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Effect of different concentrations of nitrogen on in Vitro morphogenesis of Petunia hybrida Hort.(Vilm).

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ABSTRACT:

NO_3^- and NH_4^+ are the common forms of nitrogen that all plants utilize for proper growth and development. Nitrogen significantly increases and enhances the yield and its quality by playing a vital role in biochemical and physiological functions of the plant. Both form and amount of nitrogen in nutrient medium have significant effects on the rate of cell growth and differentiation. Uniqueness of nitrogen lies in the fact that plants can use it either in anionic (NO_3^-) or in cationic form (NH_4+). This paper examines the present knowledge of the main effects that different concentrations of nitrogen have on plant morphogenesis in vitro. During the present study, effect of different concentrations of nitrogen on in vitro morphogenesis of Petunia hybrida Hort. (Vilm) was evaluated. In the present study it was concluded that maximum callus production and root formation was observed in MS Basal Medium and the medium containing half concentration of nitrogen.

Key Words: *Petunia* species, nitrogen, role, growth, development

INTRODUCTION:

Tissue culture is a technique of obtaining rapid clonal multiplication. Composition of the culture medium largely influences the growth and morphogenesis of the plant tissue under in vitro conditions. Mineral nutrients are one of the most important and basic components of plant tissue culture. The role of nitrogen assimilation in plant growth and development play a pivotal role in establishing and understanding of cell differentiation in plants. The form as well as the amount of nitrogen in the *in vitro* medium have significant effects on rate of cell growth, differentiation and cell totipotency.

The present study is on the in vitro propagation of Petunia hybrida an important ornamental plant. The most successful species to respond to the tissue culture from family Solanaceae that has been used as a model for in vitro studies. First report regarding in vitro propagation in family Solanaceae was demonstrated by Vasil and Hild in (1965) in Nicotiana tobaccum. The aim of this investigation was to search for valuable effects due to different concentrations of Nitrogen on Petunia hybrida plant. Nitrogen (N) is a unique nutrient because, unlike the other essential nutrient elements, plants can use it in either the cation form, ammonium (NH_4^+), or the anion form, nitrate (NO_3^-) (Miller and Donahue, 1990).

The assimilation of NH4+ in roots produces about 1 proton per molecule which has to be excreted into the external medium (Buchanan et al., 2002). Using ammonia as a sole source of nitrogen appears to have retarding effect on growth and morphogenises (Cousson and Tran Thanh Van, 1993; Raab and Terry, 1994, 1995; Walch et al., 2000; Carl and Richard, 2002). The negative effects of NH_4^+ have been attributed to various factors such as changes in medium pH and toxic effects of free NH_4^+. As compared with NH^+ NO_3^- tends to decrease the hydrogen ion concentration of rhizosphere and there is no risk of toxicity at alkaline pH (Marschner, 1995; Dev and Herbert, 2002). In general, maximum growth rates and plants yield can be achieved by applying the combined supply of NH_4^+ and NO_3^- (Raven, 1985; Allen et al.,1988).

Methodology:-

The present work was conducted in the tissue culture laboratory, Department of Botany, the methodology adopted in the present work i.e effect of different concentrations of Nitrogen on *in vitro* morphogenesis of *Petunia hybrid*a is as under: **3.1 Sterilization of Glassware and other equipments**

Glassware including culture vials, flasks, measuringcylinders, glass bottles, beakers, petriplates, pipettes etc were scrubbed with brush in a detergent (labolene) .They were washed thoroughly with tap water and then rinsed 2 to 3 times with distilled water.

Contaminated glass ware was first autoclaved and then soaked in detergent labolene followed by washing and finally oven dried.

Preparation of Stock Solutions: Stock Solutions were prepared in sterilized glass bottles having suitable closures, according to the composition of MS basal medium given by Murashige and Skoog(1962). The procedure for preparation of stock solution is given below:

3.2.1) Stock I (Macro Salts)

Constituents	Amount(g/l)
Ammonium nitrate(NH_4NO_3)	16.5
Potassium nitrate (KNO_3)	19.0
Calcium chloride ($Calcl_2.2H2O$)	4.4
Magnesium sulphate ($MgSO_4.7H2O$)	3.4

Final volume was made up to 100ml by adding double distilled H2O.

In order to study the effects of different concentrations of Nitrogen on *Petunia hybrid*a we prepare 5 different solutions of stock I.

Stock I (A): In this we took double concentration of NH4NO3 and rest is same as that of MS medium. I make final vol. 100 ml.

