



MEASURING OF THE ALTERATION OF RETROTRANSPOSITION IN THE RESPONSE OF SALINITY STRESS USING IRAP AND SCOT MARKERS

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ABSTRACT

Retrotransposons comprise the major part of eukaryotic genomes. They have the ability to replicate themselves through RNA intermediate via reverse transcription process. During normal development, these elements become quiescent, but they are stimulated by stresses. The availability of PCR-based techniques to detect the variation in retrotransposition rate due to salinity was tested. IRAP and SCoT markers were applied in two salinity-tolerant eukaryotic genomes: Yeast (*Saccharomyces cerevisiae* L.) and Barley (*Hordeum vulgare* L.). The genomes of the yeast strain EMCC-49 and two barley cultivars Giza-123 and Giza-2000 were extracted. Five IRAP primers with two combinations and nine SCoT primers were applied. The yeast strain was grown in the YPG media with 0.5 M, 1 M, 1.5 M NaCl or the control. The barley cultivars were irrigated with 0.25 M, 0.6 M NaCl or just distilled water. IRAP technique developed three markers in the yeast under the different levels of salinity. ScM1 IRAP primer showed a band with molecular size of 456 bp in the yeast under 0.5 and 1.5 M only. Another band with molecular size of 409 bp appeared under the control and disappeared in all salinity treatments. The third IRAP marker was shown by the ScM2 primer with molecular size of 1952 bp under the 0.5 M treatment. While, two IRAP markers appeared in barley due to high salt conditions. The 5'LTR IRAP primer showed an 886 bp band in the barley cultivar Giza-2000 under the control condition only. Sukkula IRAP primer displayed the second IRAP marker in the cultivar Giza-2000 of barley with molecular size of 330 bp under the 0.6 M only. SCoT markers

showed 17 markers in the response of salinity stress in yeast with molecular sizes ranged from 1911 to 271 bp with SCoT 31 and SCoT 26 primers, respectively. SCoT 26 primer gave the highest number of markers per SCoT primer (five different markers). In barley, 18 SCoT markers were detected under high salt conditions. They molecular sizes were between 1762 (SCoT 26) and 281 bp (SCoT 7). SCoT 32 primer showed five markers in barley under salinity as the highest number of markers per SCoT primer. The results showed different patterns between control and treatments and the high levels of salinity led to new retrotransposition. This study confirmed that PCR techniques; like IRAP and SCoT can exhibit the activation of retrotransposition due to high salt conditions. Good positive results were obtained and we recommend using these techniques for different molecular purposes due to their advantage; easy, fast, cheap and effectiveness.

Keywords: Retrotransposon, salinity, IRAP and SCoT techniques

INTRODUCTION

Transposable elements comprises about of 3% from the *Saccharomyces cerevisiae*, 15% of *Arabidopsis thaliana*, 20% of *Drosophila melanogaster*, 45% of *Homo sapiens* and 80% of *Zea mays* genomes (Kim et al 1998; Smit, 1999; Lander et al 2001; Kaminker et al 2002; Sabot and Schulman, 2006; and Maumus et al 2009). The majority components of most plant genomes are retrotransposons (Mansour, 2007). Retrotransposons are detected in all eukaryotes

(SanMiguel et al 1998). Retrotransposons are found in a random distribution in the genome (Bayram et al 2012). Retrotransposons use the "copy and paste" mechanism in its replication. They replicate via reverse transcription using an mRNA intermediate (Ikeda et al 2001; Maumus et al 2009).

Retrotransposons seem the lentiviruses in its structure and life cycle (Feschotte et al 2002; Kalendar and Schulman, 2006; Sabot and Schulman, 2006). Most retrotransposons produce proteins which are needed for their own retrotransposition (Bayram et al 2012). Both of Kalendar et al 2000; Sabot and Schulman, 2006 and Sabot et al 2006 reported that retrotransposons that didn't have these proteins use the proteins encoded from another retrotransposons. Retrotransposons integrate themselves to many loci inside the genome. They produce polymorphism among individuals (Bonchev et al 2010). The methylation is one of different mechanisms that cause inactivated majority of retrotransposons during development (Hirochika et al 2000).

