Nutrition, 14:2, 73-78, DOI: 10.1080/00380768.1968.10432012

Table 3.5 Examples of beneficial and harmful adjustments to the total nitrogen and the NO_3^- and NH_4^+ in low salt media

Basic Medium in which N modified	Plant species or variety	Type of culture and results	NO_3^- (mM)	NH_4^+ (mM)	Ratio of NO_3^- to NH_4^+	Total N (mM)	Reference
Biederman (1987)			**8.62**	**6.25**	**58:42(1.38)**	**14.87**	
Magnolia stellata		Shoot culture: maximum proliferation	10.99	6.25	1.76	17.24	Biederman (1987)
Magnolia 'Elizabeth'		Shoot culture: maximum proliferation	8.62-10.99	6.25	1.38 – 1.76	14.87 – 17.24	
Magnolia 'Yellow Bird' and '#149'		Shoot culture: maximum proliferation	7.43	6.25	1.19	13.68	
Magnolia (all vars.)		Shoot culture: death of all cultures	25.04	6.25	4.01	31.29	
Chu *et al.*, (1975) N6			**28.00**	**7.00**	**80:20(4.00)**	**35.00**	
Oryza sativa		Callus induction from immature embryos	28.00	7.00	4.00	35.00	Grimes and Hodges (1990)
		Plant regeneration after callus induction with 2,4-D	12.5–38.25	22.5-3.75	1.0 - 5.66	25.00-45.00	
		Optimum number of plants per zygotic embryo	18.75	6.25	3.00	25.00	
		Poor regeneration	100 <17.5	0 >17.5	∞ <1.0	35.00	
		Optimum plantlet growth	26.25	8.75	8.75	35.00	

George, Edwin F., Hall, Michael A., De Klerk, Geert-Jan (Eds.) Plant Propagation by Tissue Culture Volume 1. The Background

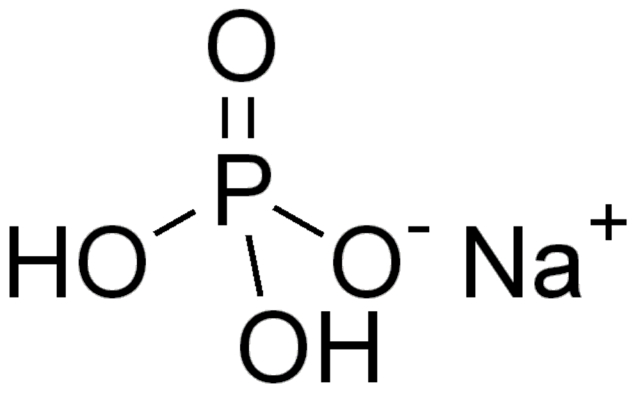
Table 3.7 Some examples of the promotion of embryogenesis by amino acids in media containing NO_3^- and NH_4^+

	Type of culture	Basal medium used	Amino acid supplements	Reference
Aesculus hippocastrum	Zygotic embryo callus	MS	CH (250 mg/l) + Proline (250 mg/l)	Radojevic (1988)
Dactylis glomerata	Suspension-derived callus	Schenk and Hildebrandt (1972)	CH (1.5 g/l)	Gray *et al.* (1984)
Daucus carota	Hypocotyl callus	Gamborg *et al.* (1968) B5	Proline (100 mM) + Serine (100 mM)	Nuti Ronchi *et al.* (1984)
Dioscorea rotundata	Zygotic embryo callus	MS	CH (1 g/l)	Osifo (1988)
Glycine max	Suspension	Kartha *et al.* (1974a)	L-asparagine (5 mM)	Finer and Nagasawa (1988)
Gossypium klotzschianum	Suspension	Gamborg *et al.* (1968) B5	Glutamine (10 mM)	Price and Smith (1979a,b)
Larix decidua	Gametophyte callus	Litvay *et al.* (1981) LM	CH (1 g/l) + Glutamine (500 mg/l)	Nagmani and Bonga (1985)
Nigella sativa	Roots or leaf callus	MS	CH (100 – 500 mg/l)	Bannerjee and Gupta (1976)
Trigonella foenum-graecum	Leaf callus	MS	CH (50 mg/l) (500 mg/l was inhibitory)	Gupta *et al.* (1987)
Triticum aestivum	Anther	Chu and Hill (1988) MN6	Serine, proline, arinine, aspartic acid and alanine (each at 40 mg/l) + glutamine (400 mg/l)	Chu and Hill (1988)
Vitis vinifera	Anther	½ MS	CH (250 mg/l)	Mauro *et al.* (1986);
Zea mays	Zygotic embryo callus	Chu *et al.* (1975) N6	Proline (20-25 mM)	Kamo *et al.* (1985) Armstrong and Green (1985)

