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The Rule of Salinity Stress in Activation of Retrotransposition Rate in some Bread Wheat Genotypes.

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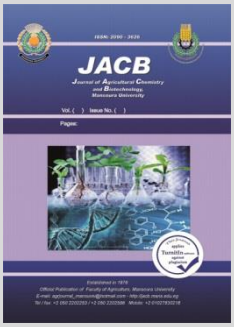

ABSTRACT

The genomes of the six wheat genotypes under study; Masr1, Shandaweel1, Line1. Line2, Giza171 and Sakha94 were extracted. Ten IRAP primers were applied for detecting the rule of salinity stress in activation of retrotransposition rate in the studied bread wheat genotypes. The wheat genotypes were irrigated with 100 mM, 150 mM and 200 mM of NaCl or distilled water for control. IRAP technique developed all the ten markers in the wheat under the different levels of salinity. IRAP-2175 primer showed a number of new bands with molecular weights ranged from 400 bp to 550 bp giving a new retrotransposition locations due to the treatment with different concentrations of salinity. The second primer (IRAP-2198) revealed that bands with molecular sizes ranged from 470 bp to 1160 bp obtained a new retrotransposition appearance. The third IRAP marker was revealed by the IRAP-2197 primer with molecular sizes of 215 bp to 342 bp under all the three levels of salinity. The results revealed different patterns between control and treatments and the high levels of salinity led to new retrotransposition. This study revealed that PCR technique; like IRAP can reflect the activation of retrotransposition due to high salt levels. The obtained results were encouraging and it is better to use this technique due to their advantage; easy, fast, cheap and effectiveness.

Keywords: Retrotransposon, salinity, IRAP techniques, bread wheat

INTRODUCTION

The retrotransposition process is divided into three types, transcription of the element into RNA, then reverse transcription to cDNA followed by reinsertion of the copied element into a new genomic. During normal development, these elements were activated by the stresses like salinity while it usually quiescent during development. The activity of retrotransposable elements can be induced by salinity stress especially long terminal repeat (LTR) retrotransposons, which are characterized by a high level of variability in the LTR sequences included in transcription, and have evolved by gaining new expression patterns mostly associated with responses to diverse stress Bennetzen 2000, Alzohairy *et al*., 2014a and b and Mohammadi, R. 2016. The majority of the LTR retrotransposons produce larger pools of transcripts in response to the stress. Nawaday it was detected that the epigenetic activation of these retrotransposons alters the expression of adjacent genes. The new insertions in or next to coding regions generate mutations that can lead to changes in gene expression and reshape the genome, both structurally and functionally Badr et al., 2020. Thus, activation of LTR retrotransposable elements can play an essential role in plant development and evolution. The availability of PCR-based techniques to detect the variation in retrotransposition rate due to salinity was tested Goudarzi M, Pakniyat H (2008). IRAP markers were applied in six bread wheat salinity-tolerant genotypes (*Triticum aestivum L.*).

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality, Chinnusamy *et al.* (2005) , Afiah et al., (2016) and Akladious and Mohamed (2018). Adverse effects of salinity on plant growth may be due to osmotic stress and ion cytotoxicity. Soil salinity is a pioneer dilemma spread, especially in arid and semiarid areas. Egypt is one of the countries that suffer severe salinity problems, Al-Naggar *et al.* (2015 a,c). Salinization is mainly due to low precipitation (<25 mm annual rainfall), high temperature (during summer, temperature reaching from 35 to 45°C), high surface evaporation (1500-2400 m/year), poor drainage system with 98% of the cultivated land under irrigated, rising water table (less than one meter below the soil surface), and irrigating with low quality water up to salinity of 4.5 dS/m, El-Hendawy *et al.* (2005), which retarded the aimed sustainable crop production, especially in the north delta of Egypt.

Transposable elements comprises about of 3% from the *Saccharomyces cerevisae*, 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 (Abou-Deif et al, 2005 and 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 , Schulman, 2006 and Abd El-Rahman et al., 2012). 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

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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 and Carvalho *et al.,* 2010). Bayram *et al* 2012 stated that activation of re- trotransposons 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. IRAP or "Inter retrotransposon amplified polymorphism" technique amplify the distance between two LTR-retrotransposons (Kalendar and Schulman, 2006).

The activation of retrotransposition rate in six bread wheat genotypes and to test the effectiveness of IRAP markers in the detection of retrotransposition as well as distinguish their banding patterns differences due to salt-activated retrotransposons.

