Differential Gene Expression and Sugar Beet Breeding Materials

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ABSTRACT

The present investigation aims at studying the possibility to use a specific molecular marker (i.e. physico-chemical properties of the purified chromatin as an indicator for transcriptional activity, and the amino acid proline content as an indicator of salinity tolerance and/or drought). In order to achieve such a purpose, five different breeding materials of sugar beet are employed. These materials are: C31/6, C12T, C39, C92 and C4612. Temperature of melting (Tm) estimated in °C and hyperchromicity were estimated at 260 nm and 280 nm, as well as the chemical compositions of chromatin; DNA; Histones; non histones and total proteins. The chemical compositions, relative to DNA showed that different chemical compositions of purified chromatin; temperature of melting at wave length 260 nm as well as at 280 nm; hyperchromicity, and repressed fraction of genome were obtained, giving a strong evidence that these molecular biomarkers might be used in breeding program of sugar beet. In addition, biochemical analysis revealed that different contents of proline were observed. In conclusion the present investigation recommends the use of physico-chemical properties and the amino acid proline as a molecular marker to measure the transcriptional activity and salinity tolerance of sugar beet materials.

INTRODUCTION

Sugar beet is a second sugar crops in Egypt. It needs a good and safe source of seeds to insure the raw materials for six or seven beet sugar factories. For this reason sugar beet breeding program has been started in Egypt by several breeders and investigators through Sugar Crops Research Institute, Agricultural Research Center, Egypt.

The main goal of sugar beet breeders is to develop sugar beet varieties with high root yield and high sugar content, better extraction yield (juice purity), higher seed germination percentages; lower tendency to "bolt" and higher resistance to leaf diseases. For that Egyptian Sugar Beet Breeding Program is concentrated on collecting different sugar beet breeding materials from different countries to achieve this goal. Sugar beet breeding materials has been exposed to different evaluating and testing experiment to select the good genotypes which have good characteristics to join the Egyptian Sugar Beet Breeding Program.

The identification of sugar beet varieties and/or genotypes became very important fruitful approach and it benefits the farmer who would thus

receive a product that provides the expected agronomic characters and also the correct identification is important for both the farmers and the seed producing companies to have techniques which help them to have quick and reliable identification of genotypes without the necessity of long-term field experiments needed for morphological characterization. Schondelmaier and Jung (1997) used twenty–four marker loci representing each of the nine linkage groups of sugar beet (*Beta vulgaris*) they assigned to nine previously produced primary trisomics. Single-copy RFLP probes were hybridized with filter-bound DNA of the trisomics. The auto radiographs were scanned and analysed by desitomeric methods. For the first time each of the linkage groups could unequivocally be assigned to one sugar beet chromosome. A standard nomenclature of the 9 chromosomes of sugar beet was suggested and discussed with respect to previous numbering systems.

Ivanov *et al.* (2002) studied a modified procedure of Random Amplified Polymorphic DNA analysis for searching open reading frames whose expression was different in N (normal) and S cytoplasm of sugar beet the S cytoplasm was characterized by changed structure and expression of the mitochondrial (mt) genome compared to the N one. EL-Manhaly *et al.* (2004) evaluated three Egyptian sugar beet genotypes Eg.6, Eg.26 and Eg.27 raised through breeding program after the success of seed production in Egypt. Potential used of Random Amplified Polymeric DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) for 18Sr DNA gene were applied in their study to detect genetic similarity and polymorphisms between and within the use three Egyptian sugar beet genotypes. Manouela *et al.* (2004) suggested that gene expression analysis, using DNA microarrays was used. They have developed DNA microarrays for profiling various germplasm and for correlating certain phonotypical characteristics with specific gene expression signatures. A subset of genes with hypothesized roles in defense response was then tested using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to quantify and verify expression level over time and their role in the defense response to C. bcticola. Lee *et al.* (2010) illustrated that solvent accessibility and the physicochemical properties of proteins are utilized to identify acetylated alanine, glycine, lysine, methionine, serine, and threonine.

Sugar beet (*Beta vulgaris* L.) is a drought and salinity tolerant species (Francois and Maas, 1994). However, soil water availability is the most limiting factor of sugar beet productivity in Mediterranean climates (Morillo-Velarde and Ober, 2006) and the provided irrigation makes these

areas prone to salinization due to the semi-arid climate (Subbarao *et al.*, 2003 and Navarro *et al.*, 2007). Under these growing conditions, plants face a combination of stresses (drought, osmotic stress, salinity), which is incorrectly characterized as water stress (Chaves *et al.*, 2002 and Munns, 2002). To cope with stress, plants produce and accumulate a wide range of organic compounds such as sugars (glucose, sucrose), proline, glycinebetaine and amino acids, which have an osmoprotective role (Gzik, 1996; Ghoulam *et al.*, 2002; Mack and Hoffmann, 2006; Pakniyat and Armion 2007; Monreal *et al.*, 2007; Huang *et al.* 2009 and Tsialtas *et al.* 2010).