Stock I (B): In this NH4 NO3 and KNO3 were absent.

Stock I(C): In this case, KNO3 was doubled; rest was same as that of MS medium. **Stock I (D):** In this concentration of both NH4NO3 as well as KNO3 were doubled, rest were same. **Stock I (E):** In this, we took half concentration of NH4 NO3 and KNO3, rest were kept same

Stock I	NH_4NO_3 +KNO_3 same as MS-medium
Stock I(A)	NH_4NO_3 + KNO_3 Double Same
Stock I(B)	NH_4NO_3 + KNO_3 Absent
Stock I(C)	KNO3 + NH4NO3 Samedouble
Stock I(D)	NH4NO3 + KNO3 Double absent
Stock I(E)	NH4NO3 + KNO3 Half Half

3.2.2) Stock Solution of II (Micro Salts):

Constituents	Amount/g
Potassium iodide (KI)	0.083
Boric acid (H3BO3)	0.62
Manganese sulphate (MnSO4. 4H2o)	2.23
Zinc sulphate (ZNSO4.7H2O)	0.86
Sodium molybedate (Na2. MoO4)	0.025
Cupric sulphate (cocl2.5H2o)	0.0025

Final volume was made up to 500ml. **3.2.3)**

Stock solution (iron source)

Constituents	Amount/g
Ferrous sulphate (Fe2So4. 7H2o)	2.78

Final volume was made up to 500ml

3.2.4) Stock solution IV

Constituents	Amount g/l
Nicotinic acid	0.50
pyridoxine Hcl	0.50
Thiamine Hcl	0.01
Glycine	0.20

Final volume was made up to 500ml. After preparation and labeling, stock solutions were stored in a deep freeze chamber.

3.3) Preparation of Media: -

For Media Preparation, all the procedures were carried out in neat and clean media preparation room. The procedure for preparation of different Media is given below: **3.3.1) Medium-Ist**For MS-Medium (100ml) we took

Stock I (Macro salts)	**10ml**
Stock II (Micro salts)	**0.5ml**
Stock III (Iron sources)	**0.5ml**
Stock IV (Inositol)	**0.5ml**
Stock V (Vitamins)	**0.5ml**
Total volume	**12 ml**

Final volume of 100ml was made by adding 88 ml of distilled water in a 100ml flask. After this 3g of sucrose was added and then stirred until fully dissolved. The pH of the medium was adjusted to 5.6-5.8 using 0.1 N NaOH or 0.1N HCl. Medium was solidified with
0.8g of agar and autoclaved at 15 lbs pressure at 120oc for 20 minutes.

At least 6 culture vials were prepared for medium containing double concentration of NH4No3.

Medium- 2nd For medium 2nd we took

Stock I (NH4NO3 & KNO3 absent)	**10ml**
Stock II	**5ml**
Stock III	**5ml**
Stock IV	**5ml**
Stock V	**5ml**

Final volume was made 100ml. Then 3g of sugar was added, pH of medium was adjusted to

5.6 – 5.8 then at last agar was added as a gelling agent. The beaker was slightly heated in order to dissolve the agar then the medium was poured in vials and finally the medium was autoclaved at 15 lbs pressure at 120oc for 20 minutes.

Medium 3rd, 4th, 5th, 6th.

For medium 3rd 4th, 5th, 6th we took 10ml of stock I(C) ,Stock I (D) stock I (E), Stock I(F) and rest were taken as same as that of medium Ist and 2nd.

RESULTS:

Effect of different treatments on callus production from leaf explants

Treatments	**Explant used**	**Callus Production**	**No. of days taken for Callus Production**	**Percent culture response**
MS basal	Leaf	Low	16 days	65 %
NH_4NO_3 & KNO_3 Half Concentration	Leaf	High	18 days	40 %
NH_4NO_3 same Conc. KNO_3 double Conc.	Leaf	Moderate	16 days	25 %
NH_4NO_3 & KNO_3 Double Conc.	Leaf	Moderate	14 days	20 %
Without Nitrogen	Leaf	Low	21 Days	5 %
NH_4NO_3 Double Conc. KNO_3 Same Conc.	Leaf	Moderate	19 Days	15%

Conclusion

From this research paper it was concluded that maximum callus production and root formation was observed in MS Basal Medium and the medium containing half concentration of nitrogen, while minimum callus percentage was seen in rest medium. The above two mediums were useful to develop efficient protocol for in vitro regenerations as it favors both callus production rooting

In the highlights of above scientific description about nitrogen from different technical aspects, we are giving conclusion that nitrogen has great role in plants

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