

IN VITRO STUDY OF CHROMOSOMAL ABERRATIONS IN CALLUS CELLS OF SUGARCANE VARIETY CoN 95132

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ABSTRACT

*An in vitro experiment was carried out to study the induction of variability using tissue culture techniques in sugarcane (*Saccharum officinarum* L.) variety CoN 95132 at Sugarcane Tissue Culture Laboratory, Regional Sugarcane Research Station, Navsari Agricultural University, Navsari during 2009-10. The various kinds of callus masses were induced on MS medium fortified with different concentrations of 2, 4-D (2,4-Dichlorophenoxy Acetic Acid). Chromosome number in callus cells developed from leaf segments varied from $2n = 90$ to $2n = 168$, while callus cells developed from stem bit, the chromosome number varied from $2n = 89$ to $2n = 168$. The percentage of cells with more than one nucleus was seen in a range from 1.78 to 2.54 per cent in fresh leaf callus, whereas it was observed in a range from 1-66 to 2.09 per cent in fresh stem callus. Different kinds of nucleated cells such as binucleate, trinucleate, quadrinucleate and pentanucleate cells were also observed during the microscopic investigation. The varying numbers of nucleoli were observed in abnormal cells ranged from 2 to 5.*

KEY WORDS: *Callus, chromosomes, nucleus, sugarcane*

INTRODUCTION

Sugarcane is an important cash crop of India. It is a tropical crop and is a major source of sugar. A basic requirement for the success of crop improvement through breeding is the availability of genetic variability. In nature, variability for desired traits is found dispersed in land races, wild and weedy relatives of the crop species. Since utilization of variability available in distinct relatives poses serious operational difficulties. In this context, callus culture helps in development of desired variations necessary for crop improvement as well as may result into newer genotype(s). Moreover, it is also possible to develop stress (biotic and abiotic) tolerance cell lines/plants by subjecting callus cells to particular stress.

Because of such importance of callus culture, this technique is also known as tissue culture breeding. Variation in chromosome numbers is observed many times in callus cells. In the present study, also the cells of sugarcane callus possessed variable chromosome numbers viz; diploid, aneuploid and mixoploid. In some other crops, viz., cotton, sunflower, barley and onion, the complete diploid, aneuploid and mixoploid callus cells were observed during cytological investigations. The majority of cell population investigated was made of diploid cells. Overall cytological heterogeneity in culture may arise because of two factors. First, the cells of various ploidies and genetic constitution of the initial explants may be induced to divide (Torrey, 1967) and secondly,

culture conditions may bring about new irregularities (Butcher *et. al.*, 1975). Mitotic abnormality likely give rise to aneuploid cells (Bayliss, 1973). Although aneuploidy has been associated with the loss of organogenic potentiality there are at least a few instances where aneuploid cells have been shown to reconstitute full plants (Koornneef *et. al.*, 1989).

MATERIALS AND METHODS

The present investigation was carried out at the Sugarcane Tissue Culture Laboratory, Regional Sugarcane Research Station, Navsari Agricultural University, Navsari during 2009-10 to study the induction of variability using tissue culture techniques in sugarcane (*Saccharum officinarum* L.) variety CoN 95132. Callus masses were induced under different treatments of 2, 4-D (2,4-Dichlorophenoxy Acetic Acid) from leaf segments as well as stem bits. Calluses were subcultured ten times on the same medium. The old calli were used for chromosome studies. In long-term callus cultures, polyploids and aneuploids were predominant. When cell and tissues are passed through a dedifferentiation phase of growth in cultures, genetic changes take place which are reflected in terms of instability in chromosomes number and structures. Keeping this in view, cytological investigations were under taken in callus cells.

Cytological study of callus cells

(i) Pretreatment and fixation

Old callus of 10th sub culture derived from leaf segments were used in this investigation. Cell masses from 10th sub culture callus were placed on the same fresh medium (0.5 mg/l NAA + 1.0 mg/l BAP + 20 g/l sucrose) to get rapid multiplication. The rapidly multiplying callus cells were drawn at 12.00 h in the morning for the arrest of metaphase stage. They were washed three times with α - bromonaphthalene and stored for 2 h at 10⁰ C. The material was further washed thrice in distilled water and fixed for 24 h in

the mixture of acetic acid and ethanol in the ratio of 1:3 (v/v), respectively. Small amount of fresh callus was also fixed without any pretreatment.

(ii) Hydrolysis, staining and squashing

After 24 h, callus cells gently heated in 2 per cent aceto-orcein for 10 minutes on spirit lamp. The cells were then transferred to a mixture of concentrated HCl and ethanol in 1:1 (v/v) ratio and further heated for 2 minutes. The cells were transferred to Carnoy's fluid-II (Glacial acetic acid: Chloroform: Ethanol; 1: 3: 6, v/v) for 4 minutes and restored in 70 per cent ethanol for squashing. Callus cells are put in a drop of 2 per cent aceto-orcein on a clean slide. It was pressed under the cover slip, slightly warmed and again pressed between several folds of blotting papers and the excess cytoplasmic stain was removed by running warm 45 per cent acetic acid under cover slip. After blotting, the slides were sealed with billing wax with the help of iron needle. The photographs of different mitotic chromosomal abnormalities were taken, while observing temporary slides under microscope. Trinocular phase contrast research microscope was connected with cable to high resolution colored Samsung 14" monitored television via adaptor (DC power supply regulator 12v). Photographs were taken by colored close circuit digital video camera METZER 795 (Zenil).