Proline is accumulated in plants under drought and salinity stress in a number of species and is thought to play an important role in plant cells for adaptation to water stress (Delauney and Verma, 1993). Several genes encoding the enzymes in the route of proline biosynthesis have been identified in several plant species and all have been reported to be up regulated in response to water deprivation and/or salinization (Hare *et al.*, 1998; Yamada *et al.*, 2005 and Ma, 2005). Geng *et al.* (2008) investigated the effect of salinity on some agro-physiological parameters in plants of one multigerm variety and five strains of sugar beet. The plants were exposed to two salt treatments, 0 and 280 mMNaCl for 16 days in water culture medium and the physiological responses were analyzed. They found that the high NaCl concentrations caused a great reduction in growth parameters such as leaf area and dry weight of plants, but leaf thickness was increased. Varietal differences were evident at high NaCl concentration for almost all of the considered parameters except the Na+ and Cl- contents in the roots and petioles.

The main objectives of the present research work are to:-

1- Use molecular marker tools such as Physico-chemical properties of purified chromatin isolated from leaves.
2- Determination of proline content as an indicator for salinity tolerance and/ or drought.

MATERIALS AND METHODS

I. Materials

Five botanical genotypes of sugar beet were kindly supplied by Sugar Crops Research Institute, Agricultural Research Center (ARC). These genotypes are shown in Table (1).

2. Methods

2.1 Sugar beet Cultivation:

Seeds were cultivated in pots. For each genotype 20 pots were used. After two months of cultivation, leaves of plants were cut and subjected to the various analyses.

2.2. Physico-chemical properties of chromatin:

Leaves (about 100g from each genotype) were collected and immediately frozen until usage.

2.2. A. Extraction and purification of chromatin:

The chromatin was isolated by the method described by Fellenberg & Schomer (1975); Seehy (1980); Seehy *et al.*, (1990) and Abdel-Fattah (2002). The frozen leaf tissues were ground in a chilled mortar with small amount of the following buffer:

0.075M NaCl
0.01 M Na-citrate
0.01M Tris-HC1, pH8.0

Then, the grounds were homogenized in the same buffer (3ml buffer for each gram material) with high-speed blender (6000 rounds per minute) for 2 minutes. The homogenate was filtered through three layers of nylon mesh (pore size of 50μ). The filtrate was saved while the residue was again homogenized in the same buffer and filtered. The residue was discarded and the combined filters were centrifuged for 30 minutes at 7000 xg. The supernatant was discarded and the pellet was resuspended in the same buffer, and centrifuged at 15000xg for 10 minutes. The last step was repeated four times using the same amount of buffer and centrifugal force. The pellet was resuspended in the following buffer:

0.03M NaCl
0.01M Tris-HCl, pH8.0,

and centrifuged for 10 minutes at 15000xg.

For chromatin extraction, the recovered pellet was gently stirred for 30 minutes in 4ml of 1MNaCl, 0.01MTris-HCl, pH7.2 buffer and the mixture was kept over-night at 2°C. Then, the chilled mixture was centrifuged at 32000xg for 30minutes and the supernatant containing the chromatin was kept in clean vial at 4°C.

Chromatin was purified by filtration through Sephadex G-25 Fine using the 1MNaCl buffer. Using the ultraviolet spectrophotometer (Shimadzuv-240) the purity of chromatin preparations was checked by the following criteria:

1- Absorbance at 260nm/absorbance at 240=or more than 1.5

2- Absorbance at 250nm/absorbance at 260= 0.8-0.9
3- Absorbance at 280nm/absorbance at 260=0.5-0.6
4- Absorbance at 320nm/absorbance at 260 less than 10%

When purified chromatin showed adequate ultraviolet absorption spectrum, then it was dialyzed three times at 0-2°C against one liter of 0.0014M NaCl, 0.00025M tris-HCl, PH 7.2 buffer.

2.2. B. Estimation of melting temperature (TM value):

Heating was carried out in diluted saline solution (0.0014M NaCl, 0.00025 M Tris -HCl, pH7.2). Melting temperature of the chromatin was recorded at 260nm as well as 280nm by ultraviolet spectrophotometer Schimadsu uv-160, while heating was done by temperature program controller, Schimadsu TB-85, giving a temperature rate of 1°C/min. in order to estimate Tm- value, the procedure was the same as that described by Bonner *et al.*, (1968); Seehy *et al.*, (1990) and Abdel-Fattah (2002). From each extraction two estimations were calculated. The absorbance of all investigated samples was recorded every minute at 260 nm as well as at 280nm. The relative absorbance was calculated as described by Seehy (1980); Seehy *et al.* (1990) and Abdel-Fattah (2002). Relative Absorbance=AG/A 50°C, where AG is the absorbance at a given temperature and A50°C is the absorbance at 50°C.