MATERIALS AND METHODS

Plant material source

This study was conducted in an experimental farm of the Agricultural Research Station in the faculty of agriculture, Benha University, Egypt. During the the seasons 2019/2020, six wheat genotypes were used. The names and proportions of the studied genotypes are listed in Table 1.

The seeds of the different genotypes were sown separately in 30 cm diameter pots containing 1:1 (v:v) sandy clay soil. Ten seeds were planted in each pot. Each genotype was planted in four replicates when the plants reached the true leaf stage. Sodium chloride was added with three different treatments (100mM, 150mM, and 200mM).

Table 1. The names and pedigree of the four wheat genotypes used in the study.

Genotype name	Pedigree and selection history	Origin
Masr 1(G1)	OASIS/SKAUZ//4*BCN/3/2*PASTOR CMss00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S	Egypt
Shandweel 1(G2)	Site/Mo/4/Nac/Th.Ac//3*Pvn/3/mirlo/Buc CMSS93 B00S 67S-72Y-010M-010Y-010M-3Y-0M-0THY-0SH	Egypt
Line1 (G3)	ATTILA50Y//ATTILA/BCN/3/STAR*3/MUSK-3. AISBW05-0043-9AP-0AP-0AP-9AP-0AP-0SD	Egypt
Line 2 (G4)	SAKHA94/MISR1 GZ2008-06DH2	Egypt
GIZA-171(G5)	SAKHA93/GEMMEIZA9 AND ITS SELECTION history is "GZ 2003-101-1GZ- IGZ-2GZ-OGZ"	Egypt
Sakha94(G6)	OPATA/RAYON//KAUZ.CMBW 90Y3180 –OTOPM-3Y-010M-010M-010Y-10M-015Y-OY-OAP-0S.	Egypt

s

DNA extraction and PCR amplification

According to the manufacturers of the QIAGEN DNeasy Plant DNA extraction Mini Kit, total genomic DNA was extracted from tissues of all the genotypes of plants that were the subject of the study. The in silico PCR tool (http://insilico.ehu.eus/PCR/) was used to evaluate the primers. Table 2 lists the expected PCR amplicons produced by the employed IRAP primers as well as their name and sequence. The 0.2 M primer with a 10 pmol concentration, 400 M of dNTP mix, 2.5 l of 10x PCR reaction buffer, 1.25 units of TAKARA Taq DNA polymerase, and 1 l of template DNA were used in the PCR reaction, which was carried out in a 25 l mixture. The final volume was adjusted with sterile double-distilled water (d.dH2O). The reactions were amplified using a PCR thermocycler at 94 °C for 5 min, 35 cycles at 94 °C for 30 sec, 56 °C for annealing for 1 min, an extension at 72 ° C for 1 min, and a final extension temperature of 72 °C for 10 min. PCR products that had been amplified were kept at -20°C for further purification and use. On 0.8% agarose gel electrophoresis, 6 l of the PCR-amplified product were loaded, stained with ethidium bromide, and then seen with a UV transilluminator (Bio RAD).

Table 2. the primers name and Sequence of and IRAP

No.	P. Name	sequence (5'→3')
1	IRAP-2175	TTAGACCCGGAACCGCCGTG
2	IRAP-2198	ATCCTTCGCGTAGATCAAGCGCCA
3	IRAP-2197	GAAGTACCGATTTACTTCCGTGTA
4	IRAP-2200	ATGTGACAGTCGACTAACCAC
5	IRAP-2202	TGGCGCTTGATCTACGCGAAGGA
6	IRAP-2204	AACTTGATCCAGATCATCTCC
7	IRAP-4334	CCATGGCGAGCAGATGTGCT
8	IRAP-4370	ATGCCGTATTCTCAGCATCC
9	IRAP-4351	CAGGCAAGAATGAGCGTCTC
10	IRAP-4340	ATGGTTGTCGAAACTCCAGC

RESULTS AND DISCUSSION

In this study, wheat cultivars were used as model of eukaryotic organisms to study the effect of salinity as an environmental stress on activation of retrotransposons. IRAP technique was used to determine if new retrotranspositions occurs under the effect due to salt stress.

IRAP primers and their combinations were used with wheat cultivars (Masr-1, Shandaweel-1, Line-1, Line-2, Giza-171 and Sakha-94) (Table 2).

