

Physico-Chemical Properties of Chromatin and Induction of Ploidy in Sugarcane

Seehy M. A.¹, Ghonema M. A.², Saleh M. S.² and Badawy O. M.²

ABSTRACT

This work was carried out at Subbhia experimental station for sugar crops. The present investigation aims at inducing polyploidy in sugar cane (*Saccharum officinarum*) and to investigate its agronomic traits. Colchicine was used to produce poly ploidy from three cultivars. These cultivars are (C9 G.T. 54-9, PH 8013 and NCO 310). Buds were treated with 0.05% colchicine solution. Treated segments were allowed to germinate until a shoot system length of 40 cm had reached. Plants were then transferred and allowed to grow in the yield. The growing plants were examined for polyploidy firstly by Guard cells of Stomata, Chloroplasts, DNA content in leaves, and chromosomes from adventitious roots initiated at the laboratory. Physico-chemical properties for chromatins and deproteinized DNA were estimated. Total soluble solids (T.S.S); plant height and plant diameter were estimated. The obtained results would be summarized as follows: The present studies aimed to produce polyploidy in cultivar C9. The obtained results showed that the average of polyploidy was found to be 9% for C9 and 4% for PH 8013. Regarding chemical composition and melting temperatures of chromatins different ratios were obtained reflecting differential transcriptional activities. However, active and repressed fractions of chromatin were estimated. With respect to the evaluated agronomic characteristics different values were observed.

INTRODUCTION

It is well known that genetic improvement of crop plants depends on the availability of desirable agronomical characters to begin with. In addition, it needs an appropriate screening method to detect these desirable characters. The scientific achievements during the last two decades in the field of biotechnology, suggest that it is now the time to apply the existing knowledge for practical problems in agriculture, specially the production and the isolation of new genetic variability in higher plants. Among these new developments, *in vitro* plant culture techniques (meristem, embryo, cali, cells and protoplasts) offer a great potential for the improvement of crop plants (Bourgin, 1983; Maliga, 1984).

All over the world, the increase of sugar production through the genetic improvement of the two most important sugar crops, i.e. sugar cane (*Saccharum*

officinarum L.) and sugar beet (*Beta vulgaris* L.), is an ongoing process, since more than 2000 years. In Egypt sugar cane is considered among the most important industrial converting crops. It is the main source of sugar industry and the sole source for molasses industry. Sugar cane is cultivated in about 270,000 fedan producing about 1,100,000 ton of sugar annually (Sugar Annual Report of Egypt, USDA-2012). In Egypt, although sugar beet crop is a supplement for sugar industry, its byproducts are used for the production of untraditional animal feed, as well as many other secondary industries. It is cultivated in about 355,000 fedan, producing about 910,000 ton of sugar annually (Sugar Annual Report of Egypt, USDA-2012). However, the genetic improvement of sucrose yield of these crops turned to be a slow and limited process. Doney, (1988) attributed this to the negative correlation between sucrose concentration and other yield component characters in sugar cane and sugar beet crops. Kholovoda *et al.*, (1985) suggested that the use of *in vitro* techniques for this purpose (increase sucrose contents) could be of great help. In such materials, it is expected to have no correlation relationship as those existing between separate organs in the whole plant. The present study was carried to induce polyploidy for any possible genetic improvement of sugar cane crop cultivated in Egypt.

MATERIALS AND METHODS

This work was planned to induce polyploidy in some sugar cane cultivars and evaluation their agronomic characters. So three cultivars were selected and used. This work was conducted at the period that ranged from 2011- 2012.

Attempts for the induction of polyploidy from these three cultivars were undertaken using cuttings with one bud each. About one hundred cuttings from each cultivar were used. Each cutting had two surfaces the first was covered by wax, while the second surface was embedded in solution of colchicine (0.05%).

In addition each bud was surrounded by a layer of cotton moistened with the same colchicine solution. After 7 days, cutting were transferred to water and allowed to grow until a shoot system of about 40 cm was observed. They were then cultivated in the field and allowed to grow under the field conditions.

¹Department of Genetics, Faculty of Agriculture, Alexandria University.

²Genetic and Breeding Department, Sugar Crops Research Institute, Agricultural Research Center.

Received October 7, 2012, Accepted December 28, 2012.

Different characteristics were measured. They were:

- 1- Number of plastids in guard cells of stoma.
- 2- Size of epidermal cells in leaves.
Point No.1 & 2 were employed for preliminary screening.
- 3- Measurement of total soluble solids, plant height and stem diameter.
- 4- Physico-chemical properties of chromatin and DNA isolated from leaves.
- 5- Investigation of meiotic chromosomes, when it was possible, and mitotic chromosomes when it was needed.

- Isolation of chromatin:

The chromatin was isolated according to the method described by Fellenberg and Schömer, (1975) and Seehy *et al.*, (1984).

- Purification of chromatin:

Chromatin was purified by filtration through Sephadex G-25 Fine using 1M NaCl buffer. The purity of chromatin preparations was checked using the criteria of Fellenberg, (1974).

-Thermal Stability and Chemical Composition of Chromatin:

When purified chromatin showed adequate ultraviolet absorption spectrum, it was then dialyzed three times at 0 - 2 °C against the following buffer (1L buffer/10 ml chromatin solution): 0.0014 M NaCl, 0.00025 M Tris – HCl, pH 7.2.

- Estimation of melting temperature (T_m-value):

The UV absorbance for the chromatin at both 260 and 280 nm was recorded at each one-degree-increase in temperature (the heating rate was 1°C / min.). Absorbances at all temperatures were calculated as relative to the absorbance at 50 °C (Spang and Platt, 1972). The relative absorbance was plotted against the temperature and the T_m-value was calculated as the temperature which gave half the overall increase in relative absorbance.

- Chemical composition of chromatin:

DNA, total protein, histones and non-histones proteins were determined as µg/ml chromatin, and then the different proteins were calculated as relative to DNA.

- Isolation of DNA:

To purified chromatin solution (soluble in 1 M NaCl), NaCl was added to a final concentration of 2.5 M, then it was gently shaken, stored at 2°C overnight. The DNA was separated from the chromosomal proteins by filtration through Sephadex G-200.

The purity of this DNA was estimated from the UV absorption spectrum at wavelengths in the range of 230-320 nm. The spectrum of DNA contaminated with proteins was distinguished from that of the deproteinized DNA most strikingly by its greater absorption at 230 nm due to the peptide bonds.

- Estimation of melting temperature of DNA:

The T_m-value of DNA was calculated in the same way described in estimation of T_m-value of chromatin.

-Estimation of thermal stability and chemical composition:

In order to estimate the thermal stability of sugar cane chromatin (against temperature) as well as the chemical composition, the following procedure was used:

- Chromatin isolation was done by the method described by Fellenberg and Schömer, (1975), and Seehy *et al.*, (1984).

- Purification of chromatin:

Chromatin in 1M NaCl was purified by chromatography on Sephadex G-25 fine (Pharmacia fine chemicals AB, Uppsala, Sweden). Purified chromatin (soluble in 1 M NaCl) was dialyzed against the following buffer: 0.0014 M NaCl + 0.00025 M Tris-HCl pH 7.2.

- Estimation of T_m-value:

The procedure was the same as that described by Bonner *et al.*, (1968). From each extraction two estimations were calculated. The absorbance of all investigated samples was recorded every minute at 260 nm as well as at 280 nm. The relative absorbance was calculated as described by Spang and Platt, (1972) and Seehy *et al.*, (1984). Relative absorbance = AG/A 50 °C, where AG is the absorbance at a given temperature and A 50 °C, is the absorbance at 50 °C.

