Differential Gene Function and Physico-Chemical Properties of Sugar Beet Chromatin

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ABSTRACT

The present investigation aims at studying the possibility to use the physico-chemical properties of the purified chromatin as an indicator for transcriptional activity. In order to achieve such a purpose, five different breeding materials of sugar beet are selected and used. These materials are: 1, 2, 3, 4 and 5. Temperature of melting (Tm) estimated in ^{0}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; temperature of melting; 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 conclusion the present investigation recommends the use of physico-chemical properties as a molecular biomarker to measure the transcriptional activity of plant materials.

INTRODUCTION

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. However, 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 (Khatab, 2001).

The identification of sugar beet varieties and/or genotypes is a fruitful approach and it benefits the farmer who would thus receive a product that provides the expected agronomic characters and also the correct identification. 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 demsitometuric 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.

The main objective of the present research work is to use Physico-chemical properties of purified chromatin isolated from leaves as a molecular marker.

MATERIALS AND METHODS

I. Materials

Five botanical genotypes of sugar beet were kindly supplied by Sugar Crops Research Institute, Agricultural Research Center (ARC).

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:

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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 Schimadzu uv-160, while heating was done by temperature program controller, Schimadzu 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). 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, non-histon, DNA

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 ^{0}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.

RERSULTS

As shown in Table (1) the tested genotype 1 was proven to display the higher value of root weight (1.530) followed by the genotype 2. However the genotype 1 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 1, 2, 3, 4 and 5. All coefficients of absorbance of chromatin were in the standard ranges for the five genotypes of *Beta vulgaris* under study. Therefore, the chromatins of these genotypes were proven to be pure enough to subject for testing the melting temperature.

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

For each chromatin type, melting profiles were applied at 260nm and 280nm wave lengths. As shown in Table (3), the Tm values at 260nm ranged from 72.5 ºC for genotype C4612 and 77 ºC for genotype C39. At 280nm, the Tm values ranged from 74.5ºC for genotype C4612 to 80.5ºC for genotype C31/6. Also, ΔTm values were found to be different from one genotype to another and ranged from 1ºC for genotype C39 to 6^{0}C for genotype C31/6.

On the other hand, the amounts of total proteins; histones and non-histones proteins were estimated in the purified chromatin of the five genotypes under study (Table, 2) and it represents non-histones % of total proteins of the purified chromatin for the five studied 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
1	U.S.A. (California)	Diploid	Polygerm	0.830	18.4
2	U.S.A. (California)	Diploid	Polygerm	0.850	17.9
3	U.S.A. (California)	Diploid	Polygerm	0.770	17.5
4	U.S.A. (California)	Diploid	Polygerm	1.530	18.2
5	**U.S.A. (**California)	**Diploid**	**Polygerm**	**1.100**	**17.4**