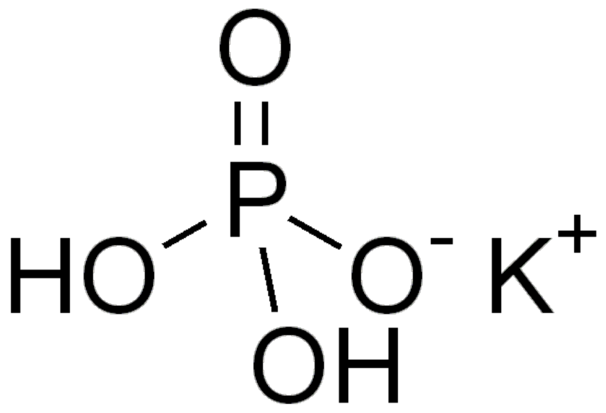
George, Edwin F., Hall, Michael A., De Klerk, Geert-Jan (Eds.) Plant Propagation by Tissue Culture Volume 1. The Background

Macronutrients : Phosphorus

- Phosphorus is absorbed into plants in the form of the primary or secondary orthophosphate anions $H_2PO_4^-$ and HPO_4^{2-} by an active process, which requires the expenditure of respiratory energy.
- It usually uses in the form of inorganic salt (phosphate salt) i.e. sodium phosphate.



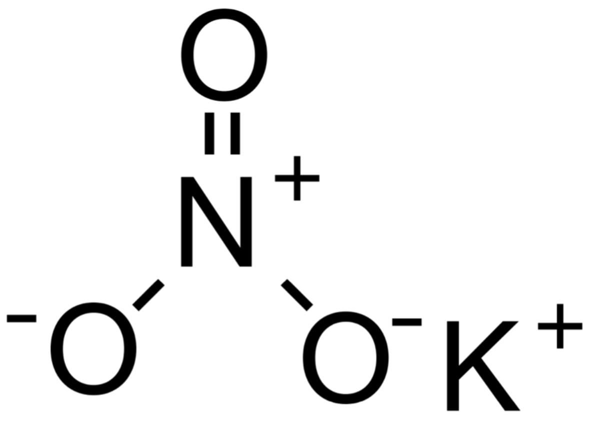
Sodium Dihydrogen Phosphate



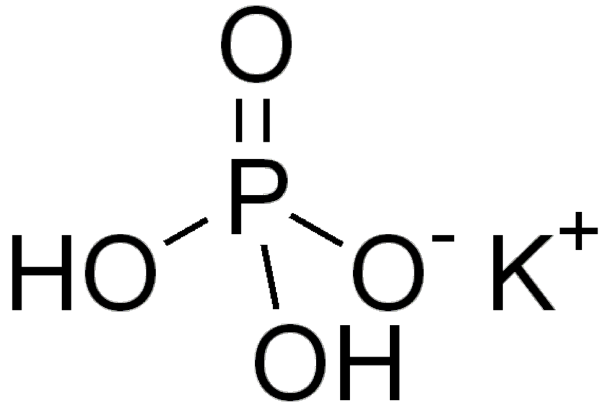
Monopotassium Phosphate

Macronutrients : Potassium

- Potassium is absorbed into plants in the form of potassium cation (K^+).
- It usually uses in the form of inorganic salt (potassium salt) i.e. potassium nitrate



Potassium Nitrate



Monopotassium Phosphate

Macronutrients : Calcium

- The Ca^{2+} ion is involved in *in vitro* morphogenesis and is required for many of the responses induced by plant growth substances, particularly auxins and cytokinin.
- Calcium is absorbed into plants in the form of calcium cation (Ca^{2+}).
- It usually uses in the form of calcium chloride ($CaCl_2 \cdot 2H_2O$) or calcium nitrate ($Ca[NO_3]_2 \cdot 4H_2O$).