- DNA estimation:

The diphenylamine method of Giles and Myers, (1965) and described by Burton (1968) was used for determination of DNA concentrations. Total protein determined by the method of Lowry *et al.*, (1951). using albumin from bovine serum (Sigma) as standard. Histones were extracted and determined by the method of Lowry *et al.*, (1951). Non-histone proteins were calculated as the differences between the total proteins and histones. Deoxyribonucleic acids, total proteins, histones and non-histones proteins were determined as µg/ml chromatin and then, the different proteins were calculated as relative to DNA. From the composition of histones and of DNA, one can calculate that if a nucleohistone in which DNA is fully complexed with basic proteins, the mass ratio of histones to DNA should be approximately 1.35 to 1 (Bonner, 1965).

RESULTS

In this work 100 cuttings for each cultivar were used. The results showed that C9 cultivar was proven to be higher than PH 8013 or NCO 310 in response to polyploid. Using C9 cultivar, 0.09 % cuttings were observed with polyploid cells, while PH 8013 gave 0.04 % with respect to cultivar NCO 310 no success was observed. Table (1) shows the results observed from this method.

Table 1. Number of cuttings used for the induction of polyploid

Cultivar	No. cuttings	No. of polyploid	%
C9	100	9	0.09
PH 8013	100	4	0.04
NCO 310	100	0	0

Figures (1, 2, 3 and 4) show the different developmental stages during colchicine treatment and induction of polyploid.

Anatomical study was carried out to compare stomata in different genotypes. It was found that stoma

in leaves of polyploid plants was found to be larger than that of diploid plants.

- Plastids in guard cell:

Table (2) shows the number of plastids in guard cell of different genotypes. It ranged from 8 –17 plastids per guard cell.

Table 2. Number of plastids in guard cell of different genotypes of sugar cane

Cultivar	No. plastids
C9	8-10
C9A	14-17

- Vascular bundles:

In this anatomical study size of vascular bundles in different genotype was recorded. Figures (5 & 6) show that vascular bundle in young leaves of polyploid plants was shown to be larger than that of diploid plants.

- Epidermal cells:

Anatomical examination of transverse section (T.S.) or young leaves showed that epidermal cells in leaf of polyploid plant were proven to be larger than that of the original cultivar.

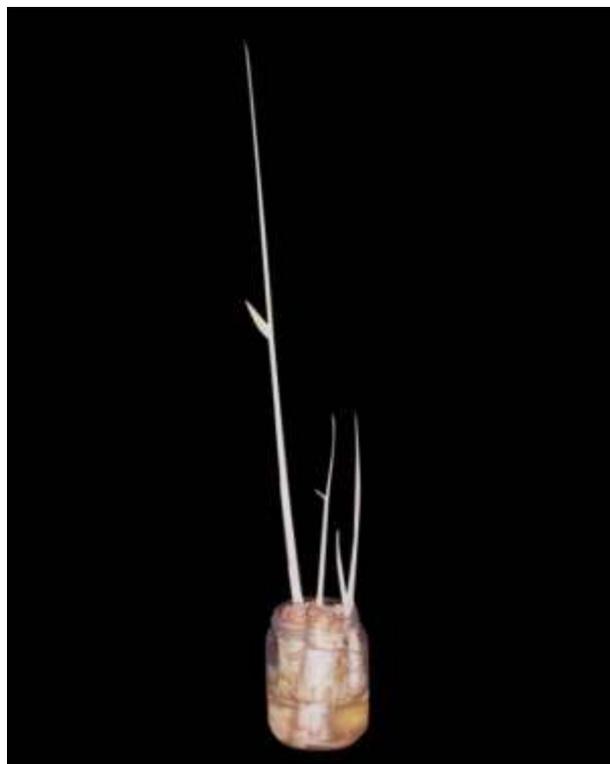


Figure 1. Photograph showing initiation of adventitious roots of C9 cuttings



Figure 2. Photograph showing initiation of adventitious roots of C9 cuttings



Figure 3. Photograph showing initiation of adventitious roots of C9A.



Figure 4. Photograph showing initiation of adventitious roots of C9A

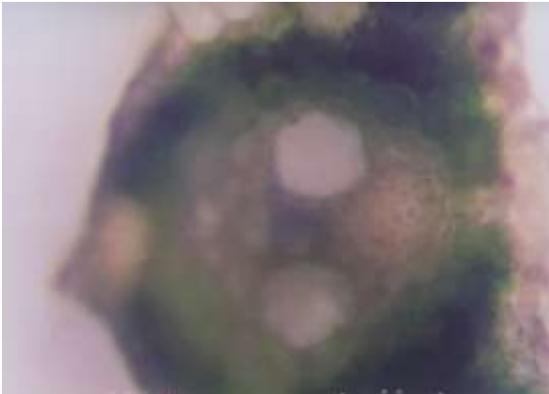


Figure 5. Photomicrograph showing Vascular bundles in a young leaf of cultivar C9

- Chromosome number:

In order to precisely detect the induction of polyploid, buds of cuttings from the observed plants were allowed to germinate under controlled conditions. Adventitious roots were treated with colchicine and chromosome complement was investigated. Chromosome number was found to be 80 chromosomes in diploid cultivar. In polyploid plants a chromosome number of 160 and 240 were observed (Figure 7 & 8 & 9).

Cytological examination of adventitious root tip cells showed that interphase nucleus of the original cultivar was found to be completely different from that of the polyploidy. Figures (10 -13) illustrate these differences where multinuclei were observed.



Figure 6. Photomicrograph showing Vascular bundles in a young leaf of genotype C9A

- Physico-chemical properties of chromatin:

In this work chromatin was isolated and purified using Sephadex G-25. Coefficients of absorbance were proven to be highly and fairly sufficient. The ultraviolet absorption spectra as well as coefficients of absorbance are shown in Tables (3, 4 and 5).

- Tm-values for chromatin:

Temperature of melting for chromatin isolated from leaves of cultivars (C9 & C9A) at (260 nm & 280 nm) and melting profiles at 260 nm as well as to 280 nm are shown in Figures (14 and 15).

Tm-value was found to be 72.5 °C for C9 at 260 nm, while it was 75.5 °C for C9A at 260 nm, Tm-value was found to be 79.5 °C for C9 at 280 nm and 77.5 °C for C9A at 280 nm.

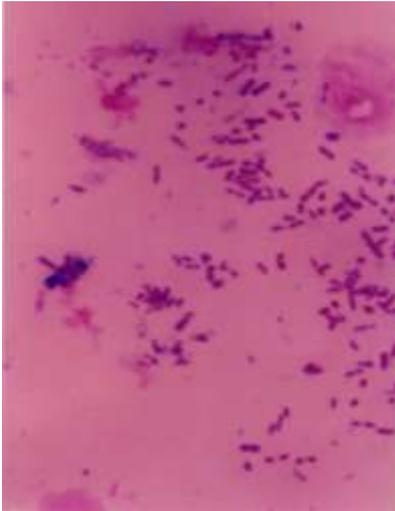


Figure 7. Photomicrograph showing chromosomes of the original cultivar C9 (80 chromosomes).

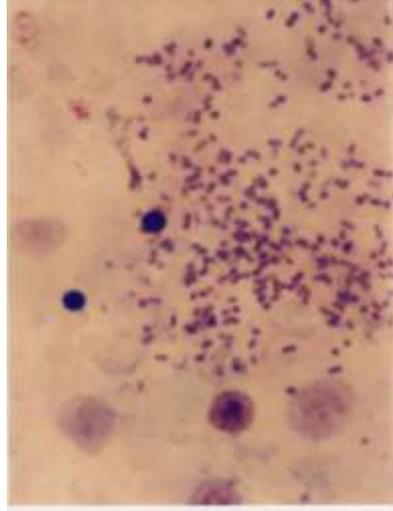


Figure 8. Photomicrograph of sugar cane genotype C9A (induced by colchicine) showing chromosomes number of 160 chromosome.