Mansour 2009 and Alzohairy et al 2012 discussed the role of stress in the enrichment of the retrotransposition rate. Stress leads to production larger pools of transcripts of retrotransposons (Mansour 2007, 2008 and Salazar et al 2007). Bayram et al 2012 stated that activation of retrotransposons can stimulate due to the effect of some stress conditions. Salazar et al 2007 found that the promoters of retrotransposons play the main role in the success of retrotransposition process.

SCoT or "start codon targeted" marker is a PCR-based technique for detection polymorphism developed by Collard and Mackill, 2009. SCoT analysis depends on the short conserved regions around the start codon. Al-qurainy et al 2015 stated that SCoT markers became one of the best choices to study the genetic diversity. Wu et al 2013 counts the advantages of the SCoT technique. They reported that SCoT marker is simple, rapid, cheap, effective, repeatable, and reproducible. IRAP or "Inter retrotransposon amplified polymorphism" technique amplify the distance between two LTR-retrotransposons (Kalendar and Schulman, 2006).

The purposes of the present study were to study the effect of salinity on the activation of retrotransposition rate in different eukaryotic organisms such as higher-flowering-plants (barley) and lowering-eukaryotic single-cell organisms (yeast) and to test the effectiveness of IRAP and SCoT

markers in the detection of retrotransposition and distinguish their banding patterns differences due to salt-activated retrotransposons.

MATERIALS AND METHODS

Yeast strain and barley cultivars

One yeast (*Saccharomyces cerevisiae* L.) strain and two barley (*Hordeum vulgare* L.) cultivars (Table 1) were used to study the effect of salinity stress on the activation of retrotransposition.

Table 1. The names and sources of the yeast strain and barley cultivars

Name	Source
EMCC-49	Microbiological Resources Centre (Cairo MIRCEN)
Giza-123	Field Crop Research Institute, Agricultural Research Center
Giza-2000	(ARC), Giza, Egypt

IRAP- and SCoT-PCR techniques

DNA extraction and PCR-amplification

Pure culture for EMCC-49 yeast strain was grown in YPG medium; yeast extract, pepton and glucose (Curran and Bugeja, 2006) on a water bath with shaker at 30°C for 48h. The control medium without any salt and treated media with high salt concentrations, 0.5 M, 1 M or 1.5 M NaCl. Then, the genomic DNA was isolated using a method described by Beringer (1974). Barley cultivars treated with high levels of salinity (0.25 M or 0.6 M NaCl) and the genomic DNA was isolated from leaves after nine days of treatment using a method described by Dellaporta et al (1983).

Five IRAP primers were used in this study. Two (ScM1 and ScM2) were applied with their combination on yeast (Table 2). While, the other three (LTR, Sukkula and WLTR 2105) with their combinations were used with the two barley cultivars as shown in Table (3).

For SCoT technique nine SCOT primers were used with both yeast and barley. Table (4) represents the names and the sequences of the nine SCOT primers.

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Table 2. The names and the sequences of yeast IRAP primers

Primer	Sequence
ScM1	5' GCTGTCATCGAAGTTAGAGG 3'
ScM2	5' AGAAGATGACGCAAATGATGAG 3'

Table 3. The names and sequences of barley IRAP primers

Primer	Sequence
5'LTR	5' ATCATTGCCTCTAGGGCATAA TTC 3'
Sukkula	5' GATAGGGTTCGCATCTTGGGCGT GAC 3'
Wltr2105	5' ACTCCATAGATGGATCTTGG TGA 3'

Table 4. The names and sequences of barley and yeast SCoT primers

Primer	Sequence
SCoT 5	5' CAACAATGGCTACCACGA 3'
SCoT 7	5' CAACAATGGCTACCACGG 3'
SCoT 18	5' ACCATGGCTACCACCGCC 3'
SCoT 22	5' AACCATGGCTACCACCAC 3'
SCoT 26	5' ACCATGGCTACCACCGTC 3'
SCoT 31	5' CCATGGCTACCACCGCCT 3'
SCoT 32	5' CCATGGCTACCACCGCAC 3'
SCoT 34	5' ACCATGGCTACCACCGCA 3'
SCoT 35	5' CATGGCTACCACCGGCC 3'

To perform PCR reactions, 10 µl master mix, 4 µl DNA, 2 µl primer and 4 µl H₂O for 20 µl total volume were used. The PCR reaction for all techniques was the same with the exception of annealing temperature as the follow: initial denaturation at 94°C for 5 minutes only on time, 35 cycles, denaturation step at 94°C for 30 second, annealing temperature; step two was 50°C or 61°C for 45 second, extension step at 72°C for 1 minute, finally the final extension was at 72°C for 10 minutes.

