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Determination of Genetic Diversity in Selected Salt Tolerance and Sensitive Wheat by Random Amplified Polymorphic cDNA

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Abstract

The aim of this study was to investigate the genetic diversity and markers associated with salinity tolerance in three genotypes of wheat created for salt tolerance by plant breeding program, as well as two Iraqi varieties using random amplified polymorphic cDNA (RAPD-PCR) with eight primers were used. The results of RAPD marker revealed that there are genetic variations in several particular segments of various sizes between the selected genotypes and the local varieties with more genetic variation except for (OPG-09) did not appear any band with the selected genotypes and local cultivars . The results of the phylogenetic tree analysis (cluster) based on the presence or absence of DNA amplified for each primer were used to classify similar genotypes into two main groups (1H, Iraq) genotypes were clustered in first group with a genetic distance of 85% and (2H, Ibaa99) genotypes clustered in second group with a genetic distance of 84%.The results of genetic similarity based on RAPD data shows the relationship between the selected genotypes and local cultivars, the highest genetic distance was 0.428571 between the selected genotypes (1H and 2H) and the local cultivar of (I), and the lowest value was 0.066667 between the selected genotype (1H and 3H). The results confirmed the efficiency of RAPD based cDNA technique of those genotypes that are studied for determination of genetic diversity and distance among them.

Keywords: *Genetic diversity, Salt tolerance, Wheat , Genotypes, RAPD*.

تحديد التنوع الوراثي في عدة تراكيب من الحنطة المنتخبة لتحمل الملوحة بإستعمال تقانة التعددية الشكلية لقِطَع الحمض النووي المُكاثرة عشوائياً (RAPD-PCR)

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الخلاصه

إن الهدف من هذه الدراسة هو التحقق من التنوع الجيني والمؤشرات المرتبطة بتحمل الملوحة في ثلاثة تراكيب وراثية من الحنطة تم استنباطها لتحمل الإجهاد الملحي بواسطة برنامج تربية النبات (التهجين)، بالإضافة إلى صنفين عراقيين باستعمال تقانة (cDNA) المعتمدة على واسم التعددية الشكلية لقِطَع الـ DNA المُكاثرة عشوائياً (RAPD–PCR) مع ثماني بادئات. أظهرت نتائج واسم RAPD أن هناك تباينات جينية في عدة قطع معينة مختلفة الأحجام من الـDNA بين التراكيب الوراثية المنتخبة والأصناف ذات التباين الوراثي الأكبر باستثناء البادئ (OPG–09) الذي لم يظهر أي نطاق مع التراكيب الوراثية المنتخبة والأصناف. تم استخدام نتائج تحليل شجرة النشوء والتطور (التحليل العنقودي) على أساس وجود أو عدم وجود قطع الحمض النووي المضخم لكل مجموعة أولية لتصنيف التراكيب الوراثية المتشابهة إلى مجموعتين رئيسيتين (1H, 2H, Ibaa99) والمجموعة الثانية (Iraqالتي تم وضعها في المجموعة الأولى بمسافة وراثية تبلغ 85٪ بمسافة وراثية 84٪، وأظهرت نتائج التشابه الجيني المبني على بيانات RAPD العلاقة بين التراكيب الوراثية المنتخبة والأصناف المحلية، وكانت أعلى مسافة وراثية 0.428571 بين التراكيب المنتخبة (1H و 2H) والصنف المحلي (I)، وكانت أقل قيمة 0.066667 هي بين التراكيب الوراثية المنتخبة (1H و 3H). أكدت النتائج كفاءة تقنية (cDNA) المعتمدة على الواسم الجزيئي RAPD لتلك التراكيب الوراثية التي تمت دراستها لتحديد التنوع الجيني والمسافة بينها.

1. INTRODUCTION

Soil salinity and irrigation severely restrict the production of crops, including wheat, in Iraq and the world, and a yield reduction of 88% has been reported in bread wheat under high irrigation salinity [1].

