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Assessment of Agronomic Traits and Sugar Content in Polyploid Plants of Sugarcane (*Saccharum officinarum*)

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ABSTRACT

The present investigation was carried out at Sabbahia experimental station for sugar crops. The present investigation aims at inducing polyploidy in sugarcane (*Sacharum officinarum*) and to investigate its agronomic traits. Colchicine was used to produce polyploidy from three cultivars. These cultivars are:

CP 36-13, CO 997 and POJ 287. Buds were treated with 0.05 % solution of colchicine. Treated segments were allowed to germinate until a shoot system length about 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 in the laboratory. Physico-chemical properties for chromatin and deproteinized DNA were estimated. Total soluble solids (T.S.S); plant height; plant diameters were estimated. The results showed that:- number of plastids in guard cells, vascular bundles shape, epidermal cells volume, physico chemical properties, and total soluble solid were proven that polyploid plants were obtained. The present study was capable in producing polyploidy in cultivar CP 36-13, and gave an average of 0.08 %, cultivar CO 997 gave 0.05 %, while for cultivar POJ 287 the study was not capable to produce polyploidy. Plastid number ranged between 9-16 plastids per each cell. The polyploidy plants had large cells, slow growth and large vascular bundles. Chemical composition analysis showed that, DNA, histones, non-histones from the polyploidy plants were increased while the T.m value for polyploidy plants was decreased at 260 nm whereas it was increased at 280 nm. It seems probable this difference was due to DNA under replication. Repressed fraction of genome was decreased in polyploid plants. So the active fraction of genome was increased in polyploid cultivars. With respect to agronomic characters an attempt was carried out to estimate some agronomic traits. Total soluble solids, plant height and plant diameter were increased in polyploidy cultivar.

***The present study was capable to produce polyploid plants from cultivar CP 36-13 and CO 997, it was failed in producing polyploid plants from cultivar POJ 287.**

INTRODUCTION

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 sugarcane is considered among the most important industrial converting crops. It is the main source of sugar industry and the sole source for molasses industry. Sugarcane is cultivated in about 200 thousand feddans producing about 800,000 tons of sugar annually. 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 40 thousand feddans in the North of the Delta, producing about 90,000 tons of sugar annually. 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. This work was planned to induce polyploid plants to obtain any possible improvement of three different cultivars of sugarcane cultivated in Egypt.

MATERIALS AND METHODS

From each cultivar about five hundred cuttings 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%). 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. Different characteristics

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were measured. Isolation of chromatin was carried out according to the method described by Fellenberg and Schömer (1975) and Seehy *et al*., (1984) and (1990). Purification of chromatin was checked using the criteria of Fellenberg, (1974). Thermal stability and chemical composition of chromatin, estimation of melting temperature (Tm-value), chemical composition of chromatin, isolation of DNA, estimation of melting temperature of DNA, estimation of thermal stability and chemical composition was done by the method described by Fellenberg and Schömer, (1975), and Seehy *et al* (1984) and (1990). Purification of chromatin was done by chromatography on Sephadex G-25 fine (Pharmcia 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 melting temperature (Tm-value) was done at 260 nm as well as at 280 nm, DNA estimation was calculated by the method of Giles and Myers, (1965) and described by Burton (1968). Total protein was determined by lowery method (Lowry *et al*., 1951) using albumin from bovin serum as standard. Non-histone proteins were calculated as the difference 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) and Seehy *et al* (1990).

RESULTS AND DISCUSSION

Anatomical study was carried out to compare stomata in different geneotypes. It was found that stomata in leaves of polyploid plants was found to be larger than that of diploid plants.

Plastid in guard cells:

Table (1) shows the number of plastids in guard cell of different genotypes. It ranged from 8-16 plastids per guard cell.

Table1. Number of plastids in guard cell of different genotypes of sugarcane

Cultivar	No. plastids
CP 36-13	8-12
CO 997	14-16

Vascular bundles:

Size of vascular bundles in different genotypes was recorded and showed the vascular bundles in young leaves of polyploid plants were shown to be larger than that of diploid plants and anatomical examination of transverse section (T.S.) of young leaves showed the epidermal cells in leaf of polyploid plant were proven to be larger than that of the original cultivar.

Chromosome number:

Chromosome number was found to be 80 chromosomes in diploid cultivar. In polyploid plants a chromosome number of 160 and 240 was observed (Figures 1 & 2 & 3).