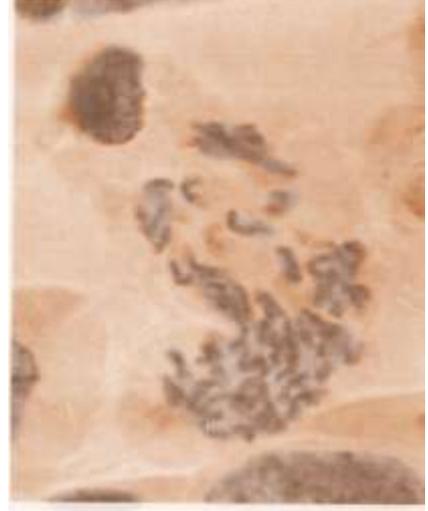
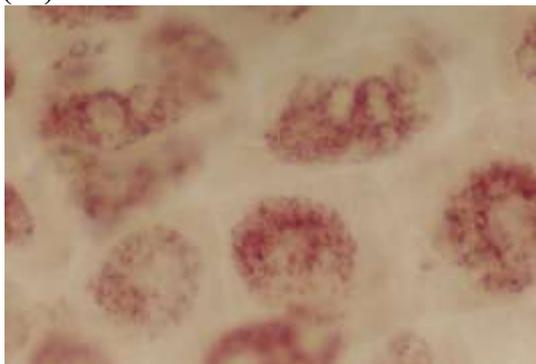


Figure 9. Photomicrograph of sugar cane genotype C9A (induced by colchicine) showing chromosomes number of 240 chromosome.

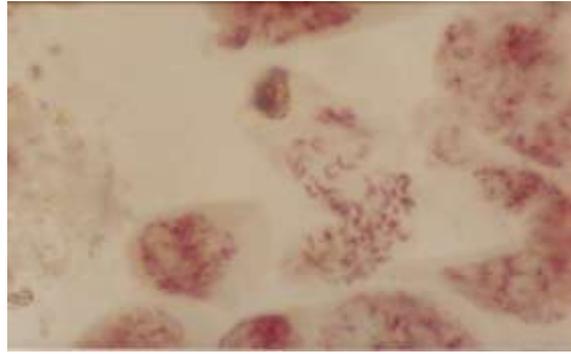


Figures 10. Photomicrograph showing Interphase nucleus of sugar cane cultivar C9 (11)



Figures 11, 12. Photomicrograph showing interphase nucleus of sugar cane genotype (C9A).

** Note: that up to 8 nucleolei were observed



Figures 13. Photomicrograph showing Metaphase stage in adventitious root-tip cells of sugar cane (C9A)

Table 3. Absolute absorbance of purified chromatin isolated from Sugar cane cultivars

Wavelength nm	230	240	250	260	280	320
C9	552	460	660	750	400	62
C9A	732	610	882	1020	540	84

Table 4. Coefficients of absorbance for purified chromatin isolated from cultivars C9 and C9A

Variety	260/240	250/260	280/260	320/260
C9	1.63	0.88	0.53	0.083
C9A	1.67	0.86	0.53	0.082

Table 5. Temperature of melting of (T_M-value) of chromatin isolated from sugar cane cultivars

Varieties	Wave length		ΔT_m (T _m 280 – T _m 260)
	260	280	
C9	72.5	79.5	7
C9A	75.5	77.5	2

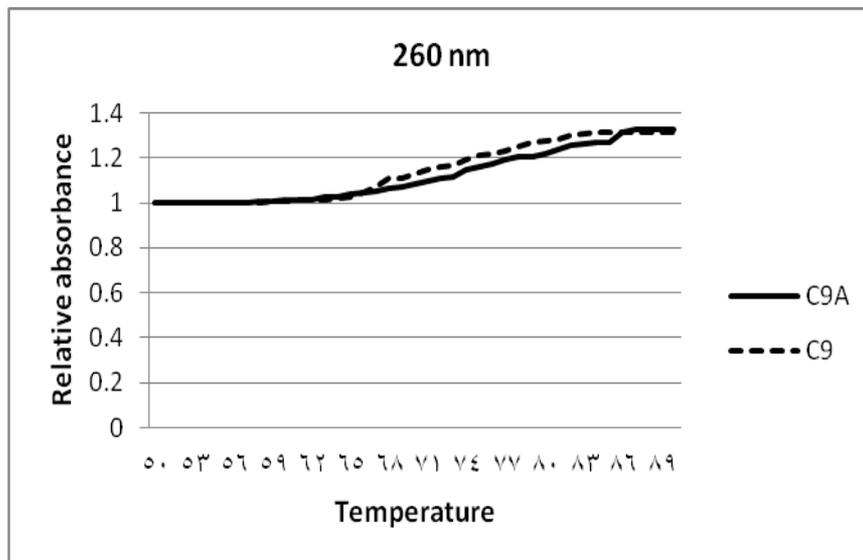


Figure 14. Melting profile of chromatin isolated from sugar cane (Cultivars C9 and C9A) at 260 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl

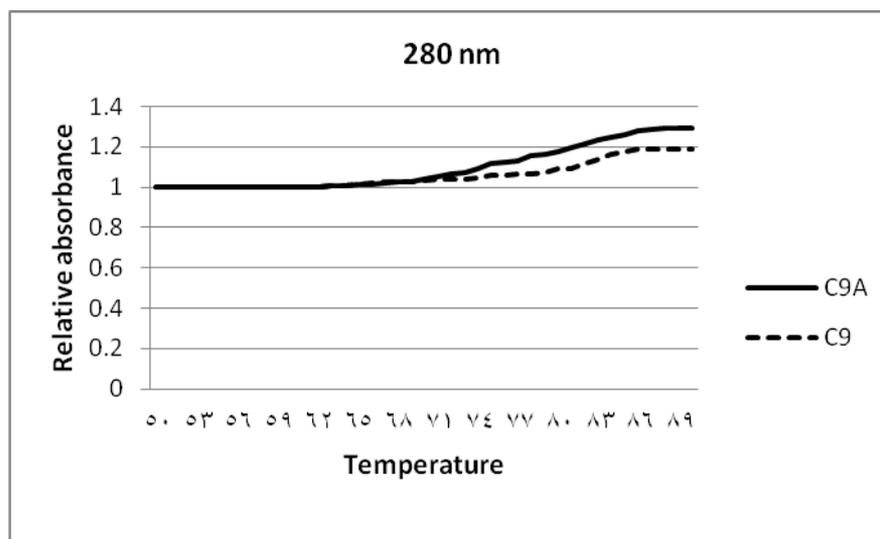


Figure 15. Melting profile of chromatin isolated from sugar cane (Cultivars C9 and C9A) at 280 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl.

- T_m-values for DNA:

Melting profiles at 260 nm as well as 280 nm are shown in Figures (16 and 17). The obtained results indicated that T_m value for deproteinized DNA of C9 was not the same as that for C9A; this difference was found to be significant. It seems probable that this difference was caused by DNA-under-replication, and this would be discussed later.

An attempt was carried out to estimate the fraction of genome which melts at 65 °C and 70 °C (Table 6) these results reflect differential structure of chromatin isolated from C9 compared with that of C9A.

Table (7, 8) show the chemical compositions of chromatin isolated from young leaves of C9 and C9A. Comparing the results shown in these tables one can conclude that C9A was proven to be higher in its transcriptional activity compared with C9 (Total acidic protein percent of total was 55.30 % versus 45.67 %).

Table (9) Repressed and active fraction of genome. The data clearly show that C9A had active fraction of genome (31.85 %) Vs (20%) for C9.

Table (10) Hyperchromicity obtained after melting of C9 chromatin. It was similar to that obtained for C9A, giving an evidence that the base composition is approximately similar.

Table 6. Fraction of Genome which melts at 65 °C and 70 °C

Source of chromatin	Fraction which melts at 65 °C		Fraction which melts at 70 °C	
	260 nm	280 nm	260 nm	280 nm
C9	11.96 %	3.46 %	25.76 %	14.18 %
C9A	8.95 %	4.18 %	41.21 %	10.80 %

An attempt was carried out to isolate DNA for chromatin. Table (11) and Figure (18) show the UV absorption spectra of DNA isolated from C9 and C9A cultivars leaves.