From the previous IRAP results, Ten primers showed different bands between the control and treatments while the other primers gave the same bands with both the control and treatments. IRAP-2175 primer with the genotype Giza 171 gave two bands, one of them with molecular size 550 bp was present only in the second treatment (150 ppm), that mean there is new retrotransposition due to this level of salinity. With the same primer and the same genotype, a new band was appeared at the molecular size 500 and was found only in the third treatment (200 ppm). At the molecular size 480 pb, new band appeared for the first treatment (100 ppm) of Masr-1 cultivar, and for the second treatment (150 ppm) of Shandaweel-1 cultivar, and all the three treatments of Line-1 and Line-2 genotypes which means a new retrotransposition appearance due to the treatment of different concentrations of salinity. For the appearance of a new band at the molecular size 450 at the control and the second and the third treartments of Giza 171 cultivar, and the first and third treatments of Shandaweel-1 which reveal a new retrotransposition appearance due to the treatment of different concentrations of salinity. While the other band was found in the control and absent from any treatments for Sakha-94, as shown in Figure (1) and Table (3). With the same primer only one band with molecular size 400 bp appeared in control and the third treatment of Giza-171, the second treatment of Shandaweel-1 and the three treatments of line-1 genotype that mean there is new retrotransposition due to these levels of salinity.

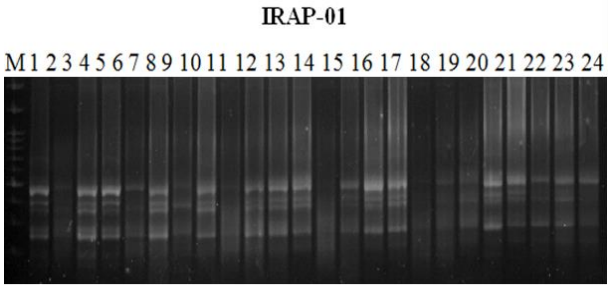

Figure 1. IRAP-2175

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

IRAP-2198 primer with all the genotypes gave one band at the molecular size 1160 bp and it was absent only in the third treatment (200 ppm). The same for the band with the molecular weight 890 pb, it was absent in the third treatment of Masr-1, the first treatment of line-1 and all the treatments of Sakha-94. For the appearance of a new band at the molecular size 810 pb at the first and second treatments of line-2, which reveal a new retrotransposition appearance due to the treatment of the two concentrations of salinity. The same idea for the band at the molecular size 665 pb in case of the third treatment of Shandaweel-1. In case of the band at the molecular weight 530 pb, it was appeared at the three treatments of Giza-171. While the other band at the molecular weight 470 pb was absent in the control as well as absent from any treatments for Sakha-94, as shown in Figure (2) and Table (4).

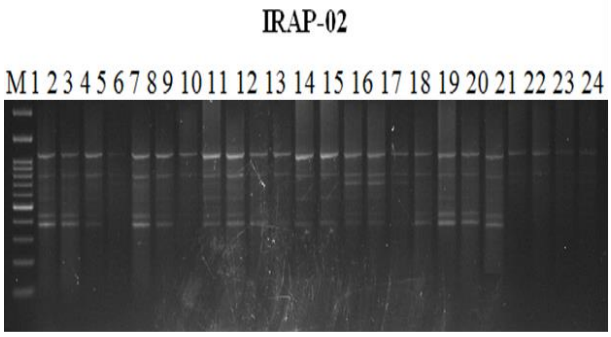

Figure 2. IRAP-2198

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

Table 3. The molecular sizes of different bands of wheat IRAP-2175 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-2175	550	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
	480	0	1	0	0	0	0	1	0	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0
	450	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	400	1	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
	348	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	242	0	1	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0	0	0	0	1	1	1	0
	200	1	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. The molecular sizes of different bands of wheat IRAP-2198 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-2198	1160	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	890	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0
	810	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	665	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	530	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
	470	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0

IRAP-2197 primer with all the genotypes gave one band at the molecular size 342 bp and it was absent only in the control of Masr-1. The same for the genotypes gave one band at the molecular size 254 bp and it was absent only in the control of Shandaweel-1 and control of Line-2. In case of the appearance of band at the molecular size 215 pb, it was absent at all the genotypes except the control of Shandaweel-1, Line-2 and Giza=171 as indicated in Figure (3) and Table (5).

