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Numerical heterogeneity of chromosomes and nucleoli in *Saccharum* spp. hybrids

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Abstract

Numerical chromosomal aberrations and their correlation with the extent of nucleolar variations was studied in seven commercial hybrids of sugarcane of subtropical India. Extensive chromosome mosaicism (2n = 92-126) and wide variation with respect to nucleolus number per cell (1-8) was observed in these clones. Numerical Aberration Factor (NAF) has been suggested to ascertain the extent of somatic chromosome variation in mosaic genotypes. The NAF value was highest in the old elite clone Co 1148. The regression analysis of NAF value on nucleoli variation per cell showed a linear relationship (r = 0.65) indicating that, intra-clonal heterogeneity of chromosome number has a direct influence (to a certain limit) on extensive nucleolar variations present in these clones.

Key words : Sugarcane, chromosome mosaicism, mitosis, nucleoli variation, Numerical Aberration Factor (NAF)

Introduction

Modem sugarcane varieties are derived from wild and cultivated species of the genus *Saccharum.* These cultivars are best described as complex interspecific aneuploids due to their highly potyploid interspecific origin and presence of chromosome mosaicism [1]. Owing to polyploid constitution and vegetative mode of propagation, segregations are complex and chromosomal anomalies tend to accumulate within the cells. As a result, the breeding behaviour of the crop remains uncertain [2]. A few commercial hybrids, which are being used in breeding programmes for subtropical India, have been studied for their chromosomal profile. Information on chromosome number and their behaviour in majority of present day cultivars is also lacking. The present investigation therefore deals with hitherto unknown numerical chromosomal aberrations and their correlation with the extent of nucleolar variations if any, in some commercial *Saccharum* spp. hybrids.

Materials and methods

Seven elite (commercial or near commercial) sugarcane clones of subtropical India namely CoJ 64, CoPant 84211, CoLk 8901 and CoS 687 (early maturing clones), CoLk 9110, LG 9223 and Co 1148 (midlate to late maturing types) were taken for the study. Single budded setts of these commercial hybrids were grown in sand culture. One cm. long, healthy root tips were excised, pretreated with 1:1 mixture of saturated aqueous solution of 2,4 para-Dichlorobenzene and 0.002 M 8-Hydroxyquinoline [3] and stained in 2% acetoorcein. Well spread squash preparations in 45% acetic acid were used to study the cytology. At least 5 plants of each clone were studied. Chromosome and nucleoli numbers were ascertained from 5-10 well spread plates per plant.

The chromosome number present in the maximum number of cells from the frequency distribution curve of different chromosome numbers observed in a particular clone, was taken as the Modal chromosome number.

Per cent numerically aberrant cells were calculated as,

$$\% \text{ Numerically aberrant cells} = \frac{\text{Number of cells with deviant chromosome numbers}}{\text{Total number of cells}}$$

(with deviant and modal chromosome numbers)

where,

deviant chromosome numbers are, chromosome numbers other than the Modal number.

Mitotic index (M.I. %) in root tips of different clones was calculated as,

$$\text{M.I.\%} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}}$$

'Numerical Aberration Factor (NAF)' has been calculated as the "proportion of cells showing numerical aberrations, taken in conjunction with the mitotic indices of different clones".

$$\text{NAF} = \frac{\% \text{ Numerically aberrant cells} \times \text{Mitotic index \%}}{100}$$

Results and Discussion

The range of numerical chromosomal variations and modal chromosome counts of different clones of sugarcane and their mitotic index (%) are given in Table 1. The clones exhibited a wide range of diploid chromosome numbers from 2n = 101 in CoLk 8901 to 2n = 115 in CoLk 9110 and Co 1148. Intra-plant chromosome number variation was quite common among various clones (from 2n = 92 to 126 /cell). The frequency of numerically aberrant cells varied from 23.91% (CoS 687) to 40.85% (Co 1148) of total dividing cells. The mitotic indices of these clones ranged between 13.97 % (CoLk 8901) to 17.88% (CoJ 64).

Chromosome mosaicism

The somatic chromosome numbers of elite cultivars (Table 1) were determined at prometaphase or

Table 1. Chromosome number variation and numerical aberration factor (NAF) in different genotypes of sugarcane

Genotypes	Modal chromosome number (2n)	Range of variation	% numerically aberrant cells	Mitotic index %	Numerical aberration factor (NAF)
Early Clones					
CoJ 64	110	100-124	36.91	17.88	6.59
CoPant 84211	104	101-110	34.48	15.10	5.20
CoS 687	104	98-108	23.91	15.71	3.76
CoLk 8901	101	92-116	37.31	13.97	5.21
Midlate & Late Clones					
CoLk 9110	115	101-125	34.92	14.33	5.00
LG 9223	104	100-108	29.30	14.71	4.16
Co 1148	115	101-126	40.85	17.50	7.14