Coefficients of absorbance for purified chromatin isolated from cultivars C9 and C9A are given in Table (12).

Fractions of DNA which melts at 65 °C and 70 °C are given in Table (13). At 280 nm values was proven to be similar where at 260 nm significant differences were obtained, giving an evidence that the DNA under-replication is achieved at A-T rich regions.

Temperature of melting for deproteinized DNA (Table 14) show that T_m-value was 73 and 70 °C at 260 nm for C9 and C9A, respectively. It was proven to be 76 and 74 °C for C9 and C9A, respectively. Such differences in T_m-value for deproteinized DNA would be caused by DNA-under-replication phenomenon.

Agronomic characters an attempt was carried out to estimate some agronomic traits. Total soluble solids, plant height and stem diameter were estimated in both genotypes (C9 and C9A). Table (15) illustrates the average of these characters.

Table 7. Chemical composition of purified chromatin isolated from leaves of Sugar cane

Cultivar	DNA	Total proteins (T)	Histone (H)	Acidic proteins (AP)	Acidic proteins; % of Total AP
C9	118	236	128	108	45.67 %
C9A	215	443	198	245	55.30 %

* $\mu\text{g} / \text{ml}$ chromatin soluble in 1 m NaCl.

Table 8. Chemical composition of purified chromatin isolated from leaves of Sugar cane

Cultivar	DNA	Total proteins (T)	Histones (H)	Acidic proteins (AP)	Acidic proteins % of Total AP %
C9	1	2	1.08	0.92	-
C9A	1	2.06	0.92	1.14	-

* Relative to its DNA.

Table 9. Repressed and active fraction of genome

Cultivar	RFG	AFG
	Repressed fraction of genome	Active fraction of genome
C9	$1.08 / 1.35 * 100 = 80$	$100 - 80 = 20 \%$
C9A	$0.92 / 1.35 * 100 = 68.14$	$100 - 68.14 = 31.85 \%$

Table 10. Hyperchromicity of purified chromatin isolated from sugar cane leaves

Source	Wavelength	
	260 nm	280 nm
C9A	31.3 %	28.7 %
C9	32.6 %	28.9 %

Table 11. Ultraviolet absorption spectra of DNA isolated from sugar cane leaves

Cultivar	230	240	250	260	270	280	290	320
C9	0.477	0.620	0.902	1.115	1.00	0.600	0.300	0.01
C9A	0.582	0.632	0.922	1.090	0.922	0.610	0.290	0.01

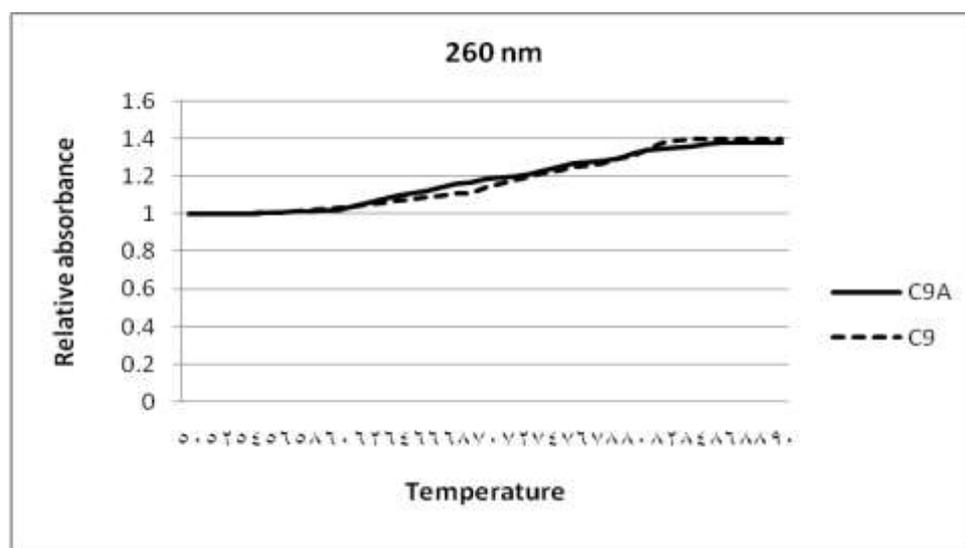


Figure 16. Melting profile of DNA isolated from sugar cane (Cultivars C9 and C9A) at 260 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl

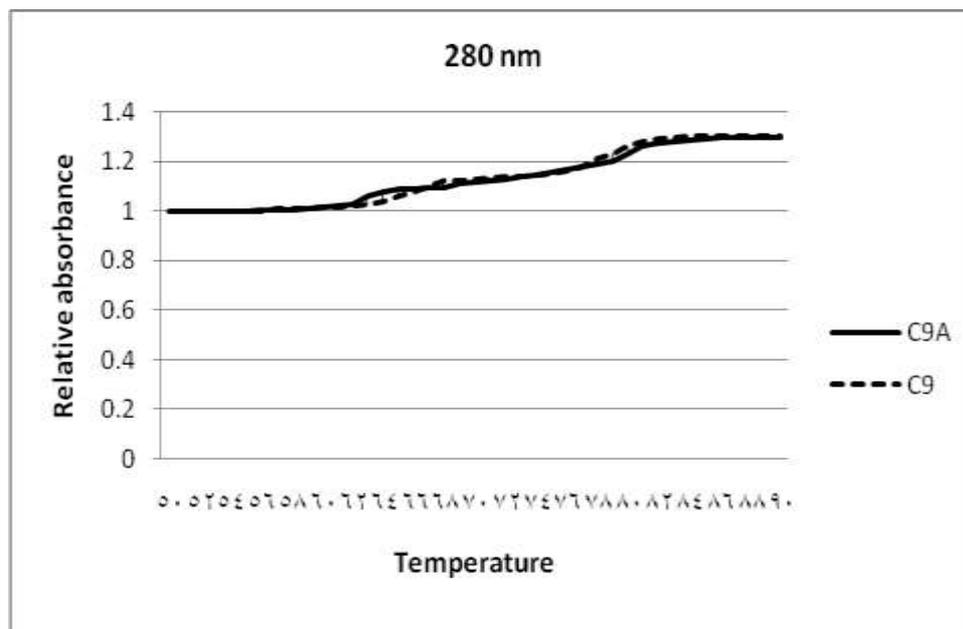


Figure 17. Melting profile of DNA isolated from sugar cane (Cultivars C9 and C9A) at 280 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl.

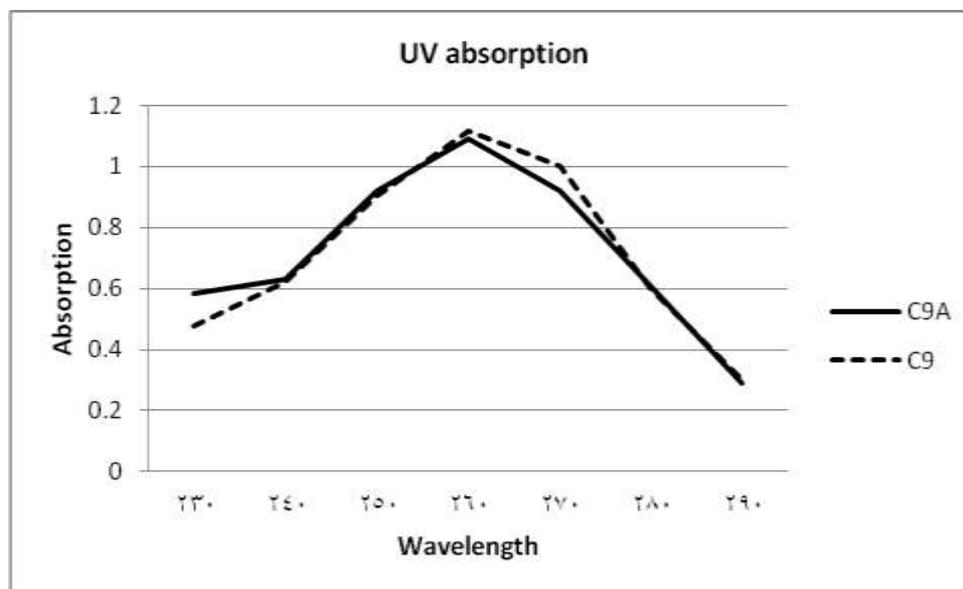


Figure 18. Ultraviolet absorption spectrum of DNA isolated from sugar cane leaves cultivars C9 and C9A.