Where for SCoT primers; for yeast and barley and IRAP for yeast the annealing temperature was 50°C whereas, it was 61°C for barley IRAP. The PCR products were running using 1.5 – 2 % agarose concentration, staining with ethidium bromide to visualize by UV.

RESULTS AND DISCUSSION

In this study, yeast strain and barley cultivars were used as models of eukaryotic organisms to study the effect of salinity as an environmental stress on activation of retrotransposons. Two techniques were used, each of them for a certain purpose. IRAP technique was used to determine if new retrotranspositions occurs under the effect due to salt stress. Whereas, SCoT markers were used to detect the effect of retrotransposition on coding regions; up or down regulation for these regions.

IRAP for yeast strain and barley cultivars

IRAP primers and their combinations were used with the yeast strain (EMCC-49) and barley cultivars (Giza-123 and Giza-2000) (Table 1). For yeast ScM1, ScM2 primers and the combination between them were used to apply IRAP technique (Fig. 1 and Table 5). Whereas, 5'LTR, Sukkula, Wltr2105 and the combination between 5'LTR and Sukkula primers were used with barley for the same purpose (Fig.1 and Table 6).

Table 5. The molecular sizes of different bands of yeast IRAP primers

Primer	Bp	C	T1	T2	T3
ScM1	456	0	1	0	1
	409	1	0	0	0
ScM2	1952	0	1	0	0

Table 6. The molecular sizes of different bands of barley IRAP primers

Primer	Bp	Giza-123			Giza-2000		
		C	T1	T2	C	T1	T2
LTR	886	0	0	0	1	0	0
Sukkula	330	0	0	0	0	0	1