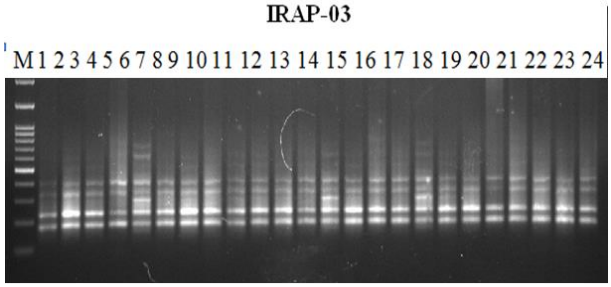

Figure 3. IRAP-2197

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

IRAP-2200 primer with all the genotypes gave one band at the molecular size 596 bp and it was absent upon first and second treatments only appeared at the third treatment of Masr-1 cultivar. Moreover, the same band appeared at the control and the second treatment of line-2 beside the first treatment of Giza-171 cultivar. The band at the molecular size 583 bp was absent only in the third treatment of Masr-1 cultivar, the control and the second treatment of Line-1, the first treatment of Giza-171. The band appeared at the molecular size 536 was detected at the second treatment of Masr-1 , Line-1 and Line-2 beside the third treatment of Line-1. The band at the molecular size 428 bp was detected at the second treatment of line-1 , the third treatment of line-2 and the first treatment of Giza-171. The band at the molecular size 207 bp was detected at the second and third treatment of Masr-1 as indicated in Figure (4) and Table (6).

IRAP-2202 primer with all the genotypes gave one band at the molecular size 332 bp and it was appeared upon third of Masr-1 and Line-1. Band at the molecular size 303 bp and it was appeared upon the three treatments of Shandaweel-1 and Giza-171 while it appeared only at the first and second treatment Line-1.third treatment of Line-2 and first treatment of Sakha-94 (Figue 5 and Table 7)

Table 5. The molecular sizes of different bands of wheat IRAP-2197 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-2197	639	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	555	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	342	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	254	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	215	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
	142	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	97	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

IRAP-04

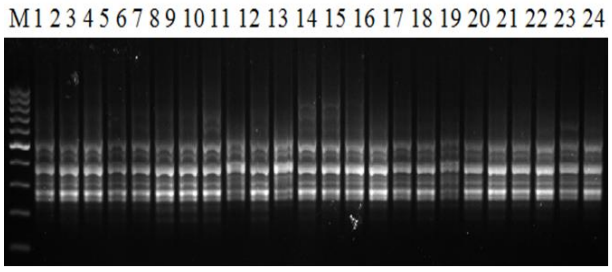

Figure 4. IRAP-2200

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

IRAP-05

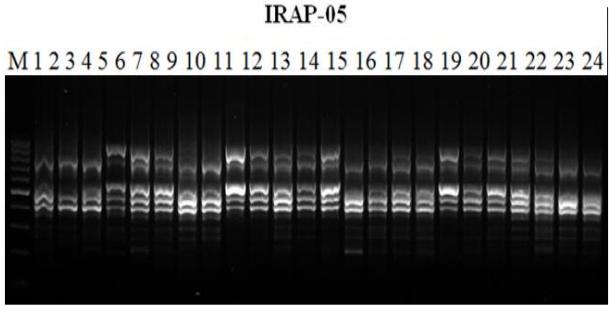

Figure 5. IRAP-2202

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

Table 6. The molecular sizes of different bands of wheat IRAP-2200 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-2200	596	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
	583	1	1	1	0	1	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1
	536	0	0	1	0	1	0	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0
	428	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0
	408	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
	321	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	254	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	220	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	207	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 7. The molecular sizes of different bands of wheat IRAP-2202 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-2202	332	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	303	0	0	0	0	1	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	0
	249	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	194	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

IRAP-2204 primer with all the genotypes gave one band at the molecular size 753 bp and it was appeared upon the control and the treatments of Masr-1 and the second and third treatment upon Shandaweel-1 and the second treatment of Line-2. Band at the molecular size 662 bp and it was appeared upon the first treatment of Shandaweel-1 and Line-1 beside third treatment in Line-1 and Line-2. All the treatments of Giza-171 showed the band. The band at the molecular size 575 bp appeared at the treatments of Nasr-1 only. Bands at the molecular size 523, 448, 315, 312 and 294 appeared in the majority of the treatments (Figue 6 and Table 8).