Table 12. Coefficients of absorbance for purified chromatin isolated from cultivars C9 and C9A

Variety	260/240	250/260	280/260	320/260
C9	1.79	0.81	0.54	0.019
C9A	1.72	0.84	0.55	0.012

Table13. Fraction of DNA which melts at 65 °C and 70 °C, %

Cultivar	260 nm		280 nm	
	65	70	65	70
C9	8	14	6	11.5
C9A	11	19	8	11.5

Table 14. Temperature of melting (Tm-value) of DNA isolated from cane leaves

Cultivar	260 nm	280 nm
C9	73	76
C9A	70	74

Table 15. average of some agronomic characters estimates in this work

Genotypes	T.S.S	Plant height	Stem diameter
C9	21.6 %	2.01 m	2.11 cm
C9A	21.81 %	2.41 m	2.82 cm

DISCUSSION

A key goal of sugarcane breeding programs is to increase sugar yield by increasing sugar production per area, which is closely associated with height, diameter and number of the stalk, along with sugar accumulation in the stalk. Sugar yields have been generally improved by increasing total biomass rather than directly increasing sugar concentration in stalks (Jackson, 2005). Regarding sugar accumulation in the cane, stalk diameter seems more important than length (Milligan *et al.*, 1990). Other important traits to be considered are vigor and productivity of the ratoon (Aitken *et al.*, 2008). Future varieties bred specifically for second-generation biofuels will be dissociated from high-sucrose yields, but instead will aim at total biomass production per area. Some traits are additive while others present dominant effects. Additive effects can be useful for breeding in early generations of the program while dominant effects are more useful in hybrids (Liu *et al.*, 2007). Increase of sugar yield has always been a major goal of breeding programs.

Most functional genomics projects performed in the 1990's focused on sucrose content, disease resistance and stress tolerance, and involved several techniques, such as EST characterization, microarray and SAGE analyses (Vettore *et al.*, 2003; Papini-Terzi *et al.*, 2005; Calsa Jr & Figueira 2007; Menossi *et al.*, 2008; Papini-Terzi *et al.*, 2009; Waclawovsky *et al.*, 2010; Iskandar *et al.*, 2011). The post-genomic era comprises the use of this information into breeding programs, with the identified markers that reveal expression profile of genes in different environmental conditions (Moore, 2005; Waclawovsky *et al.*, 2010; Khan *et al.*, 2011).

However, sugarcane is a polyan euploid species, and statistical segregation models have been developed to fit

diploid organisms interpretations (Parida *et al.*, 2010; Tabasum *et al.*, 2010; Swapna *et al.*, 2010). About 5% of publicly available sugarcane unigenes present single sequence repeats, and the frequency of perfect microsatellites is one marker for every 10.4 kb (Parida *et al.*, 2010). Considering this, many polymorphic loci obtained during crossings cannot be properly analyzed, given the difficulties due to polyploid segregation (Garcia *et al.*, 2006). In breeding of diploid species, molecular markers are significant for MAS through the use of single nucleotide polymorphisms (SNPs) for polygenic traits such as yield components and disease resistance. The polyploidy constitution of sugarcane makes it as the most difficult crop to apply MAS, that is a sugarcane breeders's dream. Finally, although many available papers report the identification of markers associated with qualitative and quantitative traits in sugarcane, it is noteworthy to mention that they have had very little impact in sugarcane breeding up to now.

The present study was carried out to induce polyploidy in sugar cane crop cultivated in Egypt. To achieve such a purpose the following was undertaken:

1. Three cultivars of sugar cane grown at Sabbahia Agricultural Research Station were chosen for their economical importance and were cultivated under conditions that stimulate flowering processes, Meiotic chromosomes of these cultivars were examined.
2. Attempts were conducted to induce polyploidy as described in the Materials and Methods part.
3. Selection for ploidy was carried out employing the different criteria.
4. A comparison between physico-chemical properties of chromatin isolated from nuclei as well as of DNA was done to investigate the transcriptional activity.

5. Some agronomic traits were measured (Total soluble solids, plant height and stem diameter)
6. Chromosome complements in mitosis of adventitious roots were obtained when needed.

Sugar cane, as a perennial grass plant, belongs to the *saccharum officinarum* species as a member of *saccharum* genus. Sugar cane (*Saccharum*) is a species of between 6-37 species (depending on taxonomic interpretation) of tall grasses (family poaceae, tribe Andropogoneae), native to warm temperate to tropical regions of the old world. They have stout, jointed fibrous stalks 2-6 m tall and sap rich in sugar. However, very little is known about sugar cane genetics. The basic chromosome number estimation in *saccharum* have been as diverse as $x = 5, 6, 8, 10$ or 12 and varied with species (Sreenivasan *et al.*, 1987). The mating systems in natural populations have not been documented. Despite these drawbacks, sugar cane is an example of very successful use of alien genetic resources. The interspecific hybridization programs that were carried out at the beginning of the century in java in India revolutionized sugar cane breeding. Modern sugar cane varieties result from interspecific hybridization and may contain more than 100 chromosomes contributed by up to five different species (Heinz 1987). It encompasses very diverse euploid and aneuploid members ($2n = 40 - 128$), Lu *et al.*, (1994). This wide range of chromosome number of sugar cane gives the breeders the chance to successfully practice his selection program.

Today's commercial sugar cane cultivars are almost exclusively back cross derivatives involving *S. officinarum* and *S. spontaneum*. However, only a few clones of these species were used this narrow genetic base of modern hybrid varieties is surely one of the principal causes of the present slow rate of sugar cane breeding process (Berding and Roach, 1987).

In Egypt, sugar cane provides about 55% of the sugar supply, while sugar beet provides 45%. There is a gap of about 850,000 tons of sugar between the consumption and the production of sugar and this amount is yearly imported from abroad and costs the country a lot of foreign currency. The plan of the government is to minimize this gap by the extension of both crops cane and beet. In sugar cane only vertical extension could be applied because horizontal extension needs a lot of irrigation water which is limited. To develop new desired sugar cane varieties and to improve the existing ones is the main goal of the breeder to achieve the vertical extension in sugar cane.

Many of the desirable characters are quantitative so multivariate statistical techniques have been suggested and utilized to a limited extent to measure genetic and phenotypic divergence among entries and genotypes to

aid in planning crosses among genotypes belonging to different clusters (Whitehouse, 1969; Goodman, 1973; Sneath, 1976; Camussi *et al.*, 1983; Kwon-Ndung and Imolehin, 2007; Amer Rasul *et al.*, 2010; Creste *et al.*, 2010; Swapna Simon & Hemaprabha, 2010; Wang *et al.* (2010) and Sanjay Kumar & Devendra Kumar, 2012). Characterization and quantification of genetic diversity, both within and between populations, has long been a major goal in evolutionary biology. In plant breeding programs, information concerning the genetic diversity within a crop species is essential for rational use of genetic resources, it is particularly useful in the characterization of individual accessions and cultivars, in detecting duplications of genetic material in collections, and as a general guide in the choice of parents for breeding hybrids.