metaphase (Fig. 1). All these genotypes possess widely different somatic chromosome counts ranging from 92 to 126/cell. The presence of chromosome mosaicism is apparent within the clone and approximately 23.91 to 40.85% of the total cells in different clones exhibit such intra-clonal chromosomal variation. Intra-plant chromosome number variation was more common in Co 1148, CoLk 8901 and CoJ 64 as evinced by the high frequency of numerically aberrant cells present in them e.g. 40.85, 37.31 and 36.91% of the total dividing cells respectively, are numerically aberrant. Most frequently occurring chromosome count was considered as modal 2n chromosome numbers. The cells undergoing division in each clone were analyzed for modal frequency of somatic chromosome counts (Fig. 2), which ranged between 2n = 101 in CoLk 8901 to 2n = 115 in CoLk 9110 and Co 1148. The intra-plant chromosomal variation is of common occurrence in commercial clones of sugarcane [4-7]. Such variation may have arisen due to non-disjunction of chromosomes at anaphase owing to spindle anomalies, chromosome

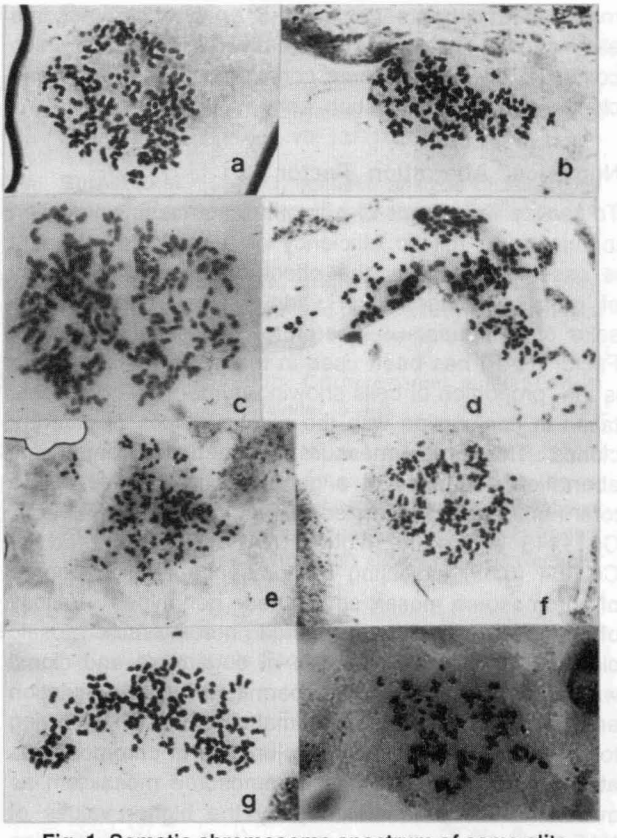

Fig. 1. Somatic chromosome spectrum of some elite genotypes of sugarcane (a) CoS 687, (b) CoJ 64, (c) CoLk 8901, (d) CoLk 8901, (e) CoPant 84211, (f) Co 1148, (g) CoJ 64, LG 9223

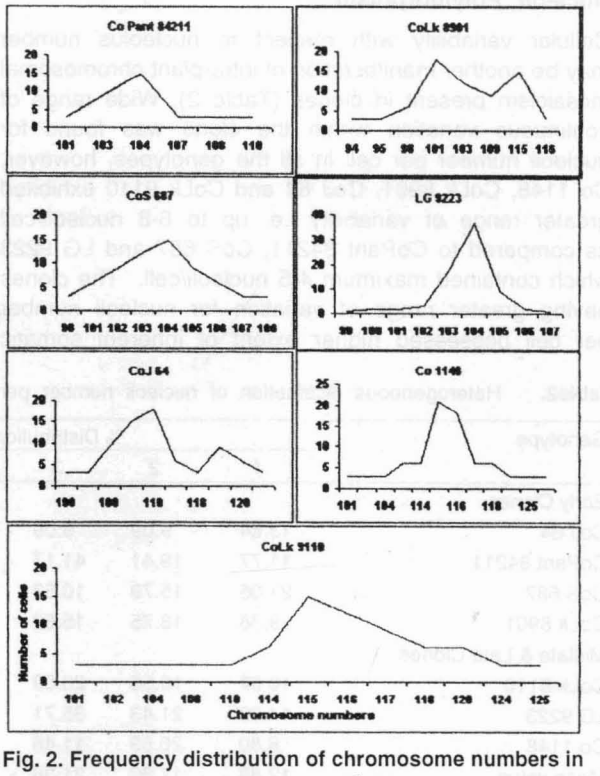

Fig. 2. Frequency distribution of chromosome numbers in sugarcane genotyes

migration and fragmentation and *en bloc* chromosome elimination. The interaction of diverse genomes which constitute the chromosome complement of these hybrid clones may result in such abnormalities.