Molecular markers play a critical role in improving the efficiency of classical breeding programs, including selection, detecting genetic variations, in accelerating the transfer and accumulation of desirable traits in the genotypes, thus increasing agricultural production, breeding for stress tolerance has proven difficult, but there are greater chances of selecting and developing superior species when there is a lot of genetic diversity [2]. The discovery of differentially expressed salt tolerance genes in wheat was accomplished using various techniques, closed systems, such as microarrays and RT-PCR, have traditionally been used extensively in plant gene expression research [3, 4]. cDNA-AFLP (cDNA-Amplified fragment length polymorphism) has been effectively utilized to measure transcript abundance and produce expression data in wheat with no requirement for prior knowledge of the organism's genome or transcriptome in open systems [5]. Other researchers [6] developed the cDNA-RAPD PCR technique to find differentially expressed genes and genetic diversity in wheat under salinity condition [7, 8], barley [9] and in soybean [10]. The adoption of the RAPD approach has shown to be an effective tool for plant breeders in improving salt tolerance in plants [11].

In an experiment conducted by [12] to estimate the genetical variation of three salt-resistant genotypes, SARC-1, SARC-5 and S-24, exposed to a saline stress, tested 101 and 50 primers,52 of them showed differences between SARC-1 and SARC-5, and 61 showed differences between SARC-5 and S-24. S-24. (SARC-5, MH-97, and S-24) showed diversity in polymorphism reached 53%, 64% and 42%, respectively.

Using RAPD approach, DNA polymorphisms were observed by using 240 primers, GLE-14 primer amplified a 970 bp polymorphic DNA fragment, and this can be used in breeding programs of wheat crop for salt tolerant [13].

The aim of this study is to determine the genetic diversity and markers associated with salinity tolerance in selected salt tolerance and sensitive wheat by Random Amplified Polymorphic cDNA techniques.

2. MATERIALS AND METHODS

2.1 Plant material

This experiment was carried out during November 2020 at plastic house in Biotechnology Research Center /Al- Nahrain University in Baghdad (Iraq). Wheat seeds were planted (three salt-tolerant:1H, 2H, 3H and two Iraqi cultivars: I= Ibaa99 and A= Iraq) in prepared saline soil reached 16 dS m^{-1} with five seeds in each plastic pot (Capacity 10 kg of saline soil) for each genotype and cultivar, and irrigated with normal water. After 30 days of salt application, leaves samples of wheat plants were collected to evaluate the level of plant salt tolerance.

2.2 RNA isolation and cDNA synthesis

Total RNA was isolated from the plant leaves using mini kit Plant depending on the manufacturer's instructions (Taiwan), and it was treated with RNase-free DNase I (Canada) for 30 min at 37°C, DNase I was inactivated at 65°C for 10 min. RNA quality was evaluated by using gel electrophoresis: (1% agarose gel containing 0.5% (v/v) ethidium bromide),. First-strand cDNA was synthesized from 500 ng of total RNA using AccuPower® RocketScriptTM RT PreMix Kit (Bioneer, Korea) with an oligo-dT15 primer. The reaction solution was used as templates for reverse transcription polymerase chain reaction (RT-PCR) [7, 14].

2.3 cDNA-RAPD

Eight primers (table-1) were used for amplification of cDNA. The primers were synthesized by (Bioneer-Korea) in lyophilized form and dissolved in sterile distilled water to obtain a final concentration of (10pmol/ml).

For hot-start PCR using cDNA template on Labnet Thermocycler (USA). PCR reaction was performed according to: 40 amplification cycles (95°C for 1 min, 43.7°C for 1 min and 72°C for 1 min). Analysis of PCR products by 1% agarose gel electrophoresis, then were visualized by staining with ethidium bromide. The comparison was made for the generated bands, then registered the differential amplified bands.

Table 1: Names and sequences of RAPD primers

Primer name	Primer sequence
OPB-01	5'-GTTTCGCTCC-3'
OPB-06	5'-TGCTCTGCCC-3'
OPC-08	5'-TGGACCGGTG-3'
OPE-13	5'-CCCGATTCGG-3'
OPE-16	5'-GGTGACTGTG-3'
OPF-20	5'-GGTCTAGAGG-3'
OPG-09	5'-CTGACGTCAC-3'
OPH-01	5'-GGTCGGAGAA-3'

Amplification of cDNA was performed with the master amplification reaction shown in table (2).

