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Mineral metabolism during *in vitro* organogenesis in *Sugarcane Var.co.740*

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Abstract- Inorganic elements are essential for growth and development of the plants grown either in vivo or in vitro. During *in vitro* differentiation, the minerals are absorbed from the basal medium and used during cell proliferation and differentiation. There are number of reports to understand mineral metabolism in sugarcane with reference to productivity studies, crop logging, mineral deficiencies and physiological role in sugar metabolism. During present investigation, major and minor elements are analyzed from the three stages (IC, IM and IR) of differentiating tissues
Key words: Sugarcane, Organogenesis, differentiation, metabolism.

Introduction

Sugarcane is one of the cash crops of India as 60% of the world sugar. Sugarcane industries is the second largest agro based industry [12]. Sugarcane (Saccharum species hybrid) issue and cell culture is widely used in sugarcane improvement and breeding programmes [13].Organogenesis is the development of adventitious organs are primordial from undifferentiated cell mass in tissue culture by the process of differentiation [15].There are number of reports to understand mineral metabolism in sugarcane with reference to productivity studies, crop logging, mineral deficiencies and physiological role in sugar metabolism[1-4].During present investigation , major and minor elements are analysed from the three stages(IC,IM and IR) of differentiating tissues. Mineral metabolism during organogenesis in *in vitro* conditions helps in understanding the developmental biology of sugarcane tissue culture at metabolic and biochemical level [14, 15-17].

Materials and Methods

Three different stages of callus, medium green callus and regenerated shoot were used for the estimation of mineral ions. The acid digestion was carried as per the method of AOAC hand book [8-9]. About 1gm of oven dried plant material was transferred into it and covered with watch glass. After initial effervesces subside the beaker was slowly heated on hot plate. As soon as the plant material was completely dissolved 20 ml of perchloric acid was added into it and condensed up to 5 ml. The beaker was cooled and the stock solution was prepared to 50ml in a volumetric flask by diluting with glass distilled water. The extract was then filtered through Whatman filter paper (No.44) the filtrate was used for estimation of mineral ions using different methods.

a) Estimation of Sodium and Potassium

These two elements are determined by using Elico flame photometer [10-11] standard NaCl and KCl was used for standards.

b) Estimation of Calcium

Calcium was estimated by titrimetric EDTA method [5-7]. In the EDTA titration a metal ion sensitive indicator is often employed. The EDTA forms a complex with Ca giving a pink colour with ammonium purpuate used as an indicator. This titration was carried out in highly alkaline conditions. About 10ml of acid digested extract, 25ml of distilled water, and 5ml of 2N NaOH were taken in an evaporating dish and a pinch of ammonim purpurate was added to observe the pink colour. The mixture was titrated against 0.1m EDTA and the end point results in a colour change purple to violet. The titration was repeated 3 times. The amount of Calcium was determined.

c) Estimation of Fe, Cu, Mn, Mg, Mo and Zn

The mineral ions like Fe, Cu, Mn, Mg, Mo and Zn were analyzed using acid digested extract. These minerals were estimated using atomic absorption spectrophotometer

Results and Discussions

a. Analysis of Major elements in the regenerating tissues of sugarcane

It is seen in Table-1 that the level of nitrogen, potassium, calcium and magnesium in the three different stages of differentiating callus into shoot buds. Nitrogen content shows linear increase in its level as callus becomes green and as it proliferates into well developed shoots. Nitrogen content is almost double in the completely regenerated shoots (IR) as compared to undifferentiated callus cells (IM). Similar trend is noticed with respect to uptake of potassium in the three stages of development. The level of potassium increases slightly during transformation of callus cells into green shoot buds, however there is a six fold increase in its content in the completely regenerated shoots. Thus depicting the higher requirements of potassium ions in the different metabolic process in an organized leaf tissue. The values of calcium content in the various stages of differentiated shoots are shown in Table-1 and Figure-2. Calcium content decreases during early phase of regeneration and again increase in well developed shoots. These results show that a calcium requirement is higher during shoot induction. The values of magnesium as shown in the same table also reports similar observation. Their requirement seems to be maximum in the green differentiated shoots and in the callus cells as compared to green shoot buds. In general it is seen that the uptake of all major elements is maximum in the completely regenerated shoots as compared to unorganized structures.