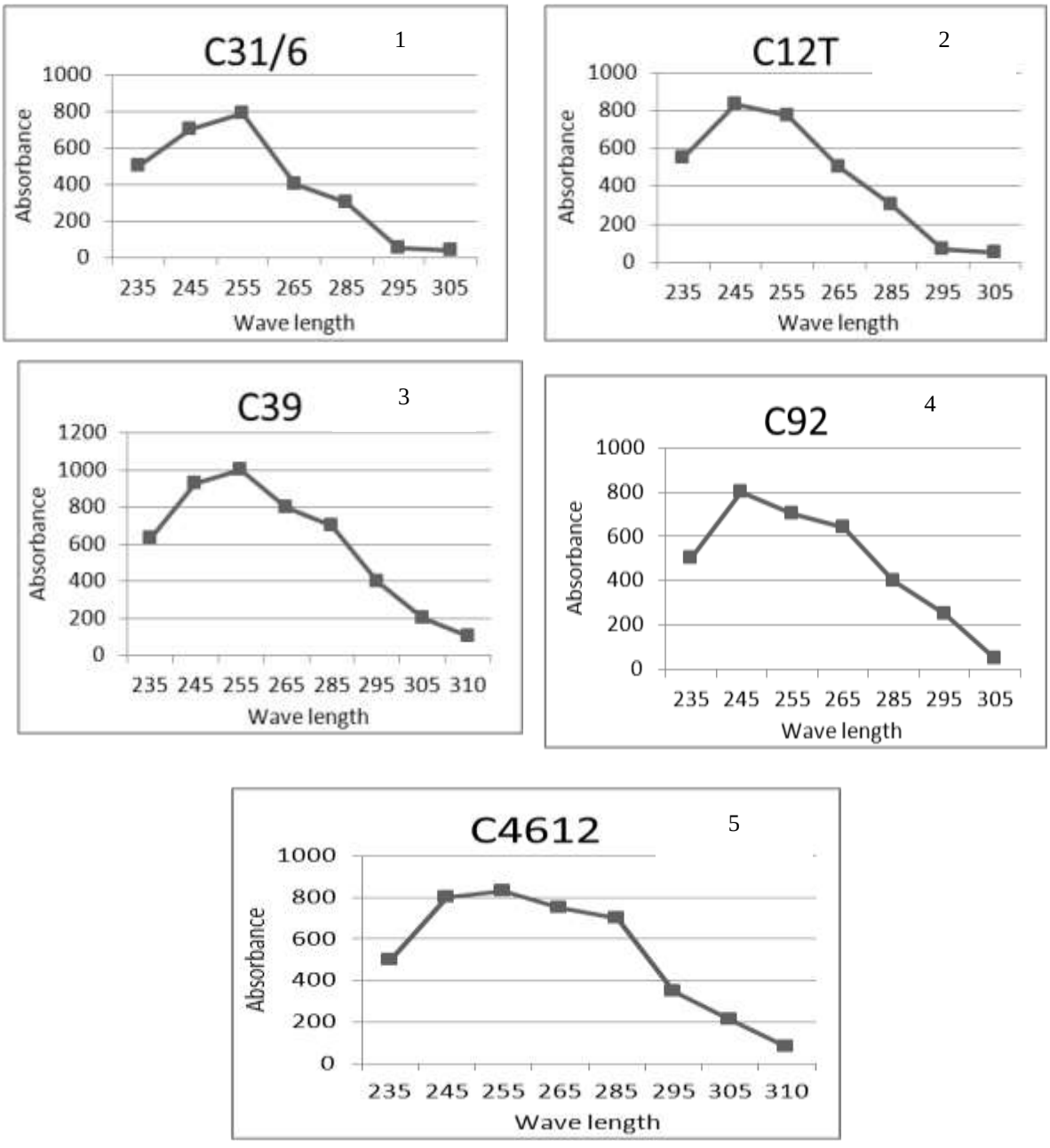

Figure 1. Ultraviolet absorption spectra of purified chromatin isolated from genotypes

Table 2. *Chemical compositions of ** purified chromatin isolated from the tested genotypes

Genotypes	DNA	Total proteins	Histones	Non-Histones	Acidic proteins % of total proteins
1	152	222	148	74	33%
2	210	383	219	164	43%
3	162	201	170	31	15%
4	190	411	220	191	46%
5	130	301	150	151	50%

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

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

Genotype	Tm260	Tm280	Δ Tm	Hyperchromicity
1	74.5	80.5	6	33.40%
2	74.5	76.5	2	28.10%
3	77	78	1	40%
4	74.5	77	2.5	46%
5	72.5	74.5	2	41.40%