RESULTS AND DISCUSSION

Cytological studies of callus cells were made for variation in chromosome number and structural abnormalities. Cells with different number of nuclei were observed during the cytology of untreated callus by spindle poisons. Any change in chromosome number and their structures shows wide spectrum of variation in the progeny of *in vitro* regenerants.

Abnormal cell containing varying number of nuclei in callus cells

The modal chromosome number of sugarcane var. CoN 95132 is $2n=98$. Cells with varying number of nuclei were observed during the cytology of untreated callus by spindle poisons (Table 1). As seen from the table that the percentage of cells with more than one nucleus ranged between 1.78 to 2.54 per cent in fresh leaf callus, whereas it was observed in a range from 1.66 to 2.09 per cent in fresh stem callus. However, most of the cells had single nucleus (> 97%). The varying numbers of nuclei were observed in abnormal cells. It ranged from 2 to 5. The binucleate, trinucleate, quadrinucleate and pentanucleate cells were also observed during the microscopic examination. (Plate 1 A-E). Frequency of the abnormal cells with two nuclei was maximum, followed by three, four and five nuclei. Similar results were reported by Pithia (1995) in Indian mustard (*Brassica juncea* L. Czern and Coss) var. Varuna.

Variation in chromosome number in callus cells

Beside the range of upper and lower chromosome numbers in cells was observed in different levels of 2, 4-D (Table 2). In long-term callus cultures, polyploids and aneuploids were predominant. In case of callus cells, all the cells had diploid chromosome number. However, some callus cells were aneuploids cells with odd chromosome numbers. Cytological studies of callus cells were made for variation in chromosome number. The variation in chromosome numbers in callus cells is depicted in Plate 2 (A-D). Chromosome number of callus cells developed from leaf segment varied from $2n = 90$ (Plate 2 A) to $2n = 168$ (Plate 2 C). While callus cells developed from stem segment varied from $2n = 89$ (Plate 2 B) to $2n = 159$ (Plate 2 D). In culture, normal mitotic abnormality occurs due to the abnormal mitotic spindle activity as affected either by the lack of physical organization of the tissues or by some

chemical constituent of the medium (Bayliss, 1973). In present study, the callus cells possessed variable chromosome numbers viz. diploid, aneuploid and mixoploid. In sugarcane, complete diploid, aneuploid and mixoploid plantlets were observed during cytological investigations by Liu and Chen (1973) and Nagai, *et al.* (1986). Similarly, Nair *et al.*, (1993) observed chromosomal variation in callus cultures of *Allium senescens* L. var. Minor. The frequency of aneuploid plant was low. The majority of cell population investigated was made of diploid cells. Plants regenerated from such cultures indicated their origin from cells whose chromosome make up is different from diploid state in sugarcane (Heinz and Mee, 1970). While studying the cells of fresh callus, multinucleate cells were observed. $2n = 306$ chromosomes were scored in suspension cells of sugarcane var. F 164 (Liu *et al.*, 1977). Heinz and Mee (1969) observed polyploids, mixoploids and diploids differentiated callus cells in sugarcane var. H 57-1627. Bajaj and Gill (1985) initiated callus from excised anthers, ovules and embryos of *Gossypium arboreum* and *G. herbacium* and showed chromosome numbers, ranging from haploids to hexaploids, and from high polyploidy to aneuploidy. Cavallini and Lupi (1987) determined chromosome mosaicism in callus derived from meristem of *Helianthus annuus*. Ziauddin and Kasha (1990) stated that the chromosome abnormalities (Polyploids) increased with time on the relative high concentrations of 2, 4-D (2.0 mg/l or more) in barley (*Hordeum vulgare*). However, the frequency of such abnormal cells was very low.

Chromosomal structural aberrations in callus cells

The chromosomal structural aberrations in callus cells are depicted in Plate 3 (A to D). The details of cells with various chromosome structures found under microscopic examination are as below:

- V – shape chromosome observed in callus cell developed from stem bit on MS medium containing 1 mg/l 2, 4-D ($2n = 98$: Plate 3-A).
- Dicentric chromosome observed in callus cell developed from leaf bit on MS medium supplemented with 3 mg/l 2, 4-D ($2n = 133$: Plate 3-B).
- C-bending chromosome observed in callus cell developed from stem bit on MS medium containing 4 mg/l 2, 4-D ($2n = 89$: Plate 3-C).
- Acrocentric chromosome observed in callus cell developed from stem bit on MS medium fortified with 5 mg/l 2, 4-D ($2n = 88$: Plate 3-D).

Callus was induced a on MS medium supplemented with 2.0 mg/l 2, 4-D leaf sheath culture of the intergeneric hybrid *Saccharum officinarum* and *Sclerostachya fusca* (Roxb.) ($2n = 55$) and cytological differentiations like dicentric and acentric chromosome structures were noticed in the cells of subclones (Sreenivassan and Sreenivassan, 1984).