Macronutrients : Magnesium

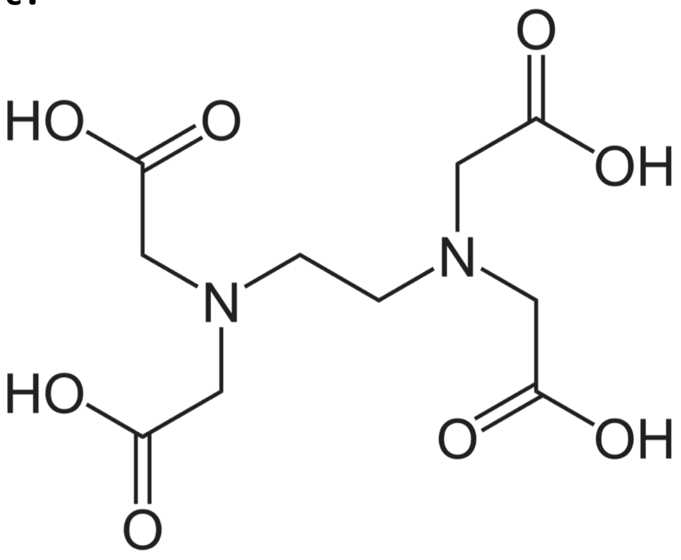
- Magnesium is an essential component of the chlorophyll molecule and is also required nonspecifically for the activity of many enzymes, especially those involved in the transfer of phosphate.
- Magnesium is absorbed into plants in the form of magnesium cation (Mg^{2+}).
- Most tissue culture media contain magnesium as magnesium sulfate ($MgSO_4 \cdot 4H_2O$).

Macronutrients : Sulfur

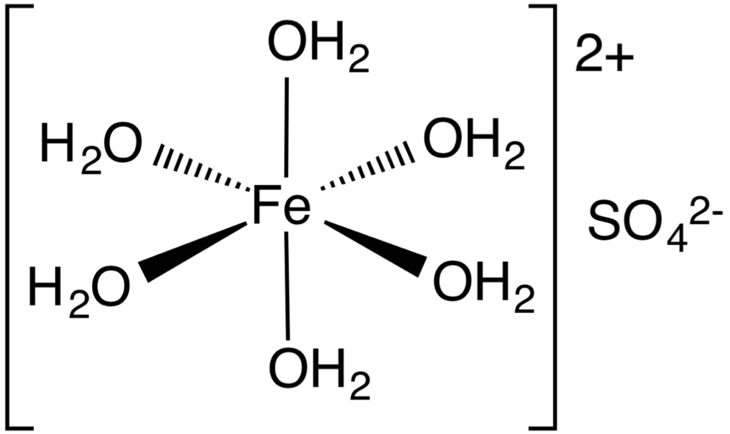
- The sulfur utilized by plants is mainly absorbed as SO_4^{2-}, which is the usual source of the element in plant culture media.
- Although sulfur is mainly absorbed by plants in the oxidized form, that which is incorporated into chemical compounds is mainly as reduced sulfhydryl, sulfide or disulfide groups.

Micronutrients : Iron

- In tissue culture media, ferrous sulfate ($FeSO_4 \cdot 7H_2O$) is often mixed with the sodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) to sequester the iron, thereby making it more readily available to the plant.



Ethylenediaminetetraacetic acid



Ferrous sulfate

Micronutrients : Boron

- Boron is involved in plasma membrane integrity and functioning, probably by influencing membrane proteins, and cell wall intactness.
- In the soil, boron occurs in the form of boric acid and it is this compound, which is generally employed as the source of the element in tissue cultures.
- But, Boric acid reacts with some organic compounds having two adjacent *cis*-hydroxyl groups (This include *o*-diphenols, hexahydric alcohols such as mannitol and sorbitol) but excludes sucrose which forms only a weak association. Once the element is complexed it appears to be unavailable to plants.

Micronutrients : Chlorine

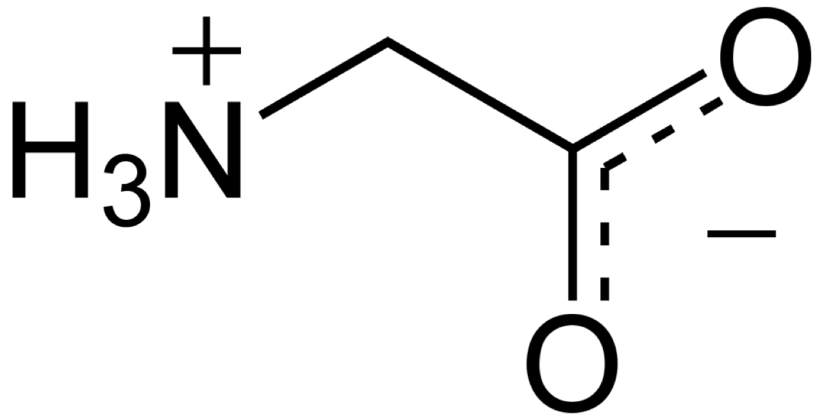
- The chloride ion (Cl^-) has been found to be essential for plant growth but seems to be involved in few biological reactions and only very small quantities are really necessary.
- Chloride is required for the water splitting protein complex of Photosystem II, and it can function in osmoregulation in particular in stomatal guard cells.
- Calcium chloride ($CaCl_2$) in the media will provide chlorine, often in larger amount than it required.