b. Analysis of major elements in the regeneration tissue of Sugarcane

Microelements like iron, manganese, zinc and Copper etc are necessary for the balanced growth and development of organs in plants. During present investigation the elements like copper, iron, manganese, zinc, molybdenum, and sodium are analyzed for their levels in regenerating tissues of sugarcane as can be seen from Table-2 and Figure 1-3. Copper, iron and sodium shows gradual increase in its contents as cell differentiated into shoots. The maximum uptake is carried by completely regenerated shoots as compared to other stages. These values are 2-3 times more in final stages of regeneration as compared to first two stages. In case of zinc and sodium there is a slight decline in its contents in the medium green shoot buds (IM) than undifferentiated callus (IC) and the level further get stimulated in the completely regenerated shoots (IR). Many of these minor elements play significant role in chlorophyll synthesis, sugar metabolism and osmoregulation and hence their optimum level in the differentiated organs has a significant role in organogenesis.

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Table 1- Major mineral components in the different stages of regenerated shoots in sugarcane variety Co.740

Stages	Calcium(g/100g)	Magnesium(g/100g)	Potassium(g/100g)	Nitrogen(g/100g)
IC	0.46(±0.21)	0.61(±0.21)	0.14(±0.03)	0.52(±0.21)
IM	0.36(±0.08)	0.49(±0.32)	0.16(±0.03)	0.82(±0.22)
IR	0.53(±0.82)	1.04(±0.07)	0.85(±0.04)	1.10(±0.18)

Table 2- Minor mineral components in the different stages of regenerated shoots in sugarcane variety co.740

Stages	Cu(mg/100g)	Fe(mg/100g)	Mn(mg/100g)	Zn(mg/100g)	Na(mg/100g)
IC	5.6(±1.1)	55.5(±0.6)	10.5(±0.6)	38.2(±2.8)	16(±2.8)
IM	5.5(±0.8)	53.0(±7.5)	13.6(±1.2)	36.0(±3.2)	15(±3.1)
IR	10.6(±1.2)	194.4(±8.5)	35.6(±6.4)	108.35(±2.1)	31(±1.2)