2.2. C. Total proteins:

Histone and non- histone proteins

Extraction of histones was carried out from purified chromatin with HCl by using 10ml chromatin in each analysis. Chromatin solution was acidified by adding 4ml of 5 HCl (kept at 2 c for 12 hours), to reach 0.4 m M HCl, then centrifuged at 6000xg for 10 minutes at 0°C. The supernatant was saved while the sediment was washed by 0.4 M HCl for 30min. at 0°C, then it was again centrifuged at 6000xg for 10 minutes at 0°C. The combined supernatants were neutralized with NaOH and the PH adjusted at 7.2. The determination of histones was done by the method of Lowry *et al* (1951).

Non- histone proteins were calculated as the difference between the total proteins and histones. Total proteins, histones and non-histone proteins were determined as µg/ml chromatin and then, the different proteins were calculated as relative to the DNA.

2.3. Proline measurement:

Proline content was estimated in the Central Laboratory, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. According to the procedure described by Bates (1973), 4ml of 3.5% sulfa salicylic acid were added, to one gram leaves, homogenized and centrifuged at 3000 rpm for

5 min. the supernatant was saved and used for proline analysis using Beckman cl Amino Acid Analyzer 119cl.

RESULTS

As shown in Table (1) the tested genotype C92 was proven to display the higher value of root weight (1.530) followed by the genotype C4612. However the genotype C92 showed high percent of sucrose (18.2%), giving a good evidence that this genotype is considered to be a suitable source for selection and/or for evaluative purpose in sugar beet breeding program.

1. Physico-chemical properties of chromatin:

Figure (1) illustrates the ultraviolet absorption spectrum of purified chromatin isolated from the genotypes C31/6, C12T, C39, C92 and C4612. As shown in Table (2), all coefficients of absorbance of chromatin were in the standard ranges for the five genotypes of *Beta vulgaris* understudy. Therefore, the chromatins of these genotypes were proven to be pure enough to subject for testing the melting temperature.

Table (3) and Figures (2) represent the melting profiles and the Tm values for each chromatin of the five genotypes of *Beta vulgaris* understudy.

For each chromatin type, melting profiles were applied at 260nm and 280nm wave lengths. As shown in Table (3) and figures (3) & (4), the Tm values at 260nm ranged from 72.5 °C for genotype C4612 and 77 °C for genotype C39. At 280nm, the Tm values ranged from 74.5°C for genotype C4612 to 80.5°C for genotype C31/6. Also, ΔTm values were found to be different from one genotype to another and ranged from 1°C for genotype C39 to 6°C for genotype C31/6.

On the other hand, the amounts of total proteins; histone and non-histone proteins were estimated in the purified chromatin of the five genotypes understudy (Table, 4).

Figure (5) represents non-histones % of total proteins of the purified chromatin for the five studied genotypes of *Beta vulgaris.*

Table (5) represents the chemical composition of purified chromatin relative to DNA isolated from the tested genotypes.

Figures (6) and (7) represent histone and non-histone of the purified chromatin relative to their DNA of the five genotypes understudy.

The repressed fraction of genome ranged from 74 to 85.70% for genotypes C4612 and C92; respectively, while the active fraction of genome ranged from 14.29 to 26% for genotypes C92 and C4612; respectively, Table (6).

2. Proline:

Five sugar beet genotypes were tested for salinity tolerance. It seems that proline accumulation in leaf is effective mechanisms for osmotic pressure adjustment and plant tolerance to salinity, a mechanism commonly seen in sugar beet ancestors. Data obtained for proline showed that the tested genotypes were significantly different regarding their leaf proline content (Table, 7). Proline was found to be ranged from 0.3 ± 0.2 to 2.4 ± 0.4. Such a result gave an evidence that these genotypes are genetically different or at least gene expression of the amino acid proline displayed differential expression. In addition, this result reflects the differential responses of the tested genotypes to tolerate draught and/or salinity.

DISCUSSION

The present investigation aims at studying a specific molecular marker (i.e. physico-chemical properties of the purified chromatin as an indicator for transcriptional activity, and proline content as an indicator of salinity tolerance and/or drought).

1. Physico-chemical properties:

The present investigation revealed that sugar beet leaves are a good source for chromatin. In addition, the method used was found to be successful for chromatin isolation.

Ultraviolet absorption spectra of all used samples of chromatin were shown to be acceptable. Melting of chromatin was carried out at 260 nm as well as 280 nm and showed differences in melting temperature of chromatin isolated from different genotypes, giving an evidence that transcriptional activity of chromatin was different Tm-value ranged from 72.5 to 77°C at 260 nm and at 280 it ranged from 74.5 to 80.5°C nm. ΔTm (280-260) ranged from 1 to 6°C.

