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C^{14} Glucose uptake during *in vitro* organogenesis of Sugarcane

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Abstract- Sugarcane tissue culture is frequently used for the developmental studies during organogenesis .Organogenesis is complicated process involving cellular, molecular and tissue level change in the metabolism. The unorganized mass of cells differentiates in to shoots by undergoing modifications in the metabolic reaction etc. It is necessary to study there metabolic change by investigating glucose utilization pattern. However there are no reports on C^{14}glucose uptakes studies in 3 organogenetic stages. During short term feeding the highest glucose activity is observed in callus stage and it declines as the tissue dedifferentiates into shoots. Similar pattern is observed during long term exposure. It indicates that C^{14} glucose utilization pattern depends on the organogenetic stage and its requirements are higher at the initial callus stage than in the completely regenerative shoots.
Key words: sugarcane, callus, organogenesis, regeneration, C^{14} glucose uptake.

Introduction

The *sugarcane Var.Co.740* is an important commercial crop in India. Many investigations are in progress to study basic and applied aspects of sugarcane physiology. Plant Tissue culture technique has become a powerful tool for studying basic and applied problems in plant biology. The establishment of viable plant tissue culture by [1-3] marked the beginning of this rapidly developing area of applied and basic research. Plant cell and tissue culture opened a number of possibilities and much literature has accumulated with new investigations over the last three decades. Organogenesis differentiation in tissue culture was observed a long time with tobacco tissue [2] and carrot explants [4]. Research on sugarcane tissue and cell culture was started in Hawai 1961 by Nickel. Tissue culture is now widely used in sugarcane improvement and breeding programmes [5]. Demonstrated for the first time that plantlet could be developed from sugarcane callus cultures. Callus can be initiated from any sugarcane tissue like shoot and root apical meristems. Young root, leaves and node tissue immature florescence and pith parenchyma [6]. xperimental investigations have concentrated on the physiological biochemical and histological aspects related to organogenesis. However, there is a need for detailed stud on the C^{14}glucose uptake during organogenesis under *in vitro* conditions. These studies will ads to our understanding of the developmental biology of sugarcane tissue culture at cellular and biochemical level. During present investigation local sugarcane variety (co.740) has been used for standardizing organogenesis protocol and its various developmental stages have been investigated for C^{14} glucose uptake green callus and whole regenerated shoots were allowed to utilize C^{14} glucose for 5 seconds and 1 hour period. C^{14} labeled compounds in ethanol soluble extracts were separated and studied by two dimensional chromatography and autoradiography [7, 8] in the 2-D chromatography (Whatman No.1) Phenol: water(80:20 v/v) was used as a first solvent whereas n –butanol :acetic acid: water(45:5:11 v/v) as a second solvent. About 0.5ml of each sample was loaded on to chromatograms and three sets of chromatogram were run for each sample. The compounds were located by developing the chromatograms with ninhydrin for amino acids, phosphomolybidilic acid for sugar phosphates, anilinepthalate for sugars and bromophenol blue for organic acids. The separated compounds on the chromatograms were marked, recovered and were used to counting the amount of radioactivity and percent distribution on the chromatograms were exposed to x ray films (Kodak make 11x14mm) for 30 days and the autoradiogram were developed. Individual chromatographic paper bands were cut into small pieces and dropped into mini glass counting vials containing 10ml of scintillation cock tail mixture. These were allowed to attain room temperature for 48 hrs with intermittent shaking once in every 2 hours. The radioactivity in each vial was measured using wallac system and percent distribution of each compound was calculated.

