African Journal of Biotechnology Vol. 7 (5), pp. 575-578, 4 March, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

^{14}C-Glucose uptake studies in the red rot toxin treated sugarcane callus

A. B. Vedamurthy[1], M. K. Sateesh[2], G. R. Naik[3]

[1]Post Graduate Department of Biotechnology, The Oxford College of Science, J.P Nagar, Bangalore-560078, India.
[2]Department of Biotechnology and Microbiology Bangalore University, Jnana Bharathi, Bangalore 560056, India.
[3]Department of Biotechnology Gulbarga University, Gulbarga-585106, India.

Accepted 8 January, 2008

Fungal toxins cause serious damage to the cellular functions of host tissue. In the present report the toxin extracted from *Colletotrichum falcatum* Went was partially purified and treatments were given to the callus of susceptible sugarcane callus variety CoC 671. The influence on ^{14}C-glucose uptake and its further utilization was investigated. The toxin treatment reduced the total uptake of glucose and also inhibited its conversion into insoluble products of cellular metabolism. Accumulation of organic acids and lowered synthesis of total sugars was mainly noticed. The susceptibility of sugarcane var. CoC 671 to red toxin seems to be due to poor efficiency and imbalance in utilization of glucose under biotic influence.

Key words: *Colletotrichum falcatum,* fungal toxin, autoradiogram, callus.

INTRODUCTION

Sugarcane var. CoC 671 is highly susceptible to the red rot disease (Naik and Vedamurthy, 1997). The pathogen *Colletotrichum falcatum* synthesizes a fungal toxin, which is responsible for the metabolic damage at cellular level. In the present investigation, an attempt has been made to investigate the uptake and metabolism of ^{14}C-glucose in the toxin treated callus cells of the cane variety CoC 671. Such fundamental studies are necessary to understand the biochemical basis and influence of disease causing fungal metabolites (Liu, 1981). Glucose is a basic precursor for many metabolic pathways in the cell and the present work investigates its probable metabolism under stress condition of fungal toxin.

MATERIALS AND METHODS

Diseased cane stalks of CoC 671 were used for the isolation of the red rot pathogen. The methodology for isolation of pathogen and extraction of red rot toxin has been reported (Vishwanathan et al., 1996). In the present work toxic metabolite extracted from sugar cane host extraction medium was used to study uptake pattern in CoC 671 callus.

The glucose uptake studies were carried out by feeding the labeled ^{14}C-glucose (0.5 mci, specific activity 42 mci/mmole) for 6 h to toxin treated callus of sugarcane variety CoC 671 and appropriate control was also maintained. The 0.5 g of CoC callus was placed in conical flasks containing 50 ml phosphate buffer (5 mM, pH 6.0). The ^{14}C-glucose measuring 0.5 ml was added to two sets of conical flasks containing callus which were floating in phosphate buffer and the flasks were kept on rotatary shaker. The experiment was carried under controlled light and temperature (25±2°C). The conical flasks were agitated continuously during the uptake of ^{14}C-glucose.

The radioactivity in the samples were counted in Wallac 1402 liquid scintillation counter. The scintillation fluid (10 ml) containing PPO (4.0 g/l), POPOP and toluene was taken in glass scintillation vials of 20 ml capacity.

^{14}C-glucose labeled compounds in ethanol soluble extracts were separated and studied by the two dimensional chromatography and autoradiography (Besnsen et al., 1950; Raghavendra and Das 1997; Patil et al., 1983). In the two dimensional chromatography (Whatman No: 1) phenol: water (80:20 v/v) was used as first solvent whereas N-butanol: acetic acid: water (44:5:11 v/v) as a second solvent. The compounds were located by developing the chromatogram with ninhydrin for amino acids, phosphomolybdic acid for sugar phosphates, anilinphthalate for sugars and bromophenol blue for organic acids.

Chromatographic paper bands were cut into small pieces and dropped into mini vials containing 3 ml of toluene with a mixture of 4

*Corresponding author. Email: vedamurthy15@yahoo.co.in

Abbreviations: PPO, 5-Diphenyl Oxazole; POPOP, 1,4-Bis(5-Phenyl Oxazolyl) Benzene.

Table 1. ^{14}C-Glucose uptake in control and toxin treated callus of sugarcane Var. CoC 671.

Callus	^{14}C-glucose uptake, soluble fractions*	^{14}C-glucose uptake, non soluble fractions*	Total uptake	Soluble / insoluble fractions
Control	468959.1 ± 1011.6	75625.0 ± 383.4	544584.1	6.2
Toxin treated (0.2%)	539755.5 ± 853.6	66152.6 ± 113.5	605908.1	8.2

*Values expressed as cpm/mg fresh weight.