Seehy *et al.* (1990) reported that an alteration 0f 0.5±°C in Tm-value is considered to be a significant difference. A comparison of Tm-values at 260nm and 280nm makes it possible to differentiate the cohesion of hydrogen bonds in deoxyribonucleic acid regions rich in adenine-thymine and guanine-cytosine, since the former more strongly absorb the wave length 260nm, while the latter absorb the wave length 280nm more strongly, (Fellenberg, 1974 and Seehy, *et al.* 1990).

Data obtained from the chemical composition of purified chromatin relative to DNA showed that the genotype C92 displayed histones higher than that of other genotypes while the genotype C4612 represents the lowest value of histones. The tested genotypes could be arranged, according to the transcriptional activity based upon non-histone proteins (relative to DNA) would be arranged as follows: C4612 > C92 > C12T >

C31/6 > C39 Furthermore, they, according to their histones (as a function for transcriptional activity) might be ranked as follows: C4612 < C12T < C39 < C31/6 < C92. These results, however, gave a strong evidence that the purified chromatin of the genotype C4612 displayed:

1- low Tm-value at 260nm as well as at 280nm;
2- low histones, relative to DNA (1), and;
3- higher non-histones (percentage of total proteins) and;
4- high active fraction of genome for transcription.

The results obtained from this part of the present investigation revealed, from a molecular genetics point of view, that these genotypes are different.

2. Proline:

It was suggested that proline is acting as a compatible cytoplasmic solute, balancing an accumulation of salts outside of the cytoplasm (Voetberg and Stewart, 2001). The results obtained from this bioassay is in agreement with that reported by several workers (e.g. Chu *et al* 1973, Aspinall & Paleg, 1976: Buhl & Stewart, 1983: Stewart *et al*; 1986; Ueda *et al*. (2001) and Voetberg & Stewart (2001).

Proline plays an important role in plants for stress tolerance, (Stewart 1978; Voetberg and Stewart 2001). Proline was increased to a steady state concentration in response to salt treatments (Voetberg and Stewart, 2001 and El-Sharnoby, 2009). These authors, stated that proline levels in salt shocked leaves, decreased to near control levels within 24 hours of relief of stress.

In general salt tolerant sugar beet genotypes combat Na toxicity by its accumulation in leaf cell vacuoles and regulate their osmotic potential under salinity stress. Besides, these genotypes accumulate higher level of proline in their leaf for their osmotic potential regulation. These findings are in agreement with those investigated in *Atriplex* which is a halophyte and belongs to Chenopodiaceae family (Glenn *et al*., 1994).

Comparing proline content of tolerant and non-tolerant genotypes, it was indicated that tolerant genotypes accumulate highe level of proline than that of non-tolerant ones. This evidence confirms the osmoregulation role of Na and proline in osmotic potential adjustment of the plant and hence it's salt tolerance. This is in conformity with Gzick (1996) who concluded that higher proline level under salt stress is related to osmotic potential regulation in sugar beet.

Osmotic adjustment undoubtedly gained considerable recognition as a significant and effective mechanism of salinity tolerance in crop plants.

Osmoregulatory effects of proline, glycine betaine and ions on water balance and salt tolerance, have been shown in spinach (Martino *et al*, 2003), wheat (Abdel-Aziz and Reda, 2000), bean (Shabala *et al*, 2000), cowpea (Freitas *et al*, 2001), sugar beet (Ghoulam *et al*, 2002; Heuer *et al*., 1981 and Pakniyat & Armion, 2007) and a halophyte sea aster (Ueda *et al*, 2003).

Regarding proline content, these genotypes were among the group genotypes which contained the highest and the lowest amount of proline content. Other tolerant and non-tolerant check genotypes followed the same trend regarding their Na and proline content.

The data obtained from this work indicated that differential gene expression with respect to Proline, at the same environmental conditions was achieved. This conclusion represents additional evidence that the treated genotypes are genetically different, giving an adequate source for selection, hybridization and consequently for breeding program.

From these results, one can conclude that genotype C4612 displayed the lowest value for Tm-value of chromatin not only at 260nm but also at 280nm. This genotype, however showed non-histone proteins (acidic chromosomal proteins) a value of 1.16 relative to DNA and 50% as a percent of total chromosomal proteins. Furthermore its leaves contain 2.4mg/g proline (the highest value).

In conclusion, the present study revealed, that the tested genotypes, at the level of this study, are genetically different. In addition these two molecular biomarkers might be used and/or employed in evaluative purposes of breeding program for sugar beet.