Table 2: The master amplification reaction

Materials	Final concentration	Volume for 1 tube
PCR pre mix	1x	5µI
DNA template	100ng	2µI
Primer	(10pmol/ µl) 10pmol /µl	2µI
Deionized D.W	-	11µI
-	-	Final Volume= 20µI

2.4 Estimation of genetic distance

Data generated from the detection of polymorphic fragments were analyzed. The amplification bands were compared and scored as 1 for presence and 0 for the same absence. Genetic distance estimates were calculated between the selected genotype and local cultivar according to [15]. The cluster analysis was performed to construct a genetic relationship tree using UPGMA method depending on Jaccard's similarity coefficients with SAHN system in NTSYSpc Version 2.2 [16]. The percentage of polymorphic bands was defined as ratio of the number of polymorphic bands amplified.

3. Results and Discussion

3.1 Genetic Properties for genotypes

In order to amplify all of the genotypes and cultivars by using RAPD approach, eight primers were utilized. The capacity of each primer to detect genotype and cultivar variations differed considerably. The results of employing eight primers (OPB-01, OPB-06, OPC-08, OPE-13, OPE-16, OPF-20, OPG-09, OPH-01) respectively to amplify three genotypes (1H, 2H, 3H) and (Ibaa99, Iraq) cultivars. The results in Figure 1 indicated the variations in band patterns between selected salt tolerance genotype (3H) and local cultivars of Ibaa99 (sensitive), as their bands appeared in five primers (OPB-01, OPB-06, OPE-16, OPF-20, OPH-01), as no bands showed in the others primers for the genotype 3H. Figure 1-A), with regard to the cultivar Ibaa99 (Figure 1-B), their bands appeared just in five primers (OPB-01, OPE-16, OPF-20, OPH-01).

In addition, the data revealed that the primers differ in their bands in patterns that emerged in these genotypes, with the genotype H3, the eight primers differed in various sizes, and there were also differences in the bands size of the genotypes (Figure 1-A: H3). With the genotype H3, the eight primers differed in various sizes, and there were also differences in the bands size of the genotypes using OPB-01 primers, they varied in two bands, one about 1000 bp and the other about 500 bp, this did not see in the sensitive cultivar (Iraq) (Figure 1-B: Ibaa99), and there are no bands in the Ibaa99 cultivar when using OPB-06, OPC-08, OPE-13, OPF-20, OPG-09 primers.

Regarding the wheat cultivar (Iraq), the results of the gel electrophoresis in (Figure 2) showed the presence of three bands very clear in the primer (OPB-01) graded from 100- 500, then 1000 bp respectively, and two bands shown by the primers (OPE-16 and OPF-20), while the other primers did not show any bands.

The results in (Figure 3) showed that the eight RAPD primers detected specific band patterns, with all primers except the OPC-08 and POG-09 primers. The selected genotype (1H, Figure 3-A) differed with local cultivars only in several bands (2-4) with (100-1000 bp) in OPB-01 and it reaches (1500 bp) in the (OPB-06, OPE-13, OPF-20) primers the other bands were similar in selected genotypes and cultivars, but the genotype 2H (Figure 3-B) differed in one band with OPB-01 primer (size 100bp) and other with OPH-01 bp primer (200 bp), while with OPG-09 primer there are no bands as in selected genotypes and local cultivars, therefore all primers gave specific bands pattern, these results are in agreement with what was found by Majeed, *et al.*, (2018) [7].