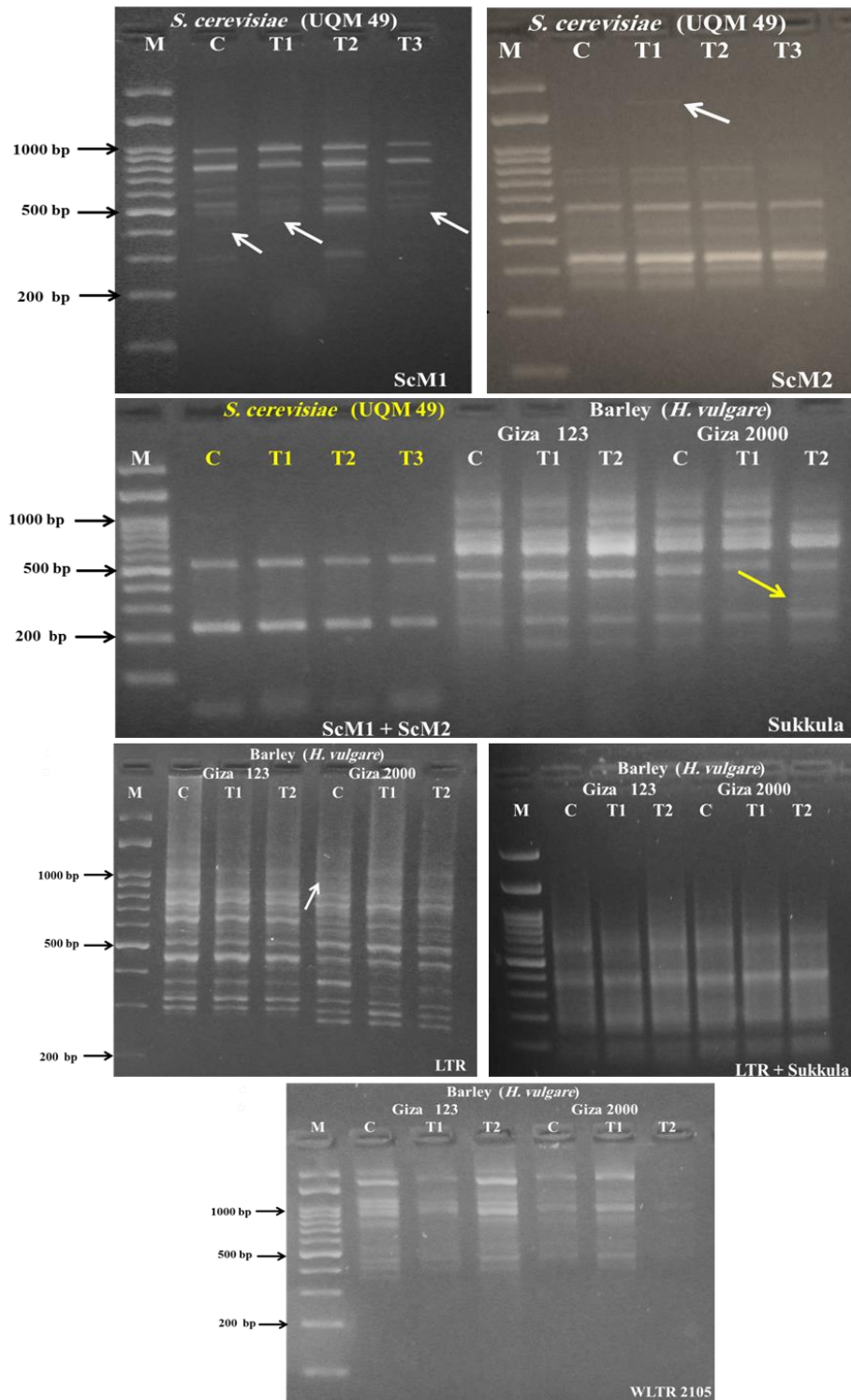


Fig. 1. IRAP banding patterns for yeast (ScM1, ScM2 and ScM1+ScM2 primers) and barley (LTR, Sukkula, Wltr2105 and LTR+Sukkula primers) under the control (C), 0.5 M (T1), 1 M (T2) and 1.5 M NaCl (T3) for yeast, whereas, under the control, 0.25 M (T1) and 0.6 M NaCl (T2) for barley cultivars; Giza-123 and Giza-2000. The arrows refer to the different "polymorphic" bands.

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From the previous IRAP results, four primers showed different bands between the control and treatments while the other primers gave the same bands with both the control and treatments such as Wltr2105 primer and the combinations LTR + Sukkula and ScM1 + ScM2. ScM1 primer with yeast gave two different bands, one of them with molecular size 456 bp was present only in T1 (0.5 M), that mean there is new retrotransposition due to this level of salinity. While the other band was found in the control and absent from any treatments, as shown in **Fig. (1) and Table (5)**. With ScM2 primer only one band with molecular size 1952 bp appeared in only in T1, that mean there is new retrotransposition due to this level of salinity. In barley, only one different band appeared with each primer. Giza-123 didn't show any different bands between the control and treatments with any primer, whereas Giza-2000 exhibit one different band with each primer (**Fig. 1 and Table 6**).

Start Codon Targeted Polymorphism (SCoT)

Is a new PCR-based technique for detection polymorphism developed by **Collard and Mackill, 2009**. SCoT analysis depends on the short conserved regions around the start codon (ATG). Nine SCoT primers (**Table 4**) were used to study the efficiency of SCoT technique in the i) detection of the differences in the transposition rate due to salinity stress, and ii) determination if these differences are related to the coding regions in the yeast and barley genomes? **Fig. (2) and Tables (7 and 8)** illustrate the different bands which appeared / disappeared under the salinity stress compared with the normal conditions for both yeast (**Fig. 2 and Table 7**) and barley (**Fig. 2 and Table 8**).

