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SHORT COMMUNICATION

CHANGES IN NUCLEIC ACID AND PROTEIN CONTENTS DURING PLANT REGENERATION FROM CALLUS IN SUGARCANE

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Total nucleic acid (DNA & RNA) and protein content were recorded during callus growth and plant differentiation in sugarcane Cv. B.O. 91. Comparison with field grown plant, revealed a rapid increase in DNA, RNA and protein content during callus growth phase. A rapid decrease in RNA and protein content was observed during plantlet differentiation while very little fluctuation was noticed in DNA.

The metabolic characteristics of *in vitro* growing tissue are manifested by alterations in carbohydrates, lipids, proteins, nucleic acid and secondary metabolism (Fuzimura *et al.*, 1980). Changes in portein and nucleic acid content are most common and have been described in a range of plant species (Raghavan, 1986) but not studied in sugarcane as yet.

The present paper describes the quantitative changes in nucleic acid and protein content during *in vitro* callus growth and plantlet differentiation.

The callus cultures of sugarcane cultivar B.O. 91 were raised from sub-apical tissue on modified Murashige and skoog (1962) medium, as previously described MS (Lal and Singh, 1991). Differentiation from callus was achieved on modified MS medium supplemented with 1.0 mg/l kinetin. All the cultures were incubated at 25 ± 3°C under cool white flourescent light (200 lux) for 14 hr photoperiod. Freshly harvested sub-apical tissue from actively growing field plants and 3 week old cultures maintained on callus growth and differentiation media were used for estimation of DNA, RNA and protein content.

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500 mg of fresh sample was homogenised with phosphate buffer (pH 7.0) and all the protein in the homogenate was precipitated with 5% TCA, dissolved in 0. 5N NaOH and total protein was estimated according to Lowry *et al.* (1951) using BSA as standard.

DNA and RNA of the samples were extracted by the method of Nieman and Paulsen (1963). The total RNA was estimated spectrophotometrically using orcinol reagent (Jenson, 1955) and total DNA was estimated by the diphenylamine reaction (Burton, 1956).

The changes in nucleic acid (DNA and RNA) and protein contents during field condition in intact plant, growing callus and differentiating callus are summarized in Table I. Results showed that the sub-apical tissues of the plant grown under field conditions have a significantly low level of protein, DNA and RNA as compared to growing and differentiating (Organogenic) callus. There was a rapid increase in DNA,RNA and protein content in the growing callus as observed at different incubation periods (Table I). The content of protein and nucleic acid at this stage actually represent the net metabolic state of the cultured tissue. Since growing callus consists of a relatively high proportion of dividing cell populations, changes in DNA and RNA are likely to occur. Intact plant (sub-apical tissue) of sugarcane contains majority of cells with a stable configuration and DNA content and the variant (of

Table I. Changes in nucleic acid and protein content* during *in vitro* callus growth and differentiation in sugarcane cv. B.O. 91.

Stage	DNA	RNA	Protein
Field grown plant (Sub-apical tissue)	0.66	14.02	1.22
Growing callus**			
7 days old	0.87	25.08	1.56
14 days old	0.89	26.61	1.83
21 days old	0.88	26.55	1.80
Differentiating callus***			
Pre-meristemoid stage	0.87	21.78	1.46
Meristemoid stage	0.85	20.56	1.31
Regenerant stage	0.85	20.01	1.14

* Values are expressed as mg/g fresh weight.
** On MS+5.0 mg/l 2,4-D medium.
*** On MS+1.0 mg/l Kn medium.

higher ploidy level) cells remain supressed. The condition become different during callus growth. It allows all the cells to divide making the cell population highly heterogenous and results in increase in DNA content due to increased number of polyploid cells. Occurrence of polyploid cells in cell cultures of sugarcane is well elucidated (Liu and Chen, 1976). The marked increase in RNA content observed in growing callus not enexpected since callus growth requires additional RNA synthesis prior to cell division (Raghavan, 1983). This agrees with relatively high RNA/DNA ratios obtained for callus growth in other plants *in vitro* (Verma and Dougall, 1978). Callus growth phase, when followed by plantlet differentiation, results into cellular differentiation, organisation and extensive lignification. This developmental change (plant differentiation) is concurrent with the sharp decline in RNA and protein levels and to a lesser extent in DNA level. In sugarcane cultivar B.O.91, regeneration takes place via somatic embryogenesis from cell cultures (Lal and Singh, 1991). This pathway requires specific regulatory and structural proteins to be synthesized and stops the formation of other proteins as reported with other embryogenic systems (Raghavan, 1986). This is achieved by lowering the RNA polymerase activity, ultimately leading to a decrease in protein content. Very little change in the DNA content is the ultimate function of the polyploidization in cell cultures coupled with genetic selection during plantlet differentiation. Gene amplification has been a common feature in cell cultures (Evans, 1989) and may be the reason for this drastic alteration in DNA content in sugarcane callus.

Since cells with normal ploidy (Stable configuration) share more in regeneration population of cell with higher ploidy level is reduced and results in decreased DNA content. This selection is further enhanced under field condition for sustainance of plant life and eliminates a very high percentage of polyploid cells. Polyploid cells are known to show slow rate of division in comparison to normal diploid cells. Changes in RNA and protien content follows a similar trend as in the case of DNA. These changes occur in consistency with most of the cases for protein and RNA content during cell division and differentiation (Raghavan, 1983), however, such a drastic change in DNA content is peculiar to sugarcane. Study of the mechanism of DNA amplification *in vitro* and regulatory proteins for cell division and differentiation in sugarcane need further research to resolve these metabolic changes and better understanding of morphogenetic control.

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