Interphase nucleous of sugarcane genotype CP 36-13 was found to contain multinucleolei (figures 4 & 5).

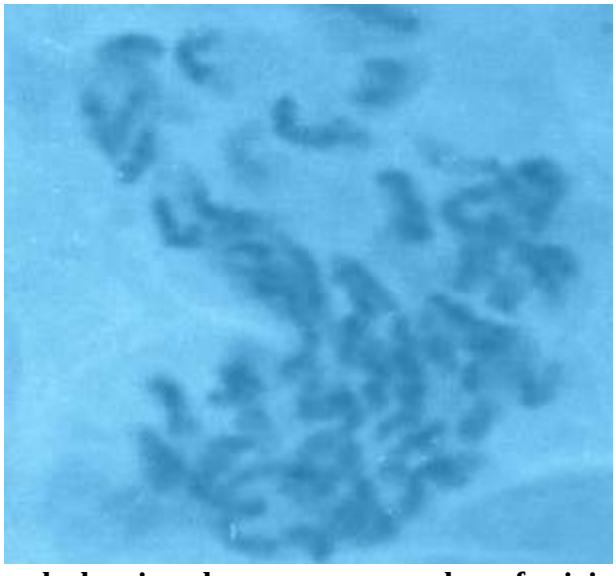


Figure1. Photomicrograph showing chromosomes number of original cultivar CP 36-13 diploid 80 chromosome

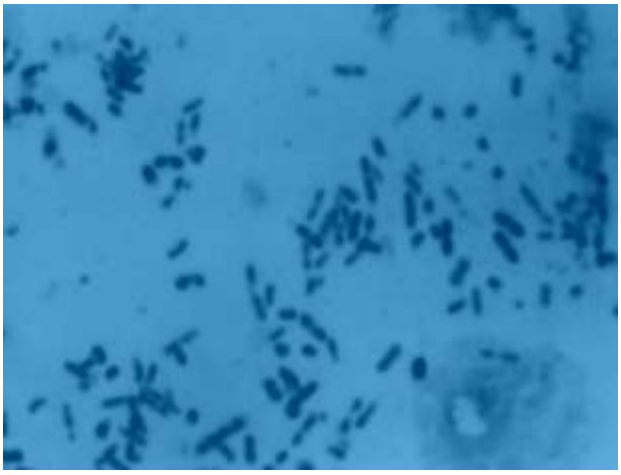


Figure2.Photomicrograph showing chromosome no. of sugarcane genotype CO 997 induced by colchicine 160 chromosomes

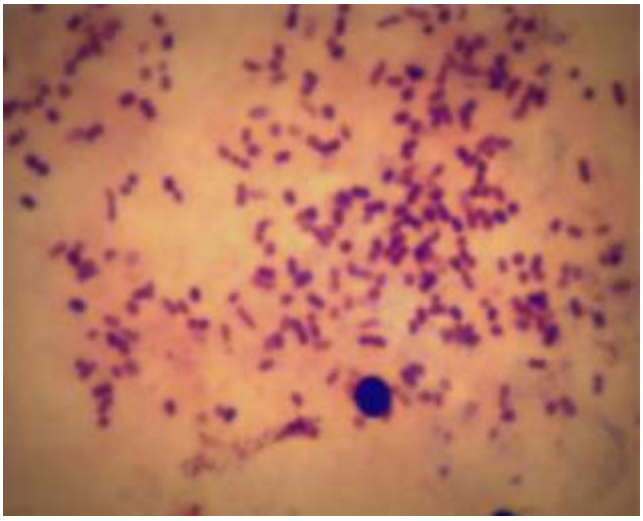


Figure 3. Photomicrograph showing chromosome number of sugarcane genotype CP 36-13 induced by colchicine 240 chromosomes