Routine methodologies were described for epicuticular wax. Stomata characters and opening (Rai, 1999): stomata characters and opening: leaf water content (Karakas *et al.*, 1997): Pyruvate orthophosphate kinase activity (Du *et al.*, 1998); regulation of C4 photosynthesis and sugar accumulation (Nose *et al.*, 1994; Madan *et al.*, 1988); chlorophyll content (Li and Zhou, 1991; Ortega *et al.*, 1984., Jagtap *et al.*, 1992) and proline content in sugarcane leaves (Jagtap *et al.*, 1992).

Stomata characters were measured following nail paints peeling technique as described by (Capellades *et al.*, 1990), by applying cellulose acetate (clear nail polish), to the upper leaf surface, which was peeled and examined microscopically for length (μ). Width (μ) and number per mm square (no mm^{-2}) of stomata.

Leaf relative water content (RWC) was determined by excising 100 mm^2 of leaf. Weighed and then re-weighed after floated on distilled water for 24 hours. Oven drying at 80°C to get the dry weight. $\text{RCC} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW})$ where FW= leaf fresh weight. DW= dry weight and TW= turgid weight (Mu-Qing and Ru-Kai, 1998). Soil moisture content was determined as described by Anderson and Ingram (1993), on dry weight basis. Three samples each of 50 g. weighing, then dried at a constant weight at 105°C . The dry weight was recorded and the moisture content by $((\text{sample wet weight} - \text{Oven dry weight}) / \text{Oven weight}) \times 100$.

Drought tolerance capacity (DTC) were determined following Na-EDTA method developed by Dwivedi *et al.*, (1998), by extracting one gram air dry leaf in 20 ml 0.025 M Na₂ EDTA and boiling it for 25-30 minutes in a water bath. After cooling, pH was measured. The percent fall in pH as compared to the control was recorded as DTC. Quantitatively, fall of $\text{pH} > 3\%$

indicates as drought tolerant genotype and fall of pH < 1.7 % as sensitive genotypes.

Chromosome number:

In order to precisely detect the induction of polyploid, buds of cuttings from the observed plants were allowed to germinate under controlled conditions. Adventitious roots were treated with colchicine and chromosome complement was investigated. Chromosome number was found to be 80 chromosomes in diploid cultivar. In polyploid plants a chromosome number of 160 and 240 were observed.

Cytological examination of adventitious root tip cells showed that interphase nucleus of the original cultivar was found to be completely different from that of the polyploidy. Multinuclei were observed; giving an evidence that nucleolar organizing region is repeated.

Water stress treatment significantly reduced stomata length ($p=0.00$), width ($p=0.00$) and increases the number per unit area ($p=0.00$). Stomata length and width decrease higher in L6 and 26 compare to L10, 22 and L14. The number of stomata per mm² was less in drought tolerant varieties compare to sensitive ones. Stomata characters play an important role in transpiration of water and gas exchange. The size and number of stomata determined the ability of the plant to lose or retain moisture. The bigger the size, the greater the amount of water is expected to be transpired from the leaves. Plate 1 shows an impression of microphotograph on nail polish impression of stomata form the upper surface of tested plants.

Cytological examination revealed that diploid, triploid, tetraploid plants were observed, giving precise evidence that inducing of polyploidy was achieved. However, evaluation of agronomic traits or characters needs propagation for the obtained plants, and this would be done. However, this step is now being carried out at Sabbahia station for sugar crops.

Anatomical observations showed that stomata size, number of plastids in guard cells, size of vascular bundles in young leaves, and size or volume of epidermal cells (of leaves) are considered an adequate tool for characterization of ploidy.

Analysis of total soluble solids (T.S.S), stem diameter; and stem length showed that there are differences between C9 cultivar and C9A Cites product from the induction of polyploidy.

With respect to the physico-chemical properties temperature of melting was proven to be at 260 nm lower than that at 280 nm, giving an evidence that DNA was shown to be in its primary structure. At 260 nm chromatin of the original cultivar was shown to be less active compared with that of its product by the induction

of polyploidy (C9A). The active fraction was shown to be 20 % and 31.85 %, respectively. This result may reflect the higher transcriptional activity detected in C9A chromatin.

Regarding the physico-chemical properties of deproteinized DNA the results showed that melting profiles as well as temperature of melting of DNA isolated from C9 was shown to be different from that of C9A. This result may reflect that DNA-under-replication was performed. However, one can assume that this DNA-under-replication was shown to be in A=T rich region. In conclusion, these measurements might be useful in evaluative purposes and breeding program.

REFERENCES

- Aitken, K.S.; Hermann, S.; Karno, K.; Bonnett, G.D.; McIntyre, L.C. & Jackson, P.A. (2008). Genetic Control of Yield Related Stalk Traits in Sugarcane, *Theoretical and Applied Genetics*, Vol.117, No.7, (November 2008), pp. 1191-1203, ISSN 0040-5752.
- Amer Rasul, Mansoor-ul-Hassan, Anjum Suhail and Shahbaz Talib Sahi (2010). The Impact of Some Physiomorphic Characters of Sugarcane Genotypes on Their Resistance Against Sugarcane Pyrilla, *Pyrilla perpusilla* WIK. (Lophopidae: Homoptera). Pak. J. Agri. Sci., Vol. 47(4), 339-344.
- Anderson, J. M. and Ingram, J. S. I. (1993). Tropical soil biology. A handbook of methods, *CAB International. Wallingford, UK 93-112*.
- Berding, N. and B. T. Roach, (1987). Germplasm collection, maintenance and use. In: D-J. Heinz (Ed.) sugar cane improvement through breeding. Elsevier press, Amsterdam, pp 143-210.
- Bonner, J. (1965). The molecular biology of development. Oxford University press. Oxford. Pp 155.
- Bonner, J., M. E. Dahmus, D. Fambrough, R. C. Huang, K. Marushige, and D. Y. H. Tuan (1968). The biology of isolated chromatin, *Sci.*, 159: 47-56.
- Bourgin, J.P (1983). Protoplasts and the isolation of plant mutants. In "protoplasts", (Potrykus et al., Eds), P. 43, Birkhauser. Basel.
- Burton, K. (1968). Determination of DNA concentration with diphenylamine. In methods in enzymol., vol. XIIB, p.163. Newyork: Academic press.
- Calsa Jr., T. and Figueira, A. (2007). Serial Analysis of Gene Expression in Sugarcane (*Saccharum* spp.) Leaves Revealed Alternative C4 Metabolism and Putative Antisense Transcripts. *Plant Molecular Biology*, Vol.63, No.6, pp. 745-762, ISSN 0735-9640.
- Camussi, A.; P. L. Spagnoletti Zeuli, and P. Melchiorre (1983). Numerical taxonomy of latin maize populations: Genetic distances on the basis of heterotic effects. *Maydica* 28: 411-424.