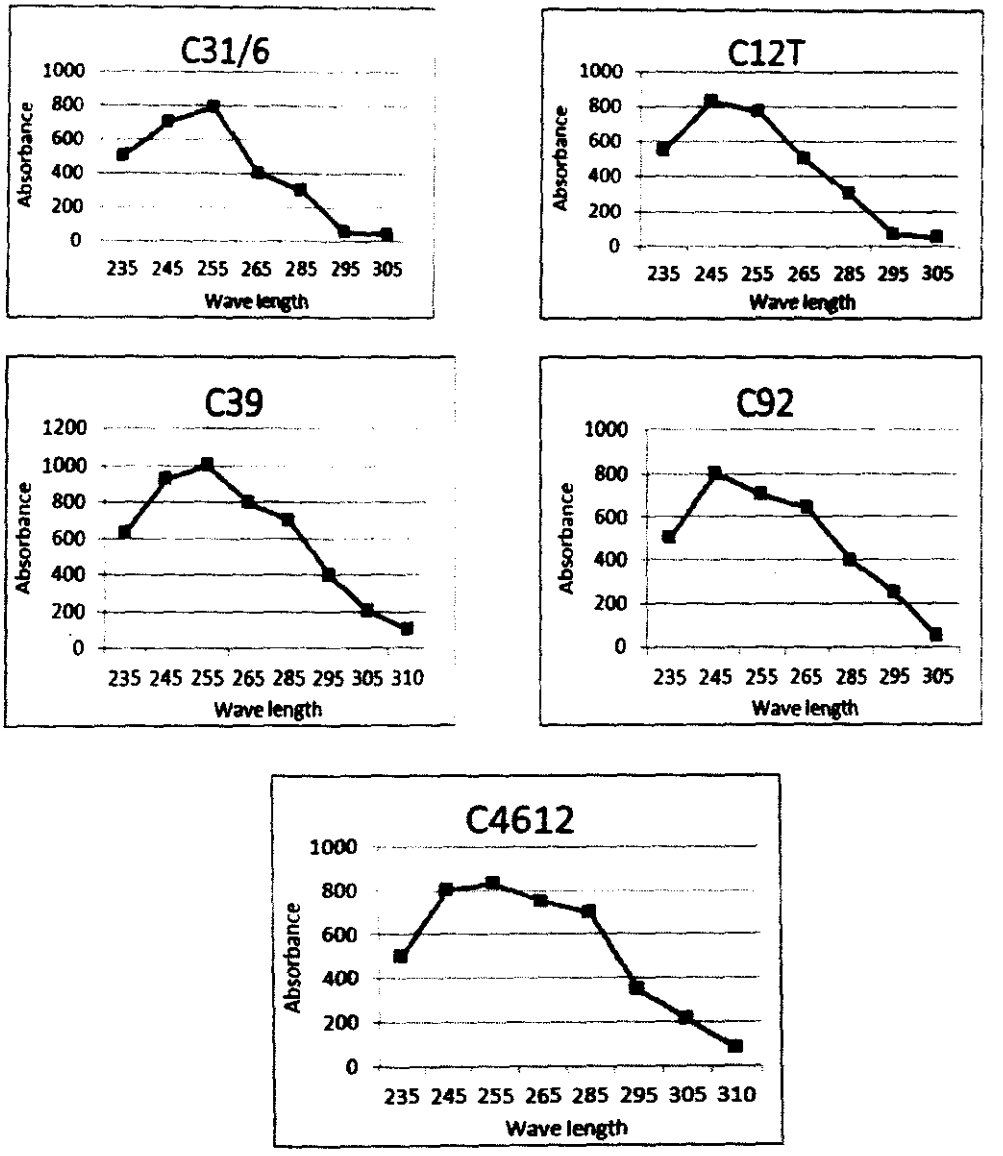

Figure (1): Ultraviolet absorption spectra of purified chromatin isolated from genotypes.