Table 2. Percent distribution of radioactivity in different compounds of sugarcane callus var CoC 671 exposed to ^{14}C-glucose for 6 h.

Compound	Control	Toxin treated
Sugars		
Fructose	23.9	0.17
Glucose	36.48	40.10
Sucrose	13.54	-
Total Sugars	73.92	40.27
Sugar phosphates		
PGA	0.20	0.30
PEP	0.06	-
Sugar Phosphate	1.03	0.40
Total sugar phosphates	1.29	0.70
Organic acids		
Citrate	0.6	3.9
Malate	0.80	5.9
Ascorbate	0.025	-
Succinate	0.04	0.08
Glycolate	0.51	-
Tartarate	0.24	7.70
Total organic acids	1.495	17.58
Amino acids		
Glutamate	4.63	19.1
Cystine	2.8	-
Valine	3.5	-
Aspartate	3.8	-
Proline	0.27	2.4
Phenylalanine	2.62	11.01
Alanine	0.43	2.20
Leucine	0.93	-
Glycine + Serine	9.77	1.8
Tyrosine	0.68	-
Hydroxyproline	2.94	-
Total amino acids	32.37	36.51

g/l PPO and 0.1 g/l POPOP cocktail. These vials were allowed to come to room temperature for 48 h and shaken for every 2 - 3 h during day time. The radioactivity in each vial was counted in a Wallac 1409 liquid scintillation counter. The X-rays films (Kodak) were used for autoradiogram on screen.

RESULTS AND DISCUSSION

To study glucose uptake metabolism, ^{14}C-glucose uptake pattern was investigated in the control and red rot toxin treated callus. The values on the rate of total uptake of ^{14}C-glucose in control and 0.2% toxin treated growing callus of CoC 671 are reported in Table 1. These values clearly show that the uptake was increased in the toxin treated callus tolerant. However, the ratio of soluble to insoluble depicts a different pattern. The toxin treated callus utilizes more glucose for the synthesis of soluble products as compared to insoluble products. In the control callus more insoluble products were detected.

The distribution of radioactivity in different fractions of control and red rot toxin treated callus of sugarcane variety CoC671 is presented in Table 2 and Figure 1. The uptake of glucose for 6 h increased the synthesis of sugars, amino acids, organic acids and sugarphosphates by 74%, 27.5%, 6.2% and 1.3%, respectively (Table 3). On the contrary the glucose metabolism in the toxin treated callus showed inhibition in its conversion into other sugar, sugar phosphates and amino acids. A marked increase in the contents of organic acids was noticed in the toxin treated callus.

The details of the assimilation of ^{14}C-glucose in the different individual compounds is shown in Table 2. The results show that the fructose and sucrose formation is drastically inhibited in the presence of toxin. Similarly the synthesis of sugar phosphates is also reduced in the callus growing in the medium containing the toxin. This treated callus show accumulation of organic acids (tartarate, malate and citrate) as compared to control callus. Similarly, increase in amino acid level is due to accumulation of glutamate, phenylalanine and alanine in the toxin treated cells were also observed.

Our results show that entry of toxin into the cells of sugarcane var. CoC 671 influences deviation in the normal pathway of synthesis and storage of amino acids. The glucose under stress conditions is not efficiently utilized for the synthesis of other essential metabolites, namely sugar phosphates and some important amino acids. Our studies have clearly indicated that the toxin resistant cells have higher efficiency to utilize glucose under stress conditions. A biological system under stress probably uses more energy than in the absence of stress. Cell culture technique provides means to study influence of biotic stress mechanism. In the present investigation