SCoT results of yeast showed that the eight from nine primers gave different bands between the control and treatments; as an example; SCoT7 showed three bands with molecular sizes 1042, 390 and 300 bp respectively, the first band was found only in treatment 1 while it was absent in the control and other treatments, the second band was found only in treatment 1 and treatment 2 while it absent in both the control and treatment 3, the third band found only in treatment 2. Each of SCoT 31, 32 and 34 displayed only one different band between the control and treatments as shown in **Fig. (2) and Table (7)**.

Table 7. The molecular sizes of different bands of yeast SCoT primers

Primer	Bp	C	T1	T2	T3
SCoT 5	1091	1	1	1	0
	313	0	1	0	0
SCoT 7	1042	0	1	0	0
	390	0	1	1	0
	300	0	0	1	0
SCoT 18	784	0	1	0	0
	462	1	0	1	1
SCoT 22	1630	1	0	0	0
	366	1	1	1	0
SCoT 26	814	1	0	0	1
	423	1	0	0	1
	350	1	0	0	1
	321	0	0	0	1
	271	1	0	0	0
SCoT 31	1911	0	1	0	0
SCoT 32	844	1	0	0	0
SCoT 34	517	1	0	1	1

Table 8. The molecular sizes of different bands of barley SCoT primers

Primer	Bp	Giza-123			Giza-2000		
		C	T1	T2	C	T1	T2
SCoT 5	462	0	0	0	1	0	0
SCoT 7	1374	1	1	0	0	1	0
	1131	1	0	0	0	1	0
	1042	0	0	0	0	1	0
	281	1	0	0	0	1	0
SCoT 26	1762	0	1	1	0	0	0
	1594	0	1	0	0	0	0
SCoT 31	564	0	0	0	0	1	1
SCoT 32	1488	0	0	0	1	0	0
	1161	0	0	0	1	1	0
	1000	0	0	0	1	0	0
	977	0	0	0	0	1	1
SCoT 34	844	1	0	1	0	0	0
	472	0	0	1	0	0	0
	437	0	1	0	0	0	0
	360	0	0	0	0	1	1
SCoT 35	332	1	0	0	0	0	0
	710	0	0	0	1	0	1

Whereas in barley, there are two primers didn't show different bands between the control and treatment, but the other primers gave different bands between them such as, SCoT 35 didn't show any different bands in Giza-123 between the control and treatments, whereas it showed one polymorphic band that appeared in both the control and treatment two but absent in treatment 1 as shown in **Fig. (2) and Table (8)**.