- Capellades, M., R. Fountarnau., C. Carulla., and P. Debergh (1990). Environmental influences anatomy of stomata and epidermal cells in tissue-cultured *Rosa multiflora* Hournal Amer. Soc. Hort. Sci. 115 (1):141-145.
- Creste, S.; Pinto, L.R.; Xavier, M.A. & Landell, M.G.A. (2010). Sugarcane Breeding Method and Genetic Mapping, In: *Sugar Cane Bioethanol: R&D for productivity and sustainability*, L.A.B. Cortez (Ed.), 353-357, Blucher, ISBN: 9788521205302, Sao Paulo.
- Doney, D. L. (1988). Selection for sucrose yield in stressed sugar beet seedlings. *Crop Science*, 28 (2): 245-247.
- Du, Y.C., Nose, Wasano, K. and Uchida, Y. (1998). Response to water stress of enzymes activities and metabolic levels in relation to sucrose and starch synthesis, the Calvin cycle and the C4 pathway in sugarcane (*Saccharum sp.*) leaves. *Aust. Journal of plant physiology* 25:253-260.
- Dwivedi, R. S., Srivastava, K. K., Solomon. S. and Singh, K. (1998). Rapid test for drought resistance in Sugarcane. *Sugar Cane*, Spring Supplement, 31-32.
- Fellenberg, G (1974). chromosomal protein. Stuttgart ulmer.
- Fellenberg, G., and Schömer, U (1975). Direct effect of IAA upon isolated chromatin of ethiolated pea saeedlings. *Z. Pflanzenphysiol.* 75 (5), 444-456.
- Garcia, A.A.; Kido, E.A.; Meza, A.N.; Souza, H.M.; Pinto, L.R.; Pastina, M.M.; Leite, C.S.; Silva, J.A.; Ulian, E.C.; Figueira, A. & Souza, A.P. (2006). Development of an Integrated Genetic Map of a Sugarcane (*Saccharum spp.*) Commercial Cross, Based on a Maximum-Likelihood Approach for Estimation of Linkage and Linkage Phases. *Theoretical and Applied Genetics*, Vol.112, No.2, (January 2006), pp. 298-314, ISSN 0040-5752.
- Giles, K. W., and A. Myers (1965). A improved Diphenyleamine method for the estimation of deoxyribonucleic acid, *Nature*, 206:93.
- Goodman, M. M. (1973). Genetic distances: Measuring dissimilarity among populations. *Yearb. Phys. Anthropol* 17: 1-38.
- Heinz D. J. (1987). Sugar cane improvement; current productivity and future opportunities, In: copersucar Breed Worksh. Copersucar, Sao Paulo, pp 57-77.
- Iskandar, H.M.; Casu, R.E.; Fletcher, A.T.; Schmidt, S.; Xu, J.; Maclean, D.J.; Manners, J.M. & Bonnett, G.D. (2011). Identification of Drought Response Genes and a Study of Their Expression During Sucrose Accumulation and Water Deficit in Sugarcane Culms. *BMC Plant Biology*, Vol.11, (January 2011), pp. 12, ISSN 1471-2229.
- Jackson, P.A. (2005). Breeding for Improved Sugar Content in Sugarcane. *Field Crops Research*, Vol.92, No.2-3, (June 2005), pp. 277-290, ISSN 0378-4290.
- Jagtap, R. A., Tawar, P. N. Sen. D. R, Pant, N. M. and Hapase, D.G. (1992). Physio-Anatomical studies in sugarcane varieties and their somaclones grown under water stress conditions. *Plant Physio & Biochem.* 19(1):42-48.
- Karakas, B., Ozias, A. P., Stushnoff, C., Suefferheld, M. and Rieger, M. (1997). Salinity and drought tolerance of mannitol-accumulating transgenic tobacco. *Plant Cell and Environment* 20:609-616.
- Khan, M. S.; Yadav,S.; Srivastava,S.; Swapna, M.; Chandra, A. & Singh, R. K. (2011). Development and Utilization of Conserved Intron Scanning Marker in Sugarcane. *Australian Journal of Botany*, Vol.59, No.1, (February 2011), pp. 38-45, ISSN 0067-1924.
- Kholovoda, V. P., V. V. Urmant seva and A. B. Meshcheryakov (1985). Sugar beet tissue culture as a model for studing physiological and biochemical processes. In : *Plant cell culture* (Edited by R. G. Butenko). 51-75 publishers corr. Merr.USSR Acad. Sci. Moscow.
- Kwon-Ndung E. H. and E.D. Imolehin (2007). Evaluation of sugarcane seedlings from biparental crosses using different growth substrates. *African Crop Science Conference Proceedings Vol. 8.* pp. 139-142.
- Li, Y.R. and Zhou, K.Y. (1991). Prediction of sugarcane yield and quality from biochemical parameters in leaves of young shoots. *Sugar Cane* 1: 12-15.
- Liu, G.F.; Zhou, H.K.; Zhu, Z.H.; Xu, H.M. & Yang, J. (2007). Genetic Analysis for Brix Weight per Stool and Its Component Traits in Sugarcane (*Saccharum officinarum*). *Journal of Zheijang University Science B*, Vol.8, No.12, (December 2007), pp. 860-866, ISSN 1673-1581
- Lu, Y. H.; A, D, Hont; D. I. T. Walker; P. S. Rao; P. Feldmannand and Glaszmann (1994). Relationships among ancestrai species of sugar cane revealed with RFLP using inle copy maize nuclear probes. *Euphytica* 78: 7-18.
- Lowry, O. H., Roebrough, N. T., Farr, A. L. and Randall, R. J. (1951). Protein measurment with the polin phenol. *J. Bio. Chem.* 193: 265-275.
- Maliga, P. (1984). Isolation and characterization of mutants in plant cell culture., *Annu. Rev. Plant. Physiol.*, 35:519-542.
- Madan, V.K., Singh, K., Soni, N., Nigam, M. and Saxena, Y.R. (1988). Foliar amylase, amylose, amylopectin and Starch patterns during growth and development of sugarcane. *Sugar Cane* 1:14-16.
- Menossi, M.; Silva-Filho M.C.; Vincentz, M.; Van-Sluis, M.A. & Souza, G.M. (2008). Sugarcane Functional Genomics: Gene Discovery for Agronomic Trait Development. *International Journal of Plant Genomics*, Vol.2008, (December 2007), pp. 458732, ISSN 16875370.
- Milligan, S.B.; Gravois, K.A.; Bischoff, K.P. & Martin, F.A. (1990). Crop Effects on Genetic Relationships Among Sugarcane Traits. *Crop Science*, Vol.30, No.4, (April 2004), pp. 927-931, ISSN 0011-183X.
- Moore, P. H. (2005). Integration of Sucrose Accumulation Processes Across Hierarchical Scales: Towards Developing an Understanding of the Gene-to-Crop Continuum. *Field Crops Research*, Vol.92, pp. 119-135, ISSN 0378-4290.