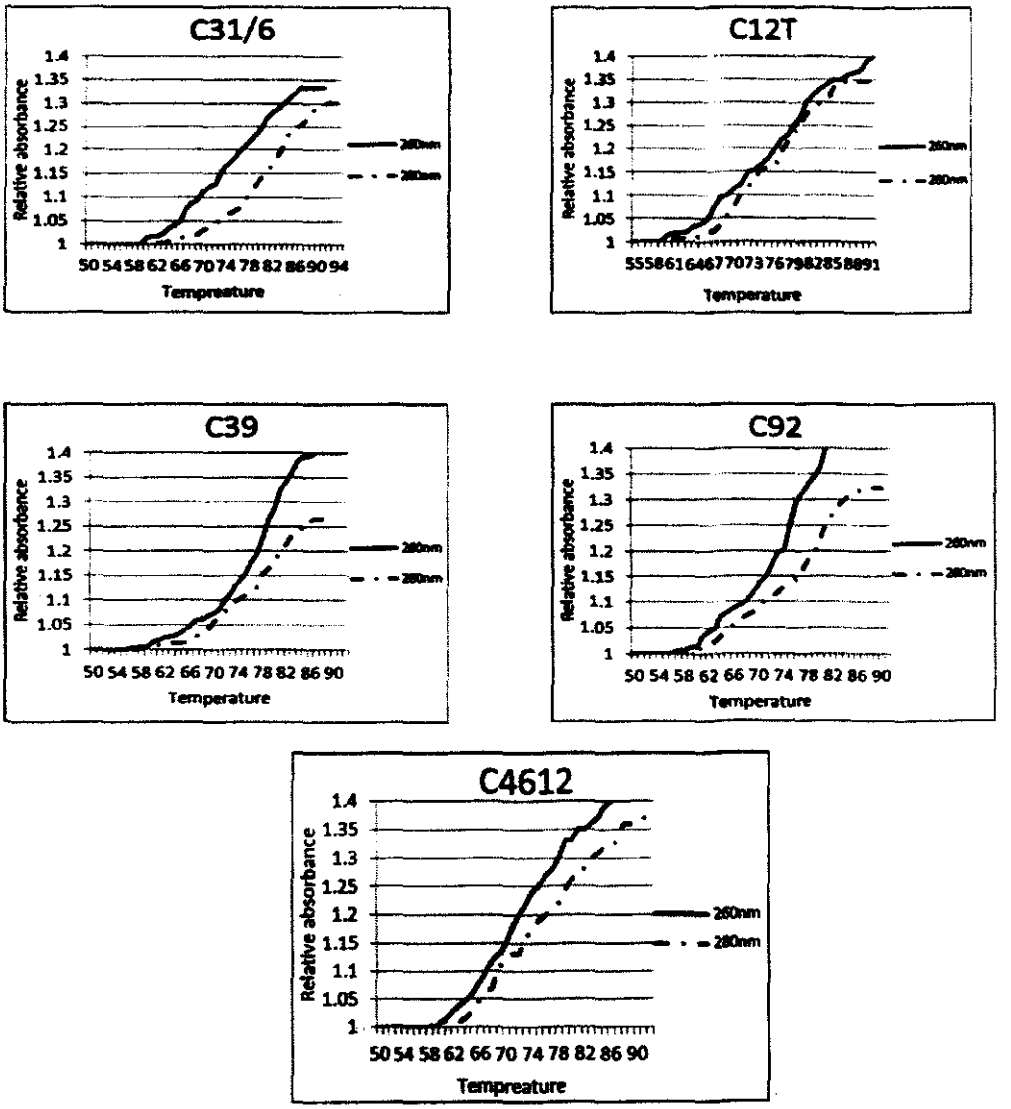

Figure (2): Melting profiles of chromatin at wave length 260 & 280nm and melting was carried out in diluted saline solution (0.0014 M NaCl).

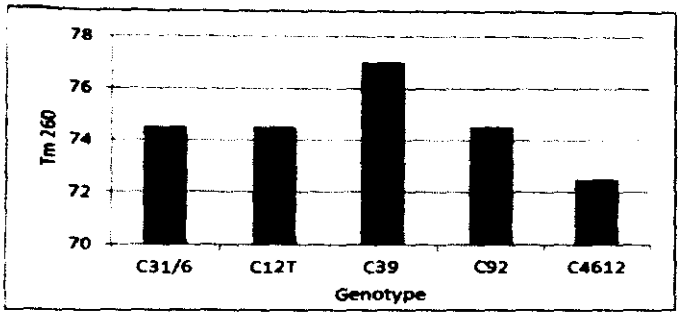

Figure (3): Tm values at 260nm for the purified chromatin of the five genotypes of *Beta vulgaris*.

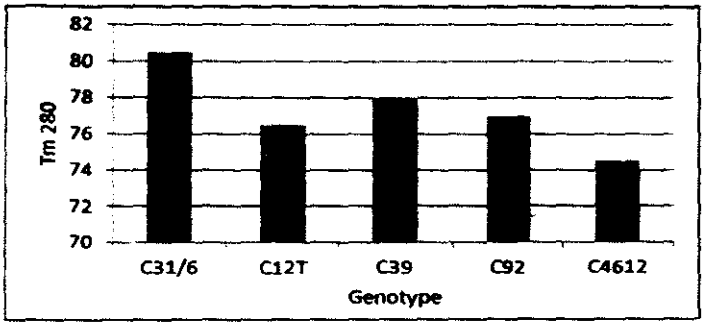

Figure (4): Tm values at 280nm for the purified chromatin of the five genotypes of *Beta vulgaris*.

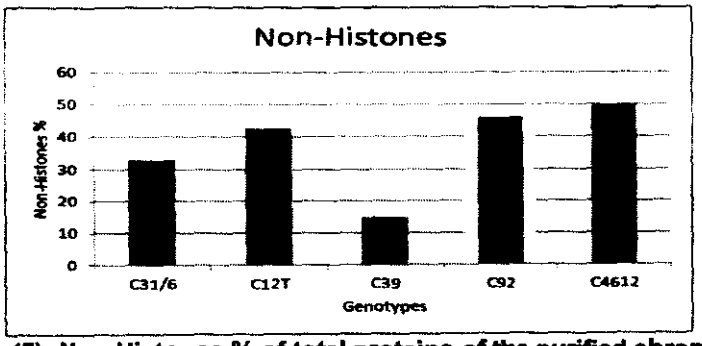

Figure (5): Non-Histones % of total proteins of the purified chromatin for the five genotypes of *Beta vulgaris*.