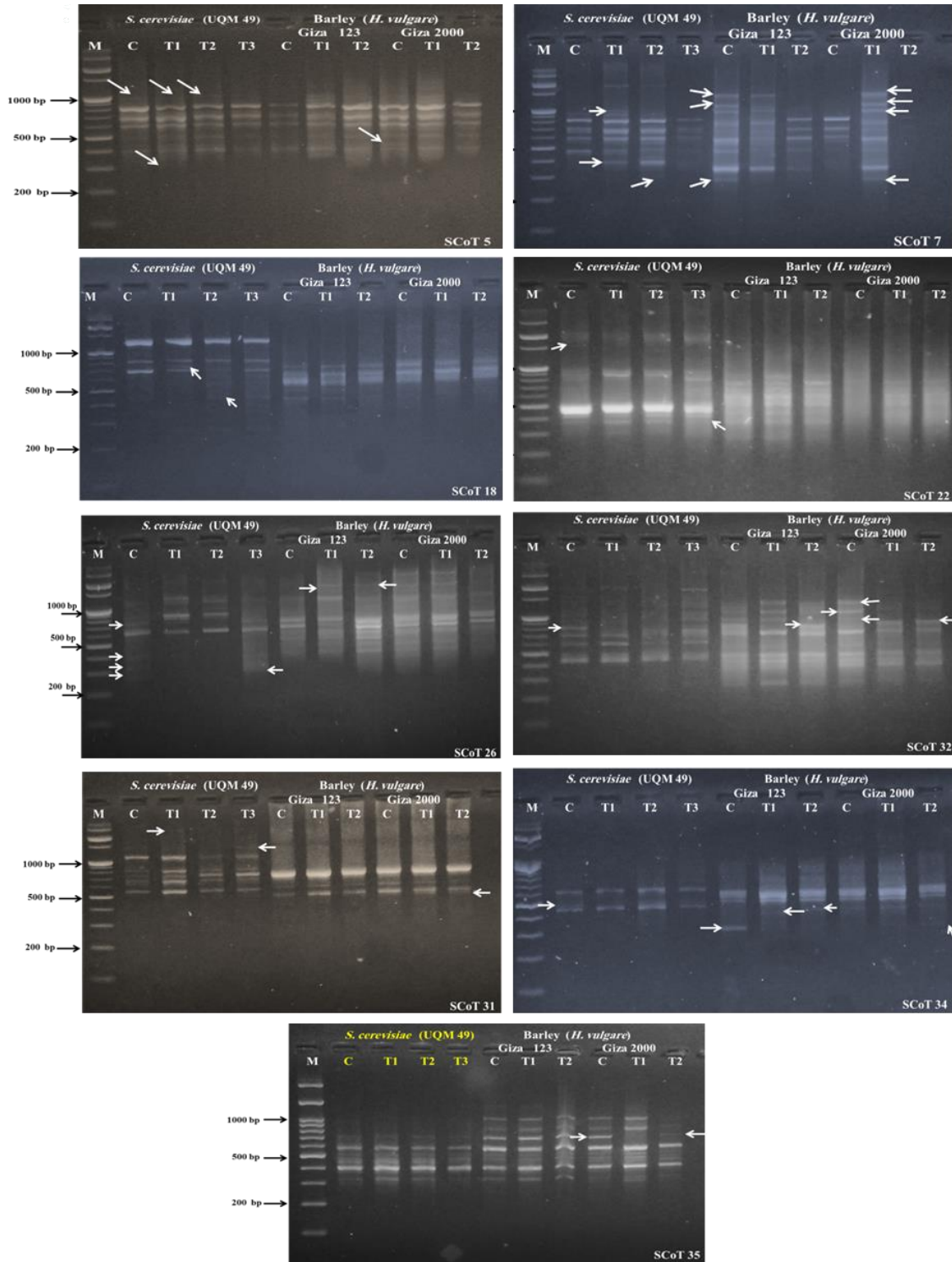


Fig. 2. SCoT banding patterns for yeast strain and barley cultivars under the control (C), 0.5 M (T1), 1 M (T2) and 1.5 M NaCl (T3) for yeast and under the control, 0.25 M (T1) and 0.6 M NaCl (T2) for barley cultivars; Giza-123 and Giza-2000. The arrows refer to the different "polymorphic" bands.

The results which obtained in this research are in agreement with **Bayram et al (2012)**, where they found activation in mobilization of Nikita retrotransposon where they used different ages of calli that originated from the same embryo and at the same time. Their results showed that the conditions of tissue culture caused this retrotransposition activation. **Kartal-Alacam et al (2014)** cultured mature barley embryos (*Hordeum vulgare* L.) for callus formation. They investigated *Sukkula* (a non autonomous retrotransposon) polymorphism in calli with different culturing time using IRAP technique, their results showed that conditions of tissue culture and the age of callus affected on the movements of *Sukkula* retrotransposon. Our results also exhibit that salinity stress cause retrotransposon movements.

On the other hand, SCoT marker is a new PCR-based technique developed for the polymorphism detection. This method based on conserved regions around the ATG regions (**Sadek and Ibrahim, 2018**). Our result showed that SCoT markers are a good method for distinguished between the different bands in the control and several treatments due to salinity stress.

Wu et al (2013) considered SCoT technique as modern way for differential expression of genes. They predict that, the SCoT markers will be a promising method to discover the novel genes. This investigating agreed with our results that showed presence of new patterns due to the stress and exhibit an effect of Retrotransposons on coding regions.

This study applied to confirm that PCR techniques can exhibit the activation of retrotransposition due to salinity stress. The obtained data from this result represents that not only real time PCR technique can detect the retrotransposition due to different stresses but we can use other techniques that be easy, fast, cheap and effectiveness for detect retrotransposition and the effect of these elements on other coding regions, like IRAP and SCoT techniques. These techniques achieve these purposes and gave good positive results and we recommend using these techniques for different purposes due to their advantage.