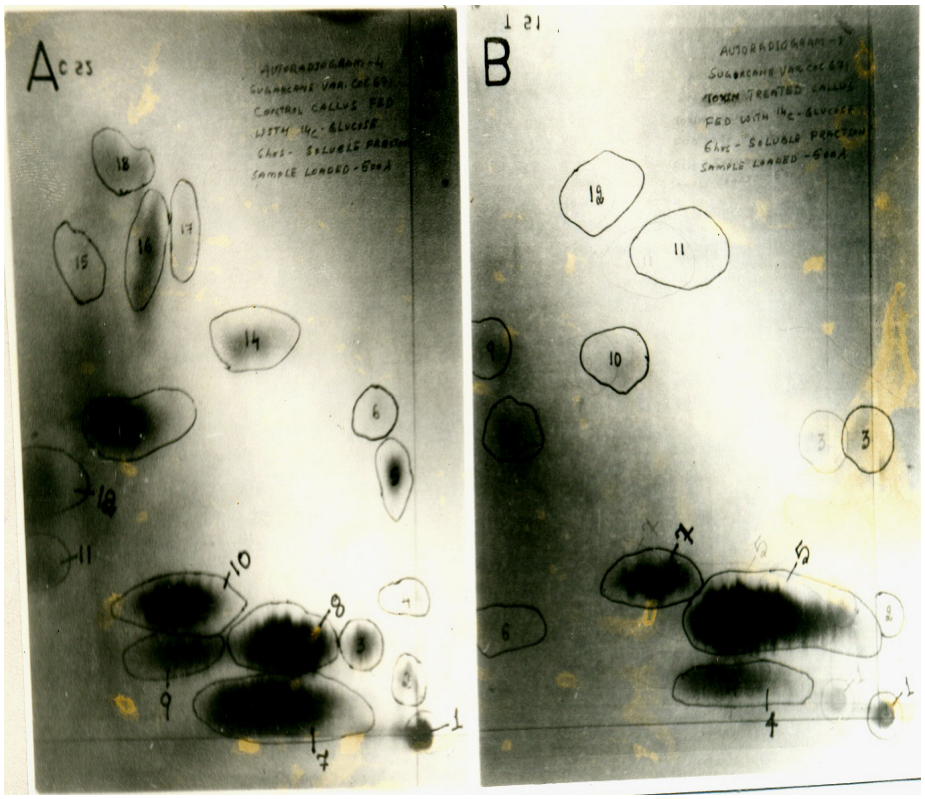

Figure 1. Autoradiogram showing distribution of radioactivity in control and toxin treated callus of CoC 671 following 6 h ^{14}C-glucose uptake. A. Control. 1. Origin. 2. PGA. 3. Sucrose. 4. PEP. 5. Citrate. 6. Glycolate. 7. Fructose. 8. Glucose. 9. Aspartate. 10. Alanine. 11. Hydroxyproline. 12. Proline. 13. Valine. 14. Malate. 15. Leucine. 16 and 17. Tyrosine. 18. Leucine. B. Toxin treated (0.2%). 1. Origin. 2. PGA. 3. Alanine. 4. Sugar Phosphates. 5. Glucose. 6. Glycine + Serine. 7. Proline. 8. Phenylalanine. 9. Glutamate. 10. Valine. 11. Tartarate. 12. Succinate.

Table 3. Total percent distribution of radioactivity in different fraction of treated sugarcane callus Var CoC 671 exposed to ^{14}C-glucose for 6 h.

Compound	Control	Treated
Sugar	73.9	40.2
Sugar phosphates	1.2	0.7
Organic Acids	6.2	36.6
Amino Acids	27.5	17.4

the toxin resistant cells showed 11.3% more efficiency for ^{14}C-glucose utilization when compared to control.

The fungal toxin causes change in the metabolism of the host cells (Agrios, 1996). This investigation analyzed the pattern of ^{14}C-glucose uptake in the control and toxin treated callus of sugarcane var. CoC 671. The results have clearly shown that the toxin treated callus has less capacity to assimilate the glucose into useful products like sugar phosphates and normal organic acids and amino acids. Instead the assimilation of some of the amino acids (glutamate, phenylalanine and alanine) and organic acids (tartarate and citrate) was observed. The results suggest that the plant with great efficiency in converting the energy to biomass under toxin stress conditions is necessary for inducing or adapting to disease resistance in sugarcane cells.

REFERENCES

Agrios GN (1997). Plant Pathology, Harcourt Publisher, 4th Edition, pp. 73-77.

Besnsen AA, Bassham JA, Calvin M, Goodale TL, Has VA, Stepke W (1950). The path of carbon in photosynthesis V. paper chromatography and radio autography of the products.J.Amer. Chem. Soc. 72: 1710.

Liu MC (1981). *In vitro* methods applied to sugarcane improvement. Plant Tissue Culture: Methods and Applications in Agriculture (ed. Thorpe, T.A), Academic Press, New York, p.29.

Naik GR, Vedamurthy AB (1997). *In vitro* evaluation of red rot toxin influence on sugarcane (*Saccharum officinarum* L) var. CoC 671. Curr. Sci, 73: 367.

Patil TM, Mirajkar PB, Hegde BA, Joshi GV (1983). Influence of Soil salinity on morphology, rate of carbon assimilation, photosynthetic products and enzyme activities in a sorghum hybrid CSH-5, Indian Plant Physiol., 26: 155.

Raghavendra AS, Das VSR (1997). Shifts in photosynthetic carbon la-

beling pattern by etiolated rice (*Oryza sativum* L) seedling during greening. New Phytol., 79: 89.
William RD (1984). Metabolic energy cost for plant cells exposed to salinity.California Agric. 38(10): 22.