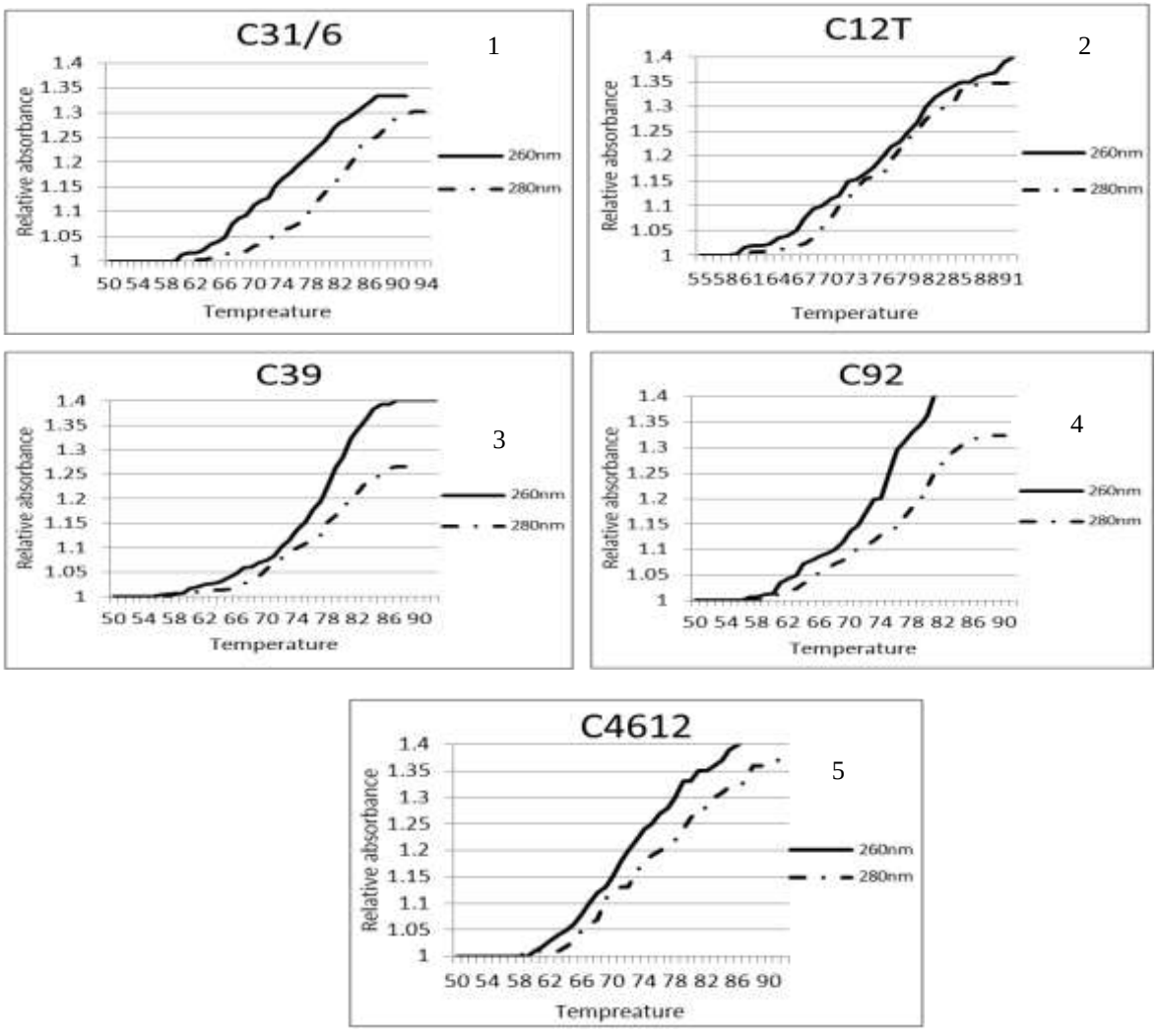

Figure 2. Melting profiles of chromatin at wave length 260 & 28and melting was carried out in diluted saline solution (0.0014 M NaCl

Table (4) represents the chemical composition of purified chromatin relative to DNA isolated from the tested genotypes.

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 (5).

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)

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 77^0C at 260 nm and at 280 it ranged from 74.5 to 80.5^0C nm. ΔTm (280-260) ranged from 1 to 6^0C.

Seehy *et al*. (1990) reported that an alteration 0f $0.5\pm^0$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.

Table 4.*Chemical compositions of ** purified chromatin isolated from the tested genotypes (Relative to DNA)

Genotype	DNA	Total proteins	Histones	Non-Histones
1	1	1.46	1.07	0.39
2	1	1.823	1.042	0.78
3	1	1.24	1.049	0.19
4	1	2.16	1.15	1.005
5	1	2.16	1	1.16

*Relative to DNA

**Chromatin soluble in 1M NaCl, PH 7.2

Table 5. Repressed and Active fraction of genome (RFG) & (AFG), (Bonner *et al*, 1968 and Seehy *et al*, 1990)

Genotype	RFG	AFG
1	79.2	20.8
2	77.18	22.814
3	77.70	22.29
4	85.70	14.29
5	74	26

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

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

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الملخص العربي

تفاوت وظيفة الجين والخواص الفيزيوكيماوية للكروماتين

مُحَمَّد الصيحى، أميرة خطاب

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.

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 موجة260 نانوميتر قيم مختلفه تراوحت مابين 72.5 م^0 و77 م^0 بينما أظهرت التحاليل ان درجة حرارة انصهار الكروماتين على طول موجة 280 نانوميتر تراوحت ما بين 74.5 م^0 و 80,5م^0 فيما بين التراكيب الوراثية الخمسة.

وكما يتضح 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.

وأظهر التحليل الكيميائي 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 على سبيل المثال الكروماتين المعزول من التركيب الوراثي C4612 أعطى أقل درجة انصهار معطياً دليلاً على القدره النسخيه العاليه كما أوضح هذا التركيب الوراثي أن محتوى البرولين كان أعلى من التراكيب الوراثية الأخرى مما يعطي دليلاً على امكانية استخدام هذين الواسمين على المستوى الجزيئي في الآغراض التطبيقيه لبرامج الانتخاب والتربيه وكذلك كانت القيم مختلفة عند حساب الجزء المثبط من الجينوم والجزء النشط من الجينوم فيما بين هذه التراكيب.

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