Numerical Aberration Factor

To realize the extent of somatic chromosome variation in relation to mitotic efficiency of a particular clone, it is essential to nullify the effect of differential efficacy of pretreating agents on various clones and for the sake of comparison. Therefore, Numerical Aberration Factor (NAF) has been used in the present study which is the "proportion of cells showing numerical aberrations, taken in conjunction with the mitotic indices of different clones". This gives a measure of numerical chromosomal aberration frequency of a genotype irrespective of the total number of dividing cells. For example in Table 1, Co 1148 shows the highest NAF (7.14) followed by CoJ 64 (6.59) indicating the presence of high degree of chromosome mosaicism in these genotypes. Validity of this assumption may be tested on some more mosaic clones. The polyploid nature of sugarcane and clonal way of its multiplication has permitted the accumulation and perpetuation of chromosomal divisional errors owing to spindle anomalies or nondisjunction of chromosomes at anaphase, resulting into chromosome mosaicism as evident in the present study by the higher values of NAF (Table 1) for the genotypes which are in cultivation since long e.g. Co 1148 and CoJ 64.

Nucleoli Polymorphism

Cellular variability with respect to nucleolus number may be another manifestation of intra-plant chromosomal mosaicism present in clones (Table 2). Wide range of continuous variation within the clone was found for nucleoli number per cell in all the genotypes, however, Co 1148, CoLk 8901, CoJ 64 and CoLk 9110 exhibited greater range of variability i.e. up to 6-8 nucleoli/cell as compared to CoPant 84211, CoS 687 and LG 9223 which contained maximum 4-5 nucleoli/cell. The clones having greater range of variation for nucleoli number per cell possessed higher extent of inherent somatic

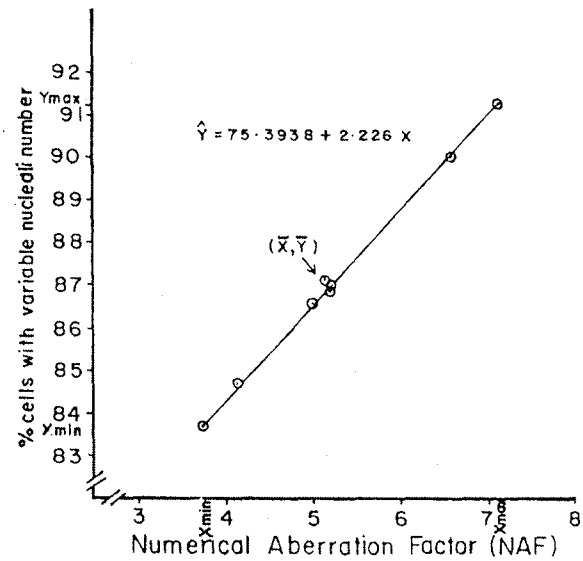

Fig. 3. Relationship between percent cells with variable nucleoli number and numerical aberration factor of different genotypes of sugarcane

chromosome mosaicism. Srivastava and Bajpai [8] reported extensive polymorphism of nucleoli number in some *Saccharum* species and commercial hybrids. They also observed that commercial cultivars exhibited greater range of variability as compared to basic species.

Numerical aberration factor *vis-a-vis* nucleolar variations

To find out if occurrence of chromosome mosaicism in a clone and in turn, the NAF, has any influence upon nucleoli variation per cell, regression analysis was performed. The relationship between NAF and percent cells with variable nucleoli number can be assumed to be linear (Fig. 3). The estimated linear regression is

$$y = 75.3938 + 2.226 \times \text{ for } 3.76 < \times < 7.14.$$

The significance test of linear regression coefficient B (=2.226) suggests that, the variation in nucleoli number for every unit change in NAF (within the range of NAF = 3.76 to 7.14) is expected to fall between 0.035 and

Table2. Heterogeneous distribution of nucleoli number per cell in different genotypes of sugarcane

Genotype	% Distribution of one to eight nucleoli/cell								Total cells with >1 nucleoli
	1	2	3	4	5	6	7	8	
Early Clones									
CoJ 64	13.64	9.09	9.09	22.73	27.27	18.18	-	-	86.36
CoPant 84211	11.77	19.41	41.17	17.65	-	-	-	88.23	
CoS 687	21.05	15.79	10.53	52.63	-	-	-	-	78.95
CoLk 8901	9.38	18.75	15.62	25.00	12.50	9.38	6.25	3.12	91.18
Midlate & Late Clones									
CoLk 9110	10.87	19.56	26.09	17.39	15.22	6.52	4.35	-	89.13
LG 9223	14.29	21.43	35.71	17.86	10.71	-	-	85.71	
Co 1148	8.80	20.59	11.46	29.41	17.65	11.77	-	-	91.18
Mean value	12.83	17.80	21.38	26.10	11.91	6.55	1.51	0.45	87.25

4.417 nucleoli/cell, 80% of the time. The strength of this linear relationship was not much, as evinced by the value of correlation coefficient i.e. r = 0.65 which indicated that the linear function of NAF can explain only 44.89% of total variation in nucleoli number per cell. Hence, intra-clonal heterogeneity of chromosome number is not the sole cause behind extensive nucleolar numerical variation present in these clones, however, to a certain limit such variation can be explained by the prevalence of hybridity and extent of mosaicism in cultivated sugarcane clones.

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