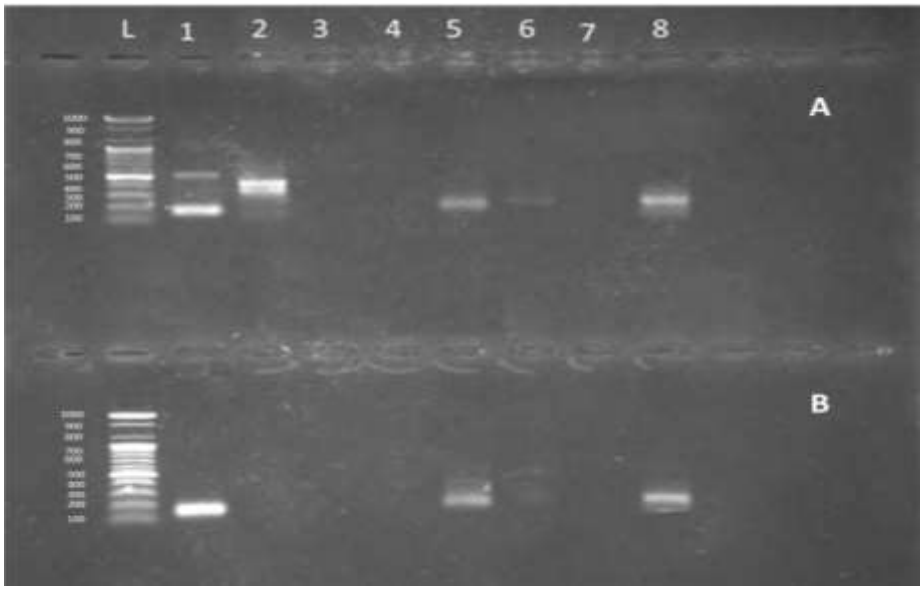

Figure 1: Agarose (1%) gel electrophoresis of PCR products generated by RAPD-PCR primers (OPB-01, OPB-06, OPC-08, OPE-13, OPE-16, OPF-20, OPG-09, OPH-01) respectively for wheat genotypes by 1% agarose gel (1X TBE, 75 v/cm, 1.15 hr) and visualized under UV light after staining with ethidium bromide 0.5µl. Lane :L= DNA ladder; 3H ; Ibaa99).

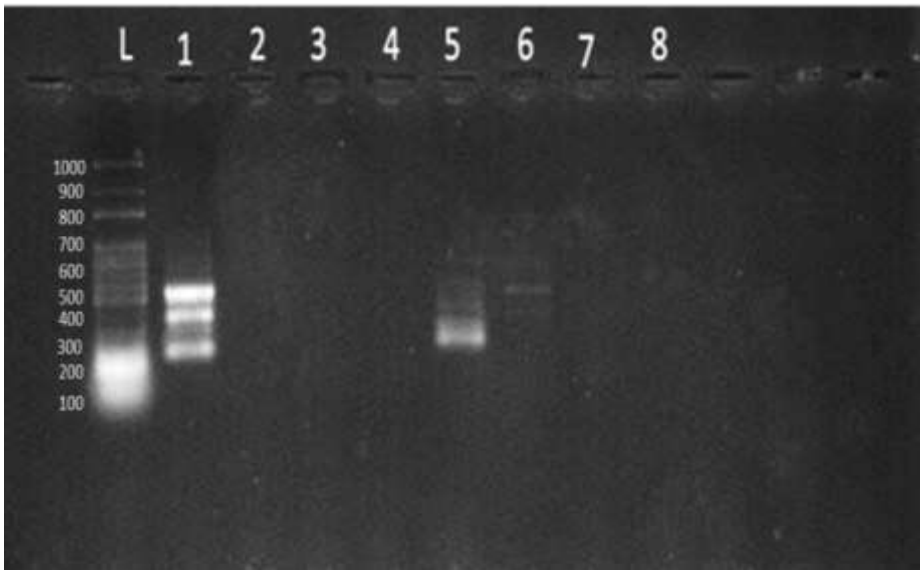

Figure 2: Agarose (1%) gel electrophoresis of PCR products generated by RAPD-PCR primers (OPB-01, OPB-06, OPC-08, OPE-13, OPE-16, OPF-20, OPG-09, OPH-01)

respectively for wheat cultivar (Iraq) by 1% agarose gel (1X TBE, 75 v/cm, 1.15 hr) and visualized under UV light after staining with ethidium bromide 0.5µl. Lanes:L= DNA ladder.