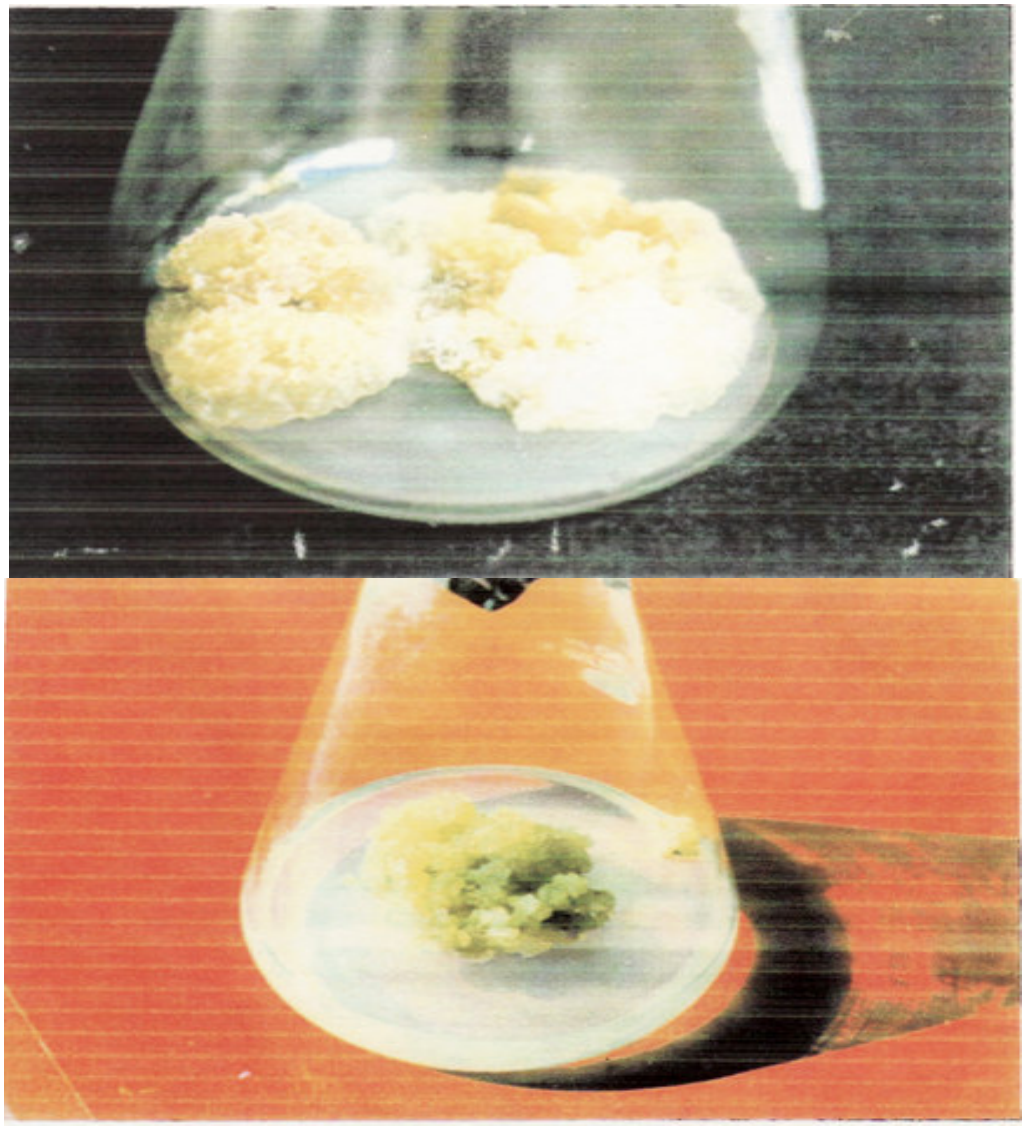

Fig. 1- Regenerated callus in sugarcane Variety Co-740

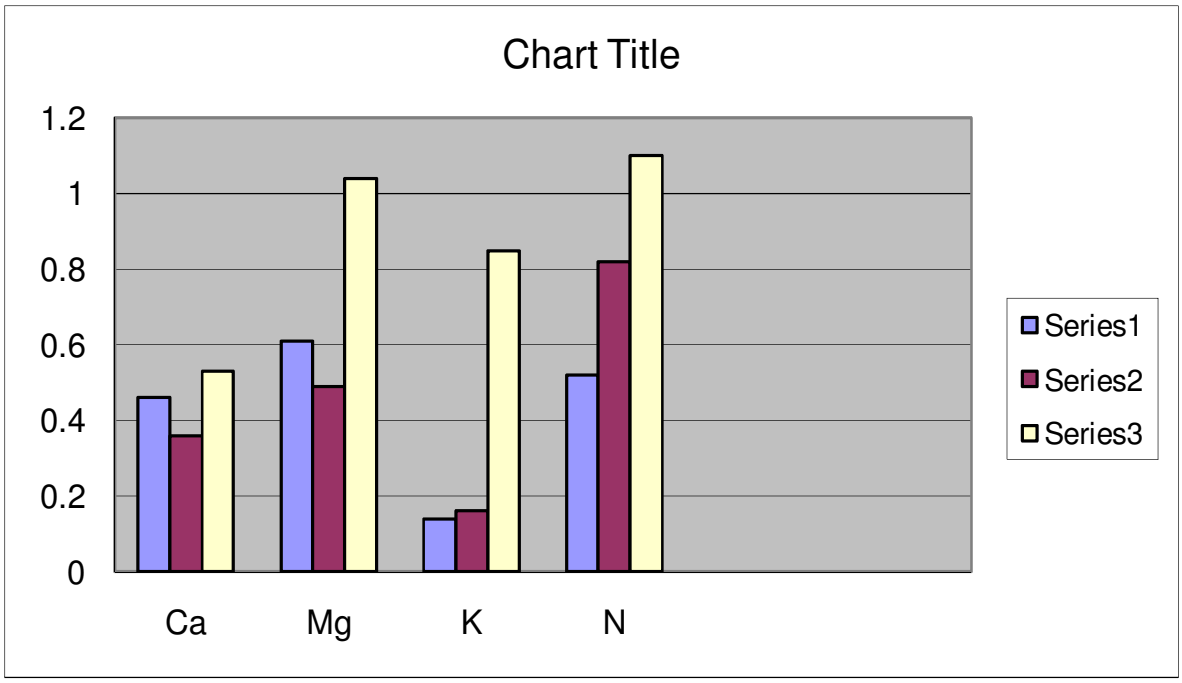

Fig. 2- Major mineral components in the different stages of regenerated shoots in sugarcane variety Co 740 (Series 1: IC, Series 2: IM and Series 3:- IR)