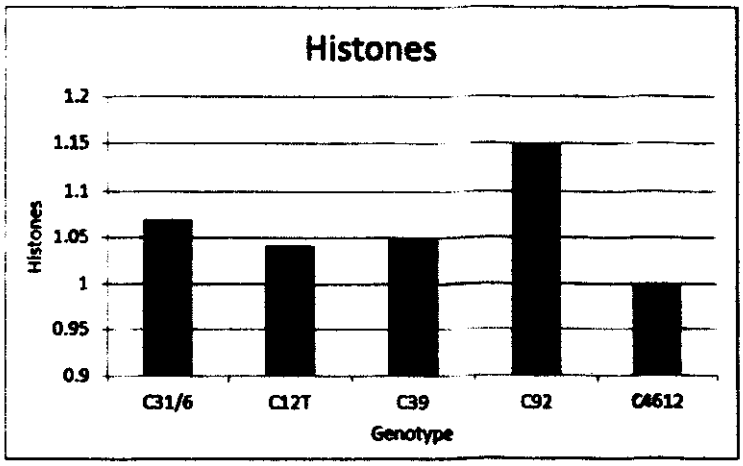

Figure (6): Histones of the purified chromatin relative to DNA for the five genotypes of *Beta vulgaris*.

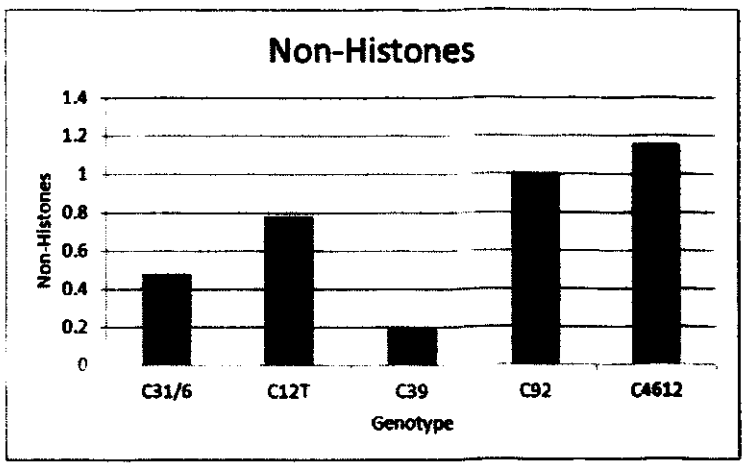

Figure (7): Non-Histones of the purified chromatin relative to DNA for the five genotypes of *Beta vulgaris*.

Table (1): Botanical genotypes of sugar beet tested in this work

Genotypes	Origin	Ploidy levels	Seed type	Root weight	Sucrose percentage
C31/6	U.S.A. (California)	Diploid	Polygerm	0.830	18.4
C12T	U.S.A. (California)	Diploid	Polygerm	0.850	17.9
C39	U.S.A. (California)	Diploid	Polygerm	0.770	17.5
C92	U.S.A. (California)	Diploid	Polygerm	1.530	18.2
C4612	**U.S.A. (California)**	**Diploid**	**Polygerm**	**1.100**	**17.4**

Table (2): Coefficients of absorbance calculated for chromatin isolated from the five *Beta Vulgaris* genotypes

Wave Length	Absolute absorbance of chromatin					Coefficient	Coefficients of absorbance				
(nm)	C31/6	C12T	C39	C92	C4612		C31/6	C12T	C39	C92	C4612
240	492	560	630	517	501	A260/A240=or more than 1.5	1.56	1.375	1.606	1.386	1.516
250	700	830	822	802	650	A250/A260 (0.8-0.9)	0.9	0.82	0.81	0.91	0.85
260	772	770	1012	717	760	A280/A260 (0.5-0.6)	0.51	0.57	0.51	0.6	0.55
280	390	440	520	462	420	A320/A260 (Less than 10%)	0.04	0.06	0.07	0.09	0.1
290	260	310	430	410	340	-	-	-	-	-	-
300	52	69	210	260	221	-	-	-	-	-	-
320	33	48	76	66	76	-	-	-	-	-	-

Table (3): Temperature of melting (Tm value)of chromatin isolated from the tested genotype at 260nm & 280nm

Genotype	Tm260	Tm280	Δ Tm	Hyperchromicity
C31/6	74.5	80.5	6	33.40%
C12T	74.5	76.5	2	28.10%
C39	77	78	1	40%
C92	74.5	77	2.5	46%
C4612	72.5	74.5	2	41.40%

Table(4): *Chemical compositions of ** purified chromatin isolated from the tested genotypes