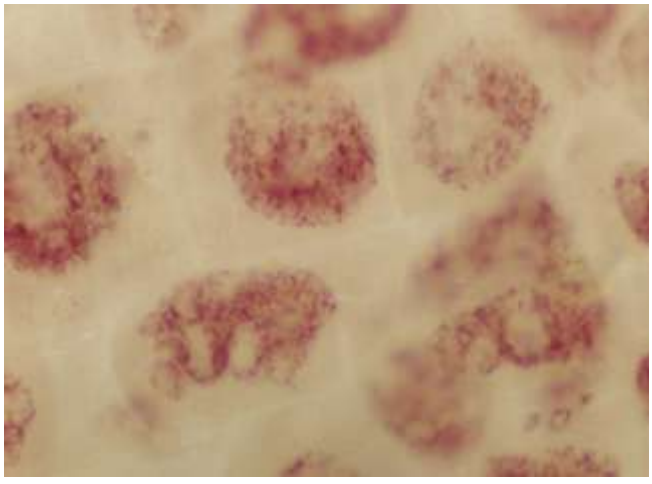


Figure 4.Photomicrograph showing Interphase nucleus of sugarcane genotype CP 36-13 that up to 8 nucleoli (induced by colchicine)

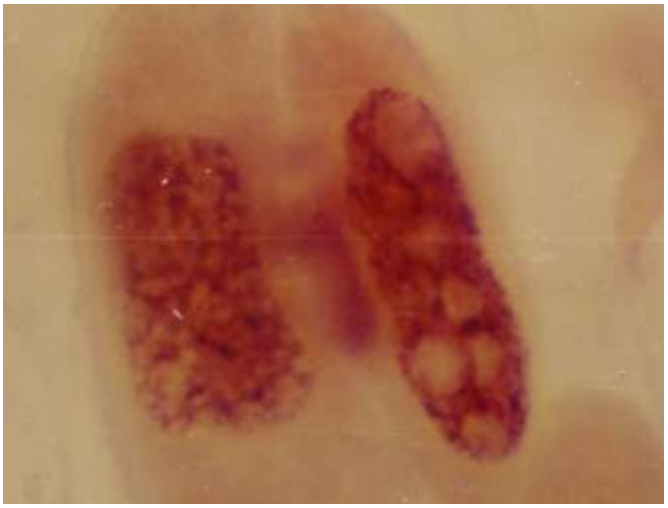


Figure 5.Photomicrograph showing Interphase nucleus of sugarcane genotype CP 36-13 induced by colchicine

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 & Figueria 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 several expression profile of genes in different environmental conditions (Moore, 2005; Waclawovsky *et al*., 2010; Khan *et al*.,2011).

However, sugarcane is a polyaneuploid 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 every10.4 kb (Parida *et al*., 2010). Considering this, many polymorphic loci obtained during crossing cannot be properly analyzed, given the difficulties due to polyploid segregation (Garcia *et al*., 2006). In breeding of diploid spices, molecular markers are significant for MAS through the use of single nucleotide polymorphism (SNPs) for polygenic traits such as yield components and difficult crop to apply MAS, that is a sugarcane breeder's dreams. 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.

Sugarcane, as a perennial grass plant, belongs to the *saccharum officinarum* species as a member of *saccharum* genus. Sugarcane (*Saccharum*) is a species of between 6-37 species (depending on taxonomic interpretation) of tall grasses (family *poaceae*, tribe *Andropogoneae*), native to warm temprate 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 sugarcane 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, sugarcane 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 sugarcane breeding. Modern sugarcane 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 aneuoploid 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.

In Egypt, sugarcane provides about 75% of the sugar supply, while sugar beet provides 25%. There is gab of about 900.000 tons of sugar between the consumption and the production of sugar and this amount is yearly imported from abroad and costs the country lot of foreign currency. The plan of the government is to minimize this gab by the extension of both crops cane and beet. In sugarcane only vertical extension could be applied because horizontal extension needs a lot of irrigation water which is limited. To develop new desired sugarcane varieties and to improve the existing ones is the main goal of the breeder to achieve the vertical extension in sugarcane.

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 and T.m Values of chromatin are given in Table (2).

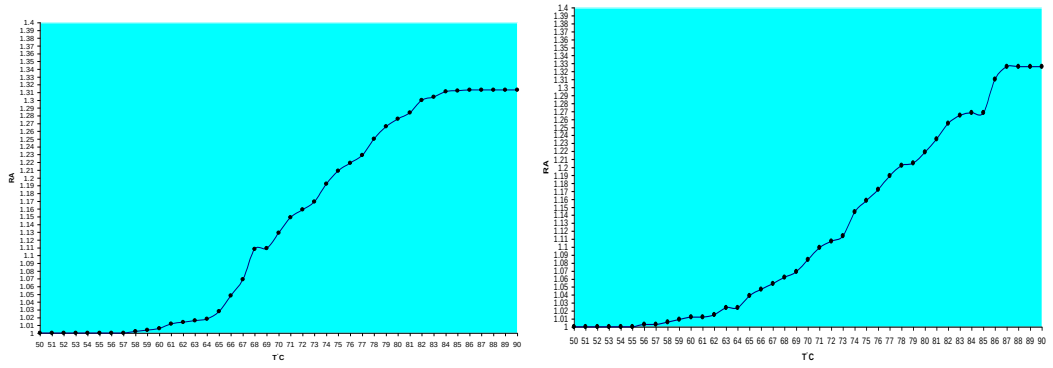
Figures (6 & 7 & 8 & 9) show the melting profiles of Chromatin and DNA.