Concerning IRAP-4334 primer, it gave one band at the first treatment with all the genotypes gave one band at the molecular size 1321 bp as it was appeared upon the control and the first treatment of Masr-1, Line-1, Line-2 and Giza-171 and the second and third treatment upon Shandaweel-1 and the second treatment of Line-2 while it appeared at the third treatment of Giza-171 and Sakha-94. Band at the molecular size 1248 bp was appeared only upon the third treatment of Masr-1. The band at the molecular size 1128 bp appeared at the second treatment of Masr-1, first and third treatment of Line-2 and the second and third treatment of Giza-171 and only appeared in the first treatment of Sakha-94. Bands at the molecular size 933 bp was detected upon the third treatment of Masr-1, first and third treatments of Shandaweel-1 and Line-1, second and third treatments of Line-2, third treatment of Giza-171 and the first treatment of Sakha-94, respectively. 779, 700, 617, 543, 430 and 360 bp appeared in the majority of the treatments (Figue 7 and Table 9).

IRAP-06

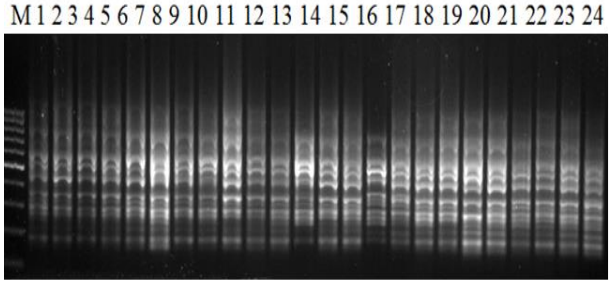

Figure 6. IRAP-2204

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

Table 8. The molecular sizes of different bands of wheat IRAP-2204 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-2204	753	1	1	1	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	662	1	0	0	0	1	1	0	0	1	1	0	1	1	0	0	1	1	1	1	1	1	0	0	0
	574	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	523	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	0
	448	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	415	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
	367	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	312	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	0	0	1	1
	294	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	222	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 9. The molecular sizes of different bands of wheat IRAP-4334 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-4334	1321	1	1	0	0	1	0	1	1	1	1	0	0	0	1	1	0	1	1	0	1	1	0	0	1
	1248	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1128	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1	1	0	0
	933	1	0	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	0	1	1	0	0	1
	779	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	1	1	1	0	1
	700	1	1	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	0	0
	617	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
	543	0	0	0	1	0	1	1	1	0	1	1	0	0	0	1	1	0	0	0	1	1	1	0	1
	468	0	0	0	1	0	1	0	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	1	1
	430	0	0	0	1	0	1	1	1	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	1
	360	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0
	251	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

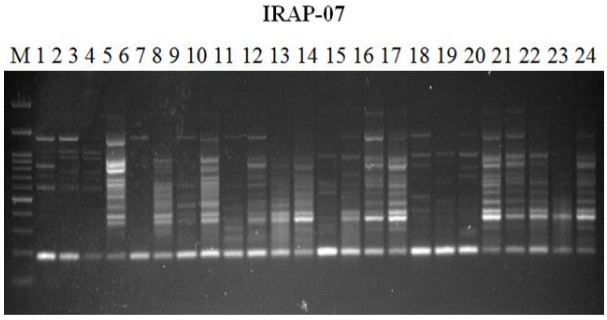

Figure 7. IRAP-4334

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

Concerning IRAP-4370 primer with all the genotypes under study, it showed one band at the molecular size 1311, 1192, 947 and 702 bp and it was appeared only upon third of Masr-1. Band at the molecular size 947 bp and it was appeared upon the third treatment of Giza-171. Band at the molecular size 545 bp appeared at the second and third treatments of Masr-1, first treatment of Shandaweel-1 and Line-1. Band at the molecular size 466 bp was detected upon the second and third treatment of Masr-1 and Line-2, the first and third treatment of Shandaweel-1 and third treatment of Giza-171 and all the treatments of Sakha-94 (Figue 8 and Table 10).