Materials and Methods

Local sugarcane variety Co 740 was obtained from the experimental field of Biotechnology Department, Gulbarga University, Gulbarga. Plant material from 3-4 months old disease free cane stand was used for *in vitro* culture. Young leaves and sub apical meristem portions were cut into pieces of 2-3 cm with sterile blade and transferred to conical flask containing distilled water added with few drops of teepol as a wetting agent. Then the material was surface sterilized in 70% alcohol for 3 mins and rewashed with distilled water thrice to remove traces of alcohol. One more sterilization was performed with 0.1% $HgCl_2$ was given. This was also shed with thrice with sterile distilled water and the material was made into pieces (3-4mm size) in sterile Petri plate containing blotting papers. The explants were inoculated on MS media immediately; otherwise it secretes polyphenol compounds which inhibit the growth of callus. The inoculated tissues were incubated at 26^0C in dark conditions. After 4-5 weeks of growth the callus was subcultured on to the same medium for more callus proliferation. The callus was continuously sub cultured in the MS medium at an interval of 3-4 weeks. The tubes stored in dark at 25oC.the highly friable callus were used for organogenesis mainly for shoot formation. The shoot forming callus at different stages of organogenesis was used for various physiological and biochemical studies during present investigation.

- Unorganized mass of callus(IC)
- II Medium green callus of 30 to 45 days old shoot buds (IM)
- III Complete regenerated shoots o 60 to 80 days OLE-IR (IR)

Three different stages were used for the investigation. The glucose residue uptake was investigated by feeding C^{14} glucose (0.5mci of specific activity and 42mci/ umol) for 1 hour to 3 stages of regenerated sugarcane callus Var 740. About 0.5gm of each stage callus sample was made into small pieces and placed into a 25ml conical flask containing 50 ml of 5Mm phosphate buffer(pH 6.0) about 0.5 ml of labeled glucose was added into each flask and was agitated on a shaker at 100 rpm at 25^0C for 6 hrs.duration. After the reaction was terminated by adding absolute alcohol after the removal of phosphate buffer. The callus bits were homogenized and soluble fractions were extracted in 80% alcohol. The extracts were vacuum evaporated and condensed to about 2ml and used for determination of total counts. The insoluble materials present on the filter paper was extracted by placing it into 250ml conical flask containing 20ml of distilled water and 1ml of conc. and the contents were condensed to about 2ml and subjected to total counts. The total counts were measured by liquid scintillation counter using the cocktail mixture consisting toluene,2,5-diphenyloxazoyl (PPO)and 1,4 bis 5-phenyloxazoyl benzene(POPOPO). About 0.1ml of each sample of soluble and insoluble fractions of all the three stages of regenerated callus samples was loaded onto a whatman paper strip and was made into small piece and placed in glass scintillating vials of 20ml capacity containing 10ml of scintillation cocktail. The liquid were measured in liquid scintillation counter. During present investigation C^{14} glucose uptake are analyzed in three stages (IC, IM IR) of differentiating tissues. The results are presented in Table-1. The plant material of *Sugarcane Var Co.740* namely callus, medium green callus and whole regenerated shoots were allowed to utilize C^{14} glucose for 5 sec and 1hr period. The ethanol soluble and insoluble fractions were analyzed for total uptake. The results are presented in Table-1 and Fig1.

Results and Discussion

The uptake of glucose for a short feeding period is highest in the callus stage (IC) and as the callus cells differentiate into shoots the counts decline. This decline is about 39% when callus gets transformed into medium green tissue and about 63% during its further development into complete green shoots. Thus as organogenesis proceeds from unorganized cell to shoot buds into regenerated shoot show interesting results with respect to C^{14}glucose uptake for 5 seconds. The results of long term feeding are also depicted in same table after 1 hr of C^{14} glucose feeding cells as compared to medium green shoots buds are regenerated shoots. However after 1 hr. uptake the completely regenerated shoots showed almost double amount of labeling as compare to cells with medium green color. The maximum uptake in the ethanol soluble fraction seen in the callus cells followed by regenerated shoots and finally into medium green cells. Similar pattern is also noticed in the insoluble fractions of the extract. In this case also maximum activity is located