Table2. Temperature of melting of (TM-value) of chromatin isolated from sugarcane cultivars

Genotypes	Wave length		ΔTm
	260	280	(Tm 280 – Tm 260)
CP 36-13	71.5	78	6.5
CO 997	74.5	77.5	3

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 (CP 36-13 A). The active fraction was shown to be 20 % and 31.85 %, respectively. This result may reflect the higher transcriptional activity detected in CP 36-13 A chromatin.

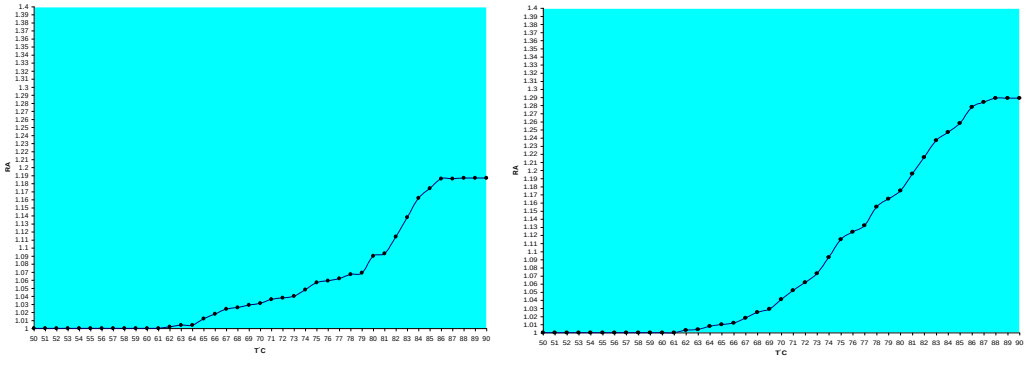
Regarding the physico-chemical properties of deproteinized DNA the results showed that melting profiles as well as temperature of melting of DNA isolated from CP 36-13 was shown to be different from that of CP 36-13 A.



CP 36-13
original cultivar

CP 36-13
Induced by colchicine

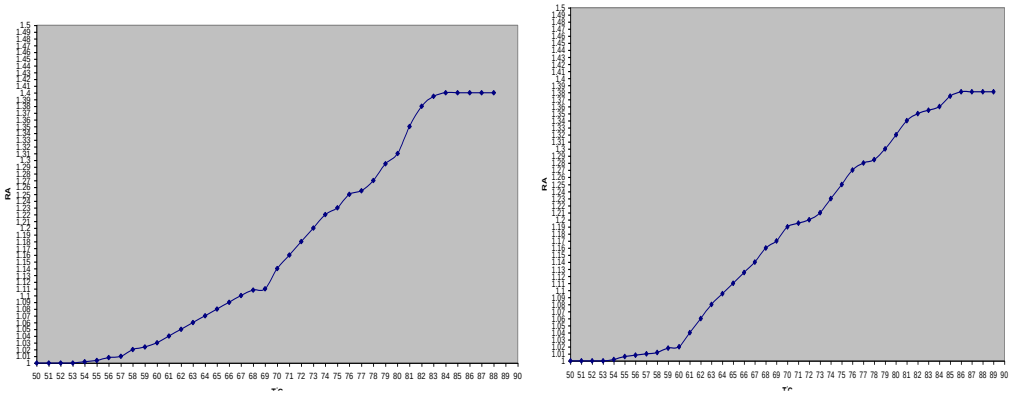
Figure 6. Melting profile of chromatin isolated from sugarcane (Genotype CP 36-13& CP 36-13 induced by colchicine) at 260 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl