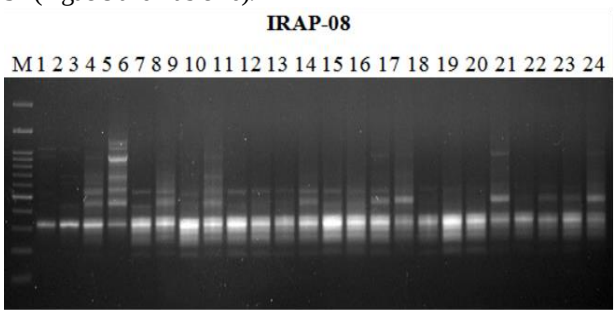

Figure 8. IRAP-4370

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

Concerning IRAP-4351 primer with all the genotypes under study, it showed one band at the molecular size 1293 bp at the third treatment of shandaweel-1, A new band appeared at the molecular size 1237 bp only upon second treatment of Line-2. Band at the molecular size 713 bp and it was appeared upon the third treatment of Masr-1 and Shandaweel-1 and Line-1 while it appeared for the second and third treatment of Line-2 and the third treatment of Giza-171 and Sakha-94. Band at the molecular size 618 bp appeared at the third treatments of Giza-171. Band at the molecular size 578 bp appeared at third treatment of Masr-1 and Line-2, second treatment of Shandaweel-1 and Line-1. Band at the molecular size 453 bp was detected upon the second and third treatment of Masr-1 and Line-2, all the treatments of Shandaweel-1 and first and third treatment of Line-1, third treatment of Giza-171 and first and third treatments of Sakha-94 (Figue 9 and Table 11). In case of band at the molecular size 435 bp was detected upon the first treatment of Masr-1 and third treatment of Line-2 and second and third treatment of Giza-171. Concerning of band at the molecular size 376 bp was detected upon the first treatment of Shandaweel , Line-1 and Giza-171 and second treatment of Sakha-94.

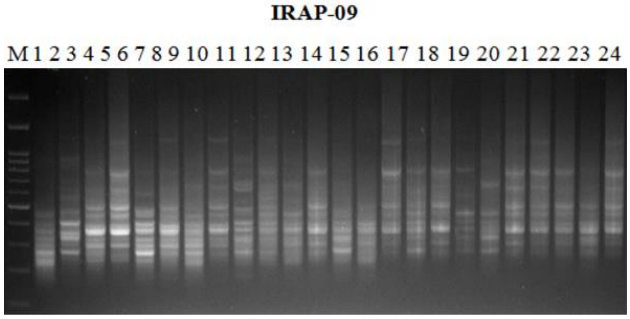

Figure 9. IRAP-4351

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res|ectively.

Table 10. The molecular sizes of different bands of wheat IRAP-4370 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-4370	1311	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1192	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	947	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	702	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	545	0	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	466	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	1	0	1	1	1
	401	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	361	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 11. The molecular sizes of different bands of wheat IRAP-4351 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
IRAP-4351	1293	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1237	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	713	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	1	1	1	0	1	1	1	0	1
	618	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	578	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	453	0	0	1	1	0	1	1	1	1	1	0	1	0	0	1	1	1	0	0	1	1	1	0	1
	435	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
	418	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	376	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0
	360	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
	339	1	1	0	0	1	1	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0
	325	1	0	1	1	1	1	1	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
	309	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0
	294	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Concerning IRAP-4340 primer with all the genotypes under study, it showed one band at the molecular size 819 bp at the third treatment of shandaweel-1 and Giza-171. A new band appeared at the molecular size 625 bp only upon second treatment of shandaweel-1. Band at the molecular size 593 bp and it was appeared upon the third treatment of Masr-1 and Shandaweel-1 and Line-1 while it appeared for the second and third treatment of Line-2 and the third treatment of Giza-171 and Sakha-94. Band at the molecular size 618 bp appeared at the third treatments of Giza-171. Band at the molecular size 578 bp appeared at third treatment of Masr-1 and Line-2, second treatment of Shandaweel-1 and Line-1. Band at the molecular size 453 bp was detected upon the second and third treatment of Masr-1 and Line-2, all the treatments of Shandaweel-1 and first and third treatment of Line-1, third treatment of Giza-171 and first and third treatments of Sakha-94 (Figue 10 and Table 12). In case of band at the molecular size 435 bp was detected upon the first treatment of Masr-1 and third treatment of Line-2 and second and third treatment of Giza-171. Concerning of band at the molecular size 376 bp was detected upon the first treatment of Shandaweel 1, Line-1 and Giza-171 and second treatment of Sakha-94.