Micronutrients : Cobalt

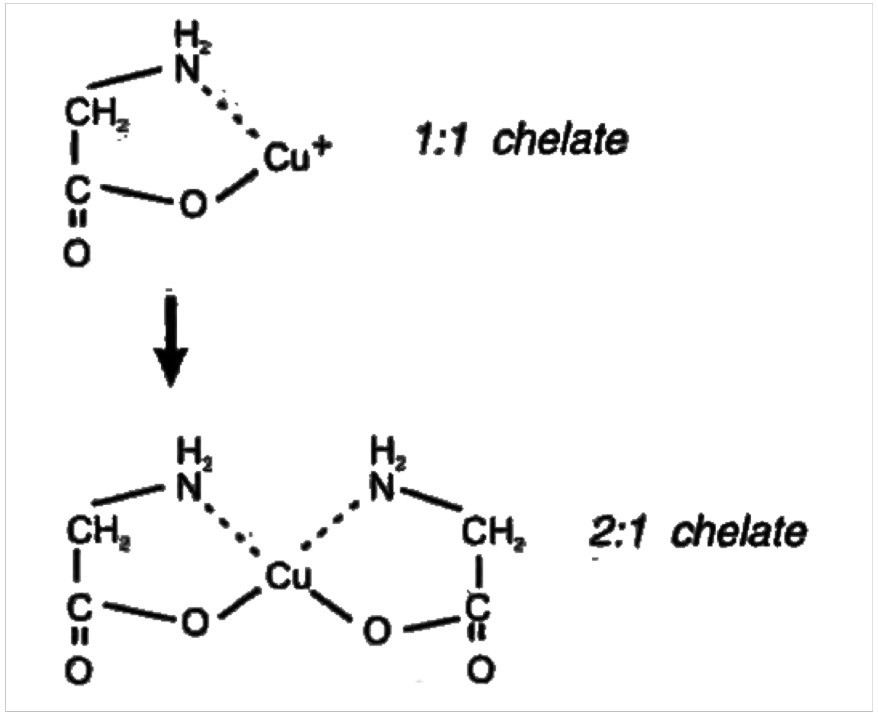
- Cobalt is a component of Vitamin B12 molecule and is essential for nitrogen fixation.
- Advantage from adding cobalt to plant culture media might be derived from the fact that the element can have a protective action against metal chelate toxicity and it is able to inhibit oxidative reactions catalyzed by ions of copper and iron.
- Cobalt chloride ($CoCl_2 \cdot 6H_2O$) is the source of cobalt in the media.

Micronutrients : Cobalt

- Metal chelate



Glycine



Copper chelated with amino acid, glycine

Micronutrients : Copper

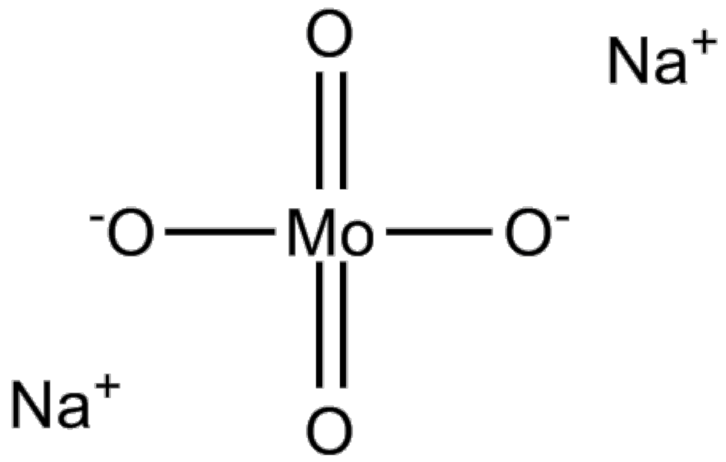
- Copper is an essential micronutrient, even though plants normally contain only a few parts per million of the element. Two kinds of copper ions exist; they are the monovalent cuprous (Cu^{+}) ion, and the divalent cupric (Cu^{2+}) ion: the former is easily oxidized to the latter; the latter is easily reduced.
- In the medium, it is added as cupric sulfate ($CuSO_4 \cdot 5H_2O$).