CP 36-13
original cultivar

CP 36-13
Induced by colchicine

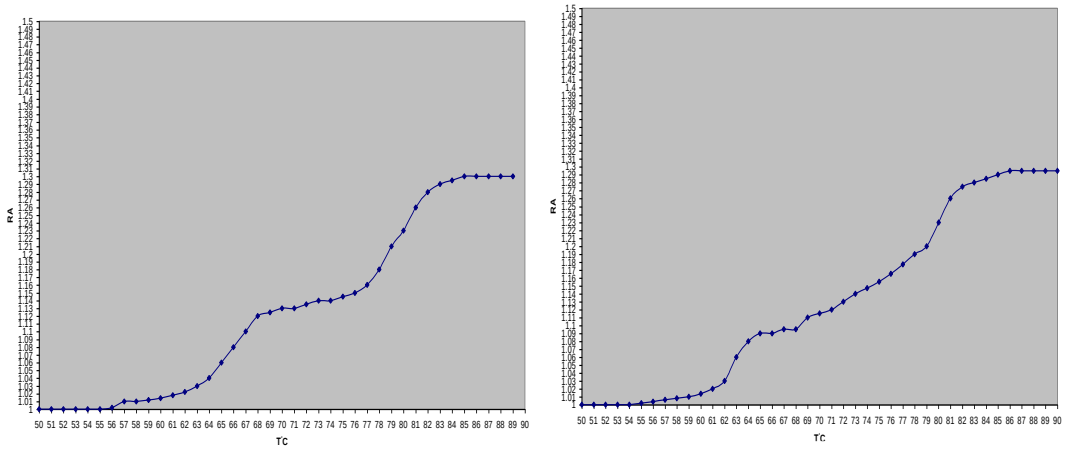
Figure7. Melting profile of chromatin isolated from sugarcane (Genotype CP 36-13 & CP 36-13 induced by colchicine) at 280 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl



CP 36-13
original cultivar

CP 36-13
Induced by colchicine

Figure 8.Melting profile of DNA isolated from sugarcane (Genotype CP 36-13& CP 36-13 induced by colchicine) at 260 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl



CP 36-13
original cultivar

CP 36-13
Induced by colchicine

Figure9.Melting profile of DNA isolated from sugarcane (Genotype CP 36-13 and CP 36-13 induced by colchicine) at 280 nm. Melting was carried out in diluted saline solution 0.0014 M NaCl

This result may reflect that DNA-under replication was performed. However, one cam assume that this DNA-under replication was shown to be in A=T rich region. In conclusion, these measurements might be useful in evaluation purposes and breeding program.

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الملخص العربي

تقييم الصفات المحصولية ومحتوى السكروز في نباتات متضاعفة من قصب السكر

محمد عبد الباعث الصيحي, أحمد السيد خالد, حسام الدين فتحي الوكيل، محمد عبد المنعم غنيمة، عمرو محمد الشيخ

تم الحصول على 3 أصناف تجارية منزرعة بمحطة بحوث المحاصيل السكرية بالصبحية بالإسكندرية وهي CP 36-13، CO 997، POJ 287 وتم تقطيعها لقطع صغيرة تحتوى كل قطعة على برعم وتمت تغطية احد الوجهين من كل قطعة بمحلول من الكولشيسين 0،05% ثم نقلت بعد ذلك إلى المعاملة بماء الصنبور ثم سمح لها بالنمو حتى يصل طول المجموع الخضري لحجم مناسب حوالي 40 سم ثم نقلت النباتات إلى الأرض المستديمة. وبالفحص تم قياس بعض الخصائص مثل عدد البلاستيدات وشكل الحزم الوعائية وحجم خلايا البشرة. كذلك تم قياس الخواص الفيزوكيماوية للكروماتين والـ DNA المنزوع من انوية الأوراق مثل كمية الـ DNA، كمية البروتينات الكروموسومية الكلية. كمية الهستونات في الأنوية. كمية البروتينات اللاهستونية.

تقدير درجة حرارة إنصهار الكروماتين وكذلك DNA خالى البروتين على طول موجة 260 nm، وطول موجة 280 nm. تقدير منحنيات الانصهار للكروماتين. تقدير الجزء المثبط والجزء النشط من الجينوم كدالة للنشاط النسخي كدلائل مبكرة على النمو والإنتاجية.

علاوة على ما تقدم فقد تمكن البحث الحالي من انتاج تضاعف للمجموعة الكروموسومية لنباتات الصنف CP 36-13، والصنف CO 997 بينما فشل إنتاج تضاعفات من الصنف POJ 287.