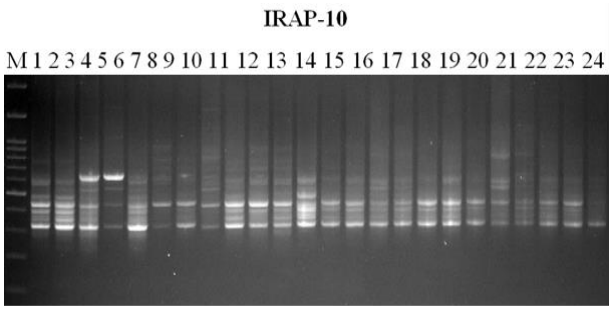

Figure 10. IRAP-4340

1=Msr1, 2=100, 3=150, 4=200, 5= Line1, 6=100, 7=150, 8=200, 9=Line2, 10=100, 11=150, 12=200, 13=Giza171, 14=100, 15=150, 16=200, 17=Shandaweel1, 18=100,19=150,20=200, 21=Sakha94, 22=100,23=150, 24=200, res[ectively.

Table 12. The molecular sizes of different bands of wheat IRAP-4340 primer

Primer	Bp	Masr-1				Shand-1				Line-1				Line-2				Giza-171				Sakha-94			
		C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C2	T1	T2	T3
IRAP-4340	819	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	625	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	593	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
	554	1	1	0	0	1	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0
	498	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	448	1	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	395	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0
	353	1	1	0	0	1	0	0	0	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	0
	310	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	1	1	1	1	0	0	0
	276	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1

From the previous IRAP results, Ten 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 IRAP-2175 for the genotype Sakha-94 at the molecular size 500 bp. Also, the genotype Line-1 at the molecular size 242 bp. Tn case of IRAP-2198, nearly all the studied genotypes at the molecular size 1160 bp. In addition to the genotype Shandaweel-1 at the molecular size 890 bp beside Line-2 and Giza=171, respectively. All the genotypes produced the same band at the molecular size 470 bp except Sakha-94. For the IRAP-2197, all the studied genotypes at the molecular size 142 and 97 bp obtain the same band and most of the genotypes at 342 and 254 bp. For the IRAP-2000, all the studied genotypes gave the same bands at the molecular size 321 and 254 and 220 bp, respectively. Concerning IRAP-2202 bp, all the studied genotypes produce the same bands at the molecular size 249 and

194 bp and most of the genotypes obtain the same band at the molecular size 303 bp. For the IRAP-2204, all the studied genotypes at the molecular size 367 bp and 222 bp gave the same band and most of the genotypes at 294 bp produce the same band. For the genotype Masr-1, the same bands appeared at the molecular size 753 bp and 574 bp, also the same band appeared at the molecular size 662 bp for the genotype Giza-171. Concerning the genotypes Shandaweel-1, Line-1 and Giza-171, the same band produced at the molecular size 523 bp for the control and treatments. Most of the genotypes obtain the same band at the molecular size 448 bp. The genotypes Line-1, Line-2 and Sakha-94 produce the same band at the molecular size 415 bp. Concerning IRAP-4334, all the studied genotypes gave the same band at the molecular size 251 bp. For the IRAP-4370, all the studied genotypes gave the same band at the molecular size 361 bp. In case of IRAP-4351, the genotypes Masr-1, Line-1, Line-2 and Sakha-94 gave the same band at the molecular size 360 bp. For the IRAP-4340, most of the studied genotypes gave the same bands at the molecular size 395 and 276 bp., that mean there is new retrotransposition due to these concentrations of salinity. While the other band was found in the control and absent from any treatments, as shown in Figure (1) and Table (3 to 12). The obtained results were in agreement with the results revealed by Shehata, Marwa et al., 2019.

Fig. 1. IRAP banding patterns for wheat (IRAP-1, IRAP-2, IRAP-3, IRAP-4, IRAP-5, IRAP-6, IRAP-7, IRAP-8, IRAP-9 and IRAP-10 primers) under the control (C), 100 mM (T1), 150 mM (T2) and 200 mM NaCl (T3) for wheat. The arrows refer to the different "polymorphic" bands. Cont, Fig. 2 IRAP and iPBS fingerprints of the six wheat genotypes tested using ten primers. Misr 1: Giza 171, Line1, Line2, shandaweel 1 and Sakha 94 genotypes.