Genotypes	DNA	Total proteins	Histones	Non-Histones	Acidic proteins % of total proteins
C31/6	152	222	148	74	33%
C12T	210	383	219	164	43%
C39	162	201	170	31	15%
C92	190	411	220	191	46%
C4612	130	301	150	151	50%

*µg/ml chromatin
** Chromatin soluble in 1 M NaCl, PH 7.2

Table (5): *Chemical compositions of ** purified chromatin isolated from the tested genotypes (Relative to DNA)

Genotype	DNA	Total proteins	Histones	Non-Histones
C31/6	1	1.46	1.07	0.39
C12T	1	1.823	1.042	0.78
C39	1	1.24	1.049	0.19
C92	1	2.16	1.15	1.005
C4612	1	2.16	1	1.16

*Relative to DNA

**Chromatin soluble in 1M NaCl, PH 7.2

Table (6): Repressed and Active fraction of genome (RFG) & (AFG), (Bonner, 1976 and Seehy *et al*, 1990)

Genotype	RFG	AFG
C31/6	79.2	20.8
C12T	77.18	22.814
C39	77.70	22.29
C92	85.70	14.29
C4612	74	26

Table (7): Proline content in leaves of the tested genotypes of sugar beet

Genotype	Concentration mg/g ± SE
C31/6	1.2 ± 0.1
C12T	0.3 ± 0.2
C39	0.8 ± 0.2
C92	1.4 ± 0.1
C4612	2.4 ± 0.4

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الملخص العربي

تباين التعبير الجيني في نبات بنجر السكر

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يهدف هذا البحث الى دراسة خصائص خمسة تراكيب وراثيه من نبات بنجر السكر وهذه التراكيب الخمسة تم الحصول عليها من معهد بحوث المحاصيل السكرية – مركز البحوث الزراعية.

ولدراسة الخواص الفيزيوكميائية للكروماتين المعزول من أنوية الخلايا والمنقى بعمود الغربلة الجزيئية تم تقدير:

1- درجة حرارة انصهار الكروماتين على طول موجة 260 نانوميتر و 280 نانوميتر، وكذلك تم تحليل المكونات الكميائية للكروماتين من بروتينات كلية وهستونات وبروتينات حامضية أي لا هستونية.

2- تحليل الحمض الاميني البرولين للتعرف على مدى وجوده في الأوراق.

وقد أظهرت النتائج أن منحنيات ومعاملات الامتصاص للكروماتين المستخلص بكلوريد الصوديوم والمنقى بعمود الغربلة الجزيئية أعطت قيما جيدة تجعلها قابلة لإجراء تجارب الانصهار والتحليل الكيموحيوي للمكونات. فقد أعطت درجة انصهار الكروماتين على طول موجة 260 نانوميتر قيم مختلفه تراوحت مابين 72.5 م^0 و 77 م^0 بينما أظهرت التحاليل ان درجة حرارة انصهار الكروماتين عل طول موجة 280 نانوميتر تراوحت ما بين 74.5 م^0 و 80.5م^0 فيما بين التراكيب الوراثية الخمسة. ويتضح من هذه النتائج ان التراكيب الوراثية قيد الاختبار تختلف اختلافاً معنوياً في درجة انصهار الكروماتين على طول موجة 260 نانوميتر وكذلك 280 نانوميتر معطية دليلاً على أن النشاط النسخي لهذه التراكيب الوراثية متفاوت. كذلك أظهرت نتائج التحليل الكميائي لمكونات الكروماتين في صورة البروتين الكلى والبروتينات الهستونية واللاهستونية نسب متفاوتة فيما بين التراكيب الوراثية الخمسة مما يظهر أن هناك نشاط نسخي متفاوت لهذه التراكيب تحت الدراسة. وكذلك كانت القيم مختلفة عند حساب الجزء المثبط من الجينوم والجزء النشط من الجينوم فيما بين هذه التراكيب. كما أظهرت نتائج تحليل الحمض الاميني البرولين ان نسب تركيزه المتفاوتة أعطت أختلافات معنوية فيما بين التراكيب الوراثية تحت الدراسة.

ومما سبق نستطيع القول أن التراكيب الوراثية الخمسة مختلفة وراثياً وقد يكون ذلك مفيد في الأغراض التقيمية في برنامج الانتخاب والتربية لنبات بنجر السكر. هذا وقد أظهرت النتائج المتحصل

عليها أن الكروماتين المعزول من التركيب الوراثي C4612 على سبيل المثال أعطى أقل درجة اصفرار معطياً دليلاً على القدره النسخيه العاليه كما أن البروتين اللاهستوني (الحامضي) مقدراً كنسبه من البروتين الكلي كان عالياً مؤكداً بذلك هذه الخلاصه. كما أوضح هذا التركيب الوراثي أن محتوى البرولين كان أعلى من التراكيب الوراثية الأخرى مما يعطي دليلاًعلى امكانية استخدام هذين الواسمين على المستوى الجزيئي في الأغراض التطبيقيه لبرامج الانتخاب والتربيه.