- Mu-Qing, Z. and Ru-Kai, C. (1998). Osmotic adjustment in lives of sugarcane in response to water stress. *Sugar Cane* 5:3-7.
- Nose, A., Uchara, M. and Kawamitsu, Y. (1994). Variation in leaf gas exchange traits of *Saccharum* including feral sugarcane, *Saccharum spontaneum* L. *Japanese Journal of Crop Science* 63:489-495.
- Ortega, D.E., Pardo, J. and Morgado, G.A. (1984). Metabolic changes in sugarcane plants subjected to water stress. *Journal Ciencia deLa Agricultura* 21:37-43.
- Papini-Terzi, F. S.; Rocha, F. R.; Vêncio, R. Z. N.; Felix, J. M.; Branco, D. S.; Waclawovsky, A. J.; Del Bem, L. E. V.; Lembke, C. G.; Costa, M. D. L.; Nishiyama Jr, M. Y.; Vicentini, R.; Vincentz, M. G. A.; Ulian, E. C.; Menossi, M. & Souza, G. M. (2009). Sugarcane Genes Associated with Sucrose Content. *BMC Genomics*, Vol.10, No.120, (March 2009), pp. 120, ISSN 1471-2164.
- Papini-Terzi, F.S.; Rocha, F.R.; Vêncio, R.Z.; Oliveira, K.C.; Felix, J. de M.; Vicentini, R.; Rocha, C. de S.; Simes, A.C.; Ulian, E.C.; di Mauro, S.M.; da Silva, A.M.; Pereira, C.A.; Menossi, M. & Souza, G.M. (2005). Transcription Profiling of Signal Transduction-Related Genes in Sugarcane Tissues. *DNA Research*, Vol.12, No.1, pp. 27-38, ISSN 1340-2838.
- Parida, S.K.; Pandit, A.; Gaikwad, K.; Sharma, T.R.; Srivastava, P.S.; Singh, N.K. & Mohapatra, T. (2010). Functionally Relevant Microsatellites in Sugarcane Unigenes. *BMC Plant Biology*, Vol.10, (November 2010), pp. 251, ISSN 1471-2229.
- Rai, R.K. (1999). Variation of epicuticular, wax content in sugar cane varieties and four species of *Saccharum*: a possible significance Cooperative – *Sugar*, 30(10):955-957.
- Sanjay Kumar and Devendra Kumar (2012). Genetic divergence in sugarcane Germplasm under sub-tropics. *Scholarly Journal of Agricultural Science* Vol. 2(8), pp. 186-189,
- Seehy, M. A.; M. Moussa and E. A. Badr (1984). Induction of reverse mutation in waxy locus of *Zea mays* pollen grains by pesticides. *Egypt. J. Genet. Cytol.*, 13: 137-142.
- Sneath, P. H.A. (1976). Some applications of numerical taxonomy to plant breeding-Z. *Pflanzenzuecht*, 76:19 - 45.
- Spang , H.A. and Platt, H. S. (1972). The effect of plant growth sybstances on the hyperchrmicity of DNA. *Physical. Plant.* 27: 321-326.
- Sreenivasan T. V., B. S. Ahloowalia & D. J. Heinz (1987). cytogenetic. In D. J. Heinz (Ed). *Sugar cane improvement through breeding*. Elsevier press. Amsterdam. Pp 211-253.
- Swapna, M.; Sivaraju, K.; Sharma, R. K.; Singh, N. K. & Mohapatra, T. (2010). Single-Strand Conformational Polymorphism of EST-SSRs: a Potential Tool for Diversity Analysis and Varietal Identification in Sugarcane. *Plant Molecular Biology Reports*, Vol.5, pp. 254-263, ISSN 0735-9640.
- Swapna Simon and Hemaprabha G. (2010). Identification of two new drought specific candidate genes in sugarcane (*Saccharum* spp.). *Electronic Journal of Plant Breeding*, 1(4):1164-1170.
- Tabasum, S.; Khan, F.A.; Nawaz, S.; Iqbal, M.Z. & Saeed, A. (2010). DNA Profiling of Sugarcane Genotypes Using Randomly Amplified Polymorphic DNA. *Genetics and Molecular Research*, Vol.9, No.1, pp. 471-483, ISSN 1676-5680.
- Vettore, A.L.; da Silva, F.R.; Kemper, E.L.; *et al.* (2003). Analysis and Functional Annotation of an Expressed Sequence Tag Collection for Tropical Crop Sugarcane. *Genome Research*, Vol.13, No.12, (November 2003), pp. 2725-2735, ISSN 1088-9051.
- Waclawovsky, A.J.; Sato, P.M.; Lembke, C.G.; Moore, P.H. & Souza, G.M. (2010). Sugarcane for Bioenergy Production: An Assessment of Yield and Regulation of Sucrose Content. *Plant Biotechnology Journal*, Vol.8, No.3, (April 2010), pp. 263-276, ISSN 1467-7644.
- Wang, J.; Roe, B.; Macmil, S.; Yu, Q.; Jan, E.; Murray, J.E.; Tang, G.; Chen, C.; Najar, F.; Wiley, G.; Bower, J.; Van Sluys, M.A.; Rokhsar, D.S.; Hudson, M.E.; Moose, S.P.; Paterson, A.H. & Ming, R. (2010). Microcollinearity Between Autopolyploid Sugarcane and Diploid Sorghum Genomes. *BMC Genomics*, Vol.11, pp. 261, ISSN 1471-2164.
- Whitehouse, R. N. H. (1969). An application of canonical analysis plant breeding. *Geet. Agrar.* 23: 61-69.

الملخص العربي

الخواص الفيزيوكيماوية للكروماتين واستحداث تضاعفات في قصب السكر

مُجدد عبد الباعث الصبيحي، مُجدد عبد المنعم غنيمه، مجدى سعد صالح، أسامة مصطفى بدوى

اللاهستونية وتقدير درجة حرارة إنصهار الكروماتين وكذلك DNA
خالى البروتين على طول موجة 260 nm، وطول موجة
280 nm.

وكذلك تم تقدير بعض الصفات المحصولية مثل المواد الصلبة الكلية
وقياس متوسط أطوال النباتات وكذلك قياس متوسط قطر سيقان
النباتات وفيما يلي ملخصاً لأهم النتائج التي تم الحصول عليها: تمكن
البحث الحالي من إنتاج تضاعفات للمجموعة الكروموسومية في
الصنف C9 بمتوسط 9 في المئة وفي الصنف PH 8013 بمتوسط 4 في
ال المئة بينما فشل إنتاج تضاعفات للمجموعة الكروموسومية لل صنف
NCO 310 . وتراوح عدد البلاستيدات الخضراء في الخلايا الحارسة
للنباتات المدروسة من 9 إلى 16 بلاستيدة لكل خلية ويعتبر الكشف
المبكر باستخدام الخلايا الحارسة للتغور (عدد البلاستيدات) وسيلة
هامية جدا لتحديد التضاعف للمجموعة الكروموسومية. وتميزت
النباتات المتضاعفة بـكبر حجم الخلايا وببطء النمو وكبر حجم الحزم
الوعائية وسمك طبقة البشرة على الأوراق. ويكون البحث الحالي قد
نجح في إنتاج نباتات متضاعفة من الصنف C9 وكذلك PH8013 إلا
أن الدراسة الحالية اقتضت على ناتج التضاعف لل صنف C9 والذي
ثم إكثاره لتقييم باقي الخواص المحصولية لهذا الصنف.

وبدراسة الاختلافات في التركيب الكيميائي ودرجة حرارة الانصهار
للكروماتين وكذلك تقدير الجزء النشط والمثبط من الجينوم فيما بين
النباتات الثنائية والمتضاعفة نجد ان جميع هذه الاختلافات تفسر لنا
اختلاف النشاط النسخي بين هذه النباتات.

كان الهدف من البحث الحالي هو استحداث تغييرات
كروموسومية (تضاعف) للهيئة الكروموسومية الخاصة بجينوم نبات
قصب السكر وذلك باستخدام مادة الكولشيسين المستخرجة من
جذور نبات اللحلاح (سيجما) ثم تقييم النباتات الناتجة على
مستوى الوراثة الجزيئية للتنبؤ بقدراتها المحصولية.

ولتحقيق هذا الغرض تم الحصول على نباتات تابعة لثلاثة أصناف
منزعة بمحطة البحوث الزراعية الخاصة بمحطة تجارب المحاصيل
السكرية بالاسكندرية وكذلك قسم الوراثة بكلية الزراعة - جامعة
الاسكندرية وهذه الأصناف المنزعة هي (C9 G.T. 54-9, PH
8013 and NCO 310).

تم تقطيع السيقان إلى قطع صغيرة حول منطقة العقد لتحتوي
القطعة الواحدة منها على برعم واحد وتم تغطية أحد أسطح القطع
بطبقة من شمع البارافين بينما طُمر السطح الآخر في محلول 0.05
% من الكولشيسين علاوة على إحاطة البرعم الموجود بقطعة من
القطن مبللة بمحلول الكولشيسين سالف الذكر. أجريت هذه المعاملة
لمدة 7 أيام، تم استخدام قرابة 100 قطعة من كل صنف نقلت بعد
هذه المعاملة إلى ماء الصنبور وسمح لها بالنمو حتى وصل طول المجموع
الخضري قرابة 40 سم، ونقلت بعدها النباتات إلى الحقل وتم قياس
خصائص كثيره كعدد البلاستيدات وشكل الحزم الوعائية. وهذه
العوامل اعتبرت ككشاف أولي لتتبع النباتات النامية.

تم دراسة الخصائص الفيزيوكيماوية للكروماتين وكذلك الـ DNA
المنزوع من أنوية الأوراق مثل كمية الـ DNA وتقدير كمية البروتينات
الكروموسومية الكلية وكمية الهستونات في الأنوية وكمية البروتينات