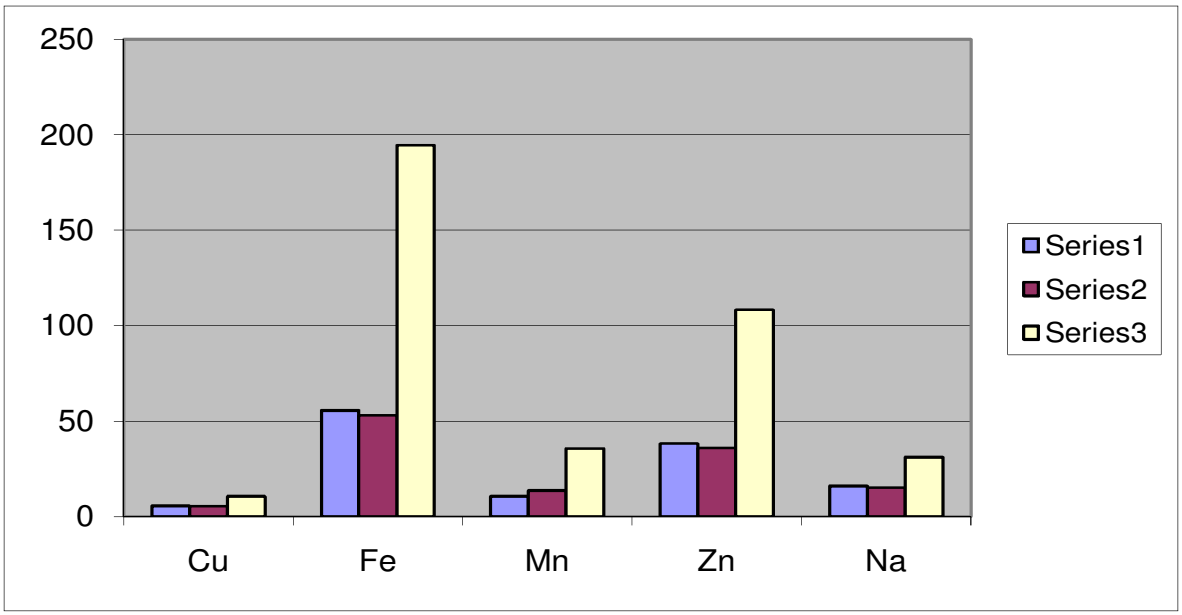

Fig. 3-Minor mineral components in the different stages of regenerated shoots in sugarcane variety Co.740 (Series 1: IC, Series 2: IM and Series 3:- IR)

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