CONCLUSION

Cytological investigation clearly indicated usefulness of the indirect organogenesis for creating variability. Variation in structure and number of chromosome in callus cells without any mutagenic treatments obviously stated potentiality of these techniques. Chromosome number in callus cells developed from leaf segments and stem portions varied from $2n = 90$ to $2n = 168$ and $2n = 89$ to $2n = 168$, respectively, in callus cells. The percentage of cells with more than one nucleus was in a range from 1.78 to 2.54 per cent in fresh leaf callus, whereas it was observed in a range from 1.66 to 2.09 per cent in fresh stem callus. Abnormal cells were ranged from 2 to 5 found in nucleoli.

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Table 1: Frequency of normal and abnormal cells in leaf and stem callus of sugarcane cv. CoN 95132

Leaf Callus

Slide No.	Number of Cells With Single Nucleus	Frequency of Abnormal Cells With Varying Number of Nuclei				Total Cells Observed	Per Cent of Cells With Single Nucleus	Per Cent of Cells With More Than One Nucleus
		Two Nuclei	Three Nuclei	Four Nuclei	Five Nuclei			
1.	1024	14	5	3	2	1048	97.71	2.29
2.	1345	15	5	4	2	1371	98.10	1.90
3.	1267	13	6	3	1	1290	98.22	1.78
4.	1150	17	7	4	2	1180	97.46	2.54
5.	1253	14	7	3	1	1278	98.04	1.96
Mean	1207.8	14.6	6	3.4	1.6	1233.4	97.91	2.09

Stem Callus

Slide No.	Number of Cells With Single Nucleus	Frequency of Abnormal Cells With Varying Number of Nuclei				Total Cells Observed	Per Cent of Cells With Single Nucleus	Per Cent of Cells With More Than One Nucleus
		Two Nuclei	Three Nuclei	Four Nuclei	Five Nuclei			
1.	1237	15	4	3	1	1260	98.17	1.83
2.	1314	16	6	4	2	1342	97.91	2.09
3.	1226	13	5	3	1	1248	98.24	1.76
4.	1339	15	6	4	1	1365	98.10	1.90
5.	1186	13	4	2	1	1206	98.34	1.66
Mean	1260.4	14.4	5	3.2	1.2	1284.2	98.15	1.85

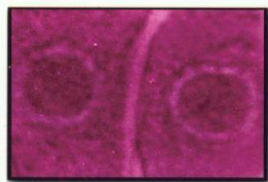
Note: Leaf and stem callus induced on MS medium supplemented with 3.0 mg/l 2, 4-D were used.

Table 2: Chromosome number variation observed in *in vitro* regenerated plantlets and callus cells subjected to different 2, 4-D levels in sugarcane cv. CoN 95132

Modal Chromosome Number: $2n = 98$

1.0 mg /l 2,4-D		2.0 mg /l 2,4-D		3.0 mg /l 2,4-D	
Leaf	Stem	Leaf	Stem	Leaf	Stem
$2n = 90$ to $2n = 110$	$2n = 89$ to $2n = 108$	$2n = 94$ to $2n = 116$	$2n = 93$ to $2n = 110$	$2n = 92$ to $2n = 131$	$2n = 91$ to $2n = 119$
4.0 mg /l 2,4-D		5.0 mg /l 2,4-D		6.0 mg /l 2,4-D	
Leaf	Stem	Leaf	Stem	Leaf	Stem
$2n = 91$ to $2n = 139$	$2n = 90$ to $2n = 136$	$2n = 94$ to $2n = 148$	$2n = 92$ to $2n = 142$	$2n = 92$ to $2n = 168$	$2n = 91$ to $2n = 159$

Plate : 1 Abnormal cells containing varying number of nuclei



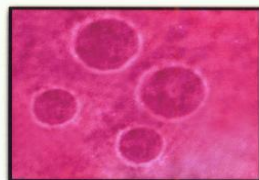
A) Uninucleate cell



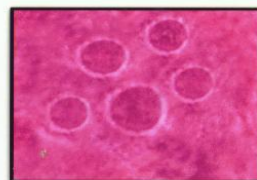
B) Binucleate cell



C) Trinucleate cell



D) Quadrinucleate cell

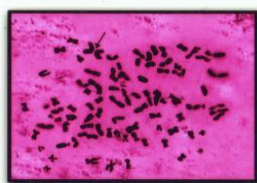


E) Pentanucleate cell

Plate : 2 Chromosomes in callus cell(Numerical Variation)



A) 2n=90, Leaf callus



B) 2n=89, Stem callus



C) 2n=168, Leaf callus



D) 2n=159, Stem callus

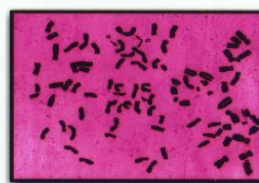
Plate : 3 Chromosomes in callus cell(Structural Variation)



A) 2n=98, V-shape



B) 2n=133, Dicentric



C) 2n=89, C-Bending



D) 2n=88, (Acrocentric)

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