CONCLUSION

Environmental stresses have been reported to activate retrotransposons. In this study, IRAP technique; retrotransposons-based technique and SCoT markers confirmed the effect of salinity

stress on the movement of retrotransposons on other coding regions. Salinity stress affect on retrotransposons movement and on coding genes. IRAP and SCoT are suitable PCR - markers for detect the retrotransposition and the effect on other regions.

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قياس التغيير في معدل الانتقال الرجعي استجابة للإجهاد الملحي باستخدام تقنيات الـ IRAP و SCoT

[207]

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الموجز

نفس البادئ في المقارنة فقط ولم تظهر في جميع المعاملات. ونتج الكشف الجزيئي الثالث في الخميرة مع البادئ ScM2 بحجم جزيئي 1952 نيوكليوتيدة تحت مستوى ملوحة 0.5 مولار فقط. وظهر كشافين جزيئيين في الشعير أحدهما ظهر مع البادئ 5'LTR بحجم جزيئي 886 نيوكليوتيدة في الصنف جيزة 2000 في المقارنة فقط، بينما أعطى البادئ Sukkula حزمة بحجم جزيئي 330 نيوكليوتيدة في نفس الصنف جيزة 2000 في مستوى ملوحة 0.6 مولار فقط. أظهرت بادئات تقنية SCoT 17 كشافا جزيئيا في الخميرة تراوحت أحجامها الجزيئية من 1911-271 نيوكليوتيدة مع البادئات SCoT31 و SCoT26 على التوالي. أوضحت هذه النتائج ظهور حزم مختلفة بين المقارنة والمعاملات وأن التركيزات العالية من الملح أدت إلى حدوث تنشيط لانتقال العناصر الوراثية المتنقلة الرجعية. وأثبتت أن التقنيات المعتمدة على PCR مثل IRAP و SCoT يمكنها الكشف عن الانتقالات الجديدة والتي يسببها الإجهاد الملحي. خلصت الدراسة إلى نتائج إيجابية جيدة وتوصي الدراسة باستخدام هذه التقنيات في الدراسات الوراثية الجزيئية نظراً لما لها من مميزات حيث أنها سهلة التطبيق قليلة التكلفة سريعة التنفيذ وعالية الفعالية.

الكلمات الدالة: العناصر الوراثية المتنقلة الرجعية، الملوحة، IRAP، SCoT

تمثل العناصر الوراثية المتنقلة الرجعية جزء كبير من جينومات الكائنات الحية حقيقية النواة، وهي العناصر التي لها القدرة علي تكرار نفسها من خلال وسيط من mRNA عن طريق عملية النسخ العكسي. أثناء مراحل النمو الطبيعية للكائن الحي تكون هذه العناصر في حالة سكون ولكن عندما يتعرض الكائن الحي للضغوط البيئية فإنها تصبح أكثر نشاطا وانتقالا. تم استخدام تقنيتي IRAP و SCoT مع اثنين من جينومات حقيقيات النواة المتحملة للملوحة هما الخميرة (*Saccharomyces cerevisiae* L.) والشعير (*Hordeum vulgare* L.). استخدمت السلالة EMCC-49 من الخميرة والصنفين جيزة 123 وجيزة 2000 من الشعير وتم استخلاص DNA من كل منهما. استخدم خمسة بادئات من تقنية IRAP وتوليفين منهما وتسعة بادئات من تقنية SCoT. تم تنمية سلالة الخميرة على بيئة YPG على تركيزات 0.5 مولار و 1 مولار و 1.5 مولار من ملح كلوريد الصوديوم بالإضافة إلى المقارنة. تم ري نباتات الشعير بتركيزات 0.25 مولار و 0.6 مولار من الملح مع المقارنة. أظهرت تقنية IRAP ثلاثة كشافات جزيئية في الخميرة تحت مستويات الملوحة المختلفة حيث أعطى البادئ ScM1 حزمة واحدة بحجم جزيئي 456 نيوكليوتيدة في الخميرة في مستوى ملوحة 0.5 مولار و 1.5 مولار، بينما ظهرت حزمة بحجم جزيئي 409 نيوكليوتيدة باستخدام

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