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in the callus cells and then in the medium green and green shoot buds. The table also shows the ratio of soluble to non soluble fractions in all these three stages of organogenesis development. The ratio of soluble to insoluble is 0.05 in the organized mass of callus cells and it increases in the medium green cells to about 645, further the ratio increases at the level of 1:1 in the completely regenerated shoots. Thus the present experiment with respect to C^{14}glucose utilization shows variation depending on the developmental stage organogenesis and pattern of utilization. Both short term and long term results indicate programmed and controlled reaction pattern during shoot development. The experiment was further continued to analyze the distribution of utilized compounds in the various fractions of organic metabolites like sugars amino acids and organic acids using autoradiography and chromatography. The plant material in 3 organogenesis stages was used for C^{14}glucose uptake studies. During short term feeding the highest glucose activity as observed. In the callus stage and it declines the tissue as the tissue dedifferentiates into shoots. During long term exposure of the material to the C14glucose 3 also depicted maximum uptake in the callus a compared to the medium green cells and regenerated shoots. The experiment conducted indicates that the C^{14}glucose utilization pattern depends on the organogenetic stage and its requirements are higher at the initial callus stage than in the completely regenerated shoots.

References

[1] Gautheret R.J. (1939) *C.R. Hebd. Seances Acad. Sc.*, 208, 118-120.
[2] White N.J. (1939) *Am. J. Bot.* 26, 59-64.
[3] Nobecourt P. (1973) *Phytomorphology*, 21:109-112.
[4] Nobecourt P. (1939) *Compt. rends. Soc. bioi.* 130, 1270-71 224.
[5] Barba R. and Nickell L.G. (1969) *Planta*, 89:299–302.
[6] Liu M.C. and Chen W.H. (1974) *Proc. Int. Soc. Sugarcane Technol*, 15:118–121.
[7] Ouellet C. and Benson A.A. (1952) *J. Exp. Bot*, 3: 237 - 245.
[8] Raghavendra A.S., Rao I.M. and Das V.S.R.Z. (1976) *Pflanzenphysiol.* 80, 36–42.

Table 1- C^{14} glucose uptake in the three different stages of regenerated shoots in sugarcane var.co 740

Stages	5 seconds	1 hour soluble	1 hour insoluble	Total counts	Ratio soluble/ insoluble
IC	593.3 (±24.7)	95.7(±12.0)	1848.6 (±57.6)	1944.3 (±69.6)	0.052
IM	364.0 (±31.6)	46.8(±9.9)	227.6 (±17.3)	374.4 (±27.2)	0.645
IR	136.0(±20.0)	354.9 (±27.5)	338.9 (±18.3)	692.9 (±45.8	1.1

*Cpm/unit volume of extract

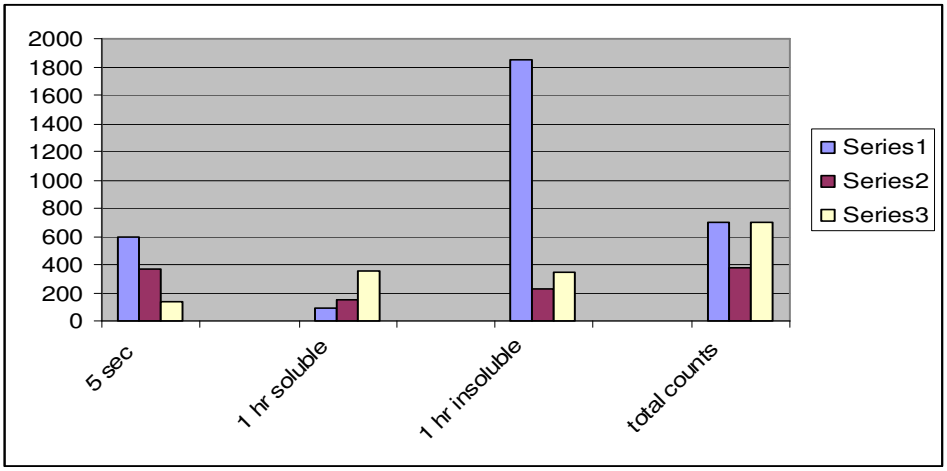

Fig. 1- C^{14} glucose uptake in three different stages of regenerated sugarcane shoots in sugarcane var.co-740 (Series 1: IC, Series 2: IM and Series 3:- IR)