Micronutrients : Iodine

- It is often added to the media as potassium iodide (KI)
- The practice of including iodide in plant culture media began with the report by White (1938) that it improved the growth of tomato roots cultured *in vitro*.
- Hannay (1956) obtained similar results and found that root growth declined in the absence of iodine which could be supplied not only from potassium iodide, but also from iodoacetate or methylene iodide, compounds which would only provide iodide ions very slowly in solution by hydrolysis.

Micronutrients : Molybdenum

- Plants utilize hexavalent molybdenum and absorb the element as the molybdate ion (MoO_4^{2-}).
- It is added to tissue culture media as sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)



Sodium Molybdate

Micronutrients : Manganese

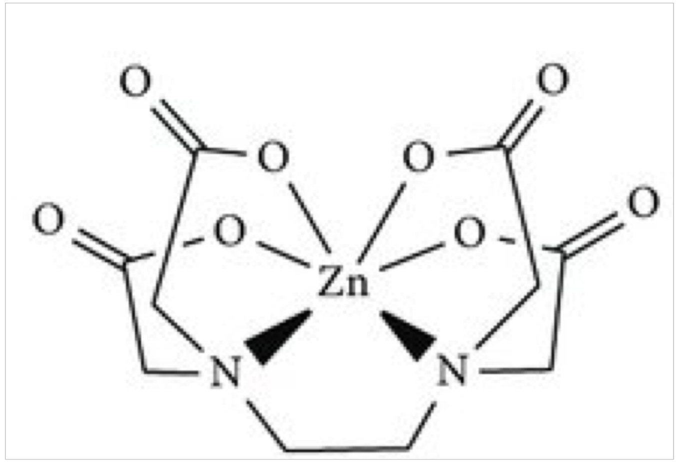
- Manganese has similar chemical properties to Mg^{2+} and is apparently able to replace magnesium in some enzyme systems.
- The most probable role for Mn is in definition of the structure of metalloproteins involved in respiration and photosynthesis.
- Magnesium sulfate or Magnesium chloride provide the necessary manganese for tissue culture media.

Micronutrients : Nickel

- The nickel ion is a component of urease enzymes which convert urea to ammonia.
- In tissue cultures the presence of 0.1 mM Ni^{2+} strongly stimulates the growth of soybean cells in a medium containing only urea as a nitrogen source.
- Nickel sulfate and Nickel chloride provide the necessary nickel for tissue culture media.

Micronutrients : Zinc

- Zinc is a component of stable metallo-enzymes with many diverse functions.
- Traces of zinc are included in the media as zinc sulfate ($ZnSO_4 \cdot 7H_2O$) or $Na_2ZnEDTA$



$Na_2ZnEDTA$

M. Butkus, Joann & O'Riley, Shelby & S. Chohan, Balwant & Basu, Swarna. (2016). Interaction of Small Zinc Complexes with Globular Proteins and Free Tryptophan. International Journal of Spectroscopy. 2016. 1-12. 10.1155/2016/1378680.

EDTA = Ethylenediaminetetraacetic acid

Murashige Minimal Organics Medium recipe (MMOM)

Inorganic salts	mg/L
NH_4NO_3	1,650.00
KNO_3	1,900.00
$CaCl_2$ (anhydrous)	332.20
$MgSO_4$ (anhydrous)	180.70
KH_2PO_4	170.00
Na_2EDTA	37.25
$FeSO_4.7H_2O$	27.80

https://www.apsnet.org/edcenter/K-12/TeachersGuide/PlantBiotechnology/Documents/PlantTissueCulture.pdf

Murashige Minimal Organics Medium recipe (MMOM)

Inorganic salts	mg/L
H_3BO_3	6.20
$MnSO_4.H_2O$	16.90
$ZnSO_4.H_2O$	5.37
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4$ (anhydrous)	0.016
$CoCl_2$ (anhydrous)	0.014
Sucrose	30,000.00
i-Inositol	100.00
Thiamine.HCl	0.40

The pH is adjusted to 5.7 using 0.1 M HCl or NaOH.

Conclusion

- Plant absorbs essentials nutrients in ion form, in this case, the inorganic salt (-Cl, -SO_4 ,etc.) of essential elements can be used.
  - Or in common term, when it dissolved in water, it can break down into ion plant really need. (in slide 2-3)
- Sometimes as a vitamins or a protein.
  - Such as Cysteine for sulfur source or Vitamin B12 for cobalt source.
- Sometimes as a complex compound.
  - Such as $Na_2ZnEDTA$, Ferrous phosphate.

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