CONCLUSION

This study revealed that the possible use of saline water (NaCl-simulated salinity of soil) for irrigating different varieties of bread wheat. This study explain how to use molecular markers retrotransposon to differentiate between the tolerant and sensitive wheat genotypes to salt stress. The results revealed that Masr-1, Shandaweel-1, Line-1 and Sakha-94 genotypes of wheat can be selected to grow under salt stress conditions. In contrast, Line2 and Giza 171 were the most sensitive genotypes. Retrotransposon-based analysis (IRAP) tagged each genotype successfully with specific unique bands and detected molecular genetic markers related to salt tolerance in wheat crops. We recommend using Masr-1, Shandaweel-1 and Sakha94 tolerant genotypes in areas suffering from salt stress.

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دراسات على دور الاجهاد الملحى على تنشيط معدل ل retrotransposition فى بعض أصناف قمح الخبز

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الملخص

تشتمل Retrotransposons على الجزء الرئيسي من الجينومات حقيقيات النواة. تتكون عملية إعادة التحول من ثلاثة أنواع ، نسخ العنصر في الحمض النووي الريبوزى ، ثم النسخ العكسي إلى [سى دنا] متبوعًا بإعادة إدخال العنصر المنسق في موقع جيني جديد. أثناء التطور الطبيعي ، تم تنشيط هذه العناصر من خلال الضغوط مثل الملوحة بينما عادة ما تكون هادئة أثناء التطور. يمكن أن يحدث نشاط العناصر القابلة للتنشيط من خلال إجهاد الملوحة وخاصة تكرار الطرفية الطويلة Retrotransposons (LTR) ، والتي تتميز بمستوى عالٍ من التباين في تسلسل LTR المشاركة في النسخ ، وتطورت باكتساب أنماط تعبير جديدة في الغالب مع الاستجابات محفزات التوتر المتنوعة. غالبية LTR Retrotransposons تنتج حمامات أكبر من النصوص استجابة للإجهاد. Nawaday تم اكتشاف أن التنشيط اللاجيني لهذه Retrotransposons يغير التعبير عن الجينات المجاورة. تولد الإدراج الجديد في مناطق الترميز أو بجانبها طفرات يمكن أن تؤدي إلى تغييرات في التعبير الجيني وإعادة تشكيل الجينوم ، من الناحية الهيكلية والوظيفية. وبالتالي ، يمكن أن يلعب تنشيط العناصر القابلة للتنقل LTR دورًا أساسيًا في تطور النبات والتطور. تم اختبار توافر التقنيات القائمة على PCR للكشف عن التباين في معدل إعادة النقل بسبب الملوحة. تم تطبيق واسمات ال IRAP في ستة تراكيب وراثية من القمح المتحملة للملوحة (L Triticum aestivum.). جينومات التراكيب الوراثية القمح الستة ؛ Line-1 ، Shandaweel-1 ، MASR-1. تم استخراج السلالة 2 و Giza-171 و Sakha-94. تم تطبيق عشرة من البادئات IRAP. تم رى التراكيب الوراثية للقمح بـ 100 مم و 150 مم و 200 مم من كلوريد الصوديوم أو الماء المقطر فقط للتحكم. طورت تقنية IRAP جميع البادئات العشرة في القمح تحت مستويات مختلفة من الملوحة. أظهر Primer IRAP-2175 عددًا من الحزم الجديدة ذات الأوزان الجزيئية تراوحت بين 550 حجم جزيئى إلى 400 حجم جزيئى مما يعطي مواقع إعادة نقل جديدة بسبب العلاج بتركيزات مختلفة من الملوحة. كشف التمهيدي الثاني (IRAP-2198) أن الحزم ذات الأحجام الجزيئية تراوحت من 1160 حجم جزيئى إلى 470 حجم جزيئى حصلت على مظهر جديد لإعادة التحول. تم الكشف عن علامة IRAP الثالثة من قبل IRAP-2197 التمهيدي بأحجام جزيئية من 342 حجم جزيئى إلى 215 حجم جزيئى تحت جميع المستويات الثلاثة من الملوحة. بينما ، أكدت هذه الدراسة أن تقنيات PCR ؛ مثل IRAP يمكن أن يظهر تنشيط إعادة النقل بسبب مستويات الملوحة المرتفعة. تم الحصول على نتائج إيجابية جيدة ونوصي باستخدام هذه التقنيات لأغراض جزيئية مختلفة بسبب ميزتها ؛ سهلة وسريعة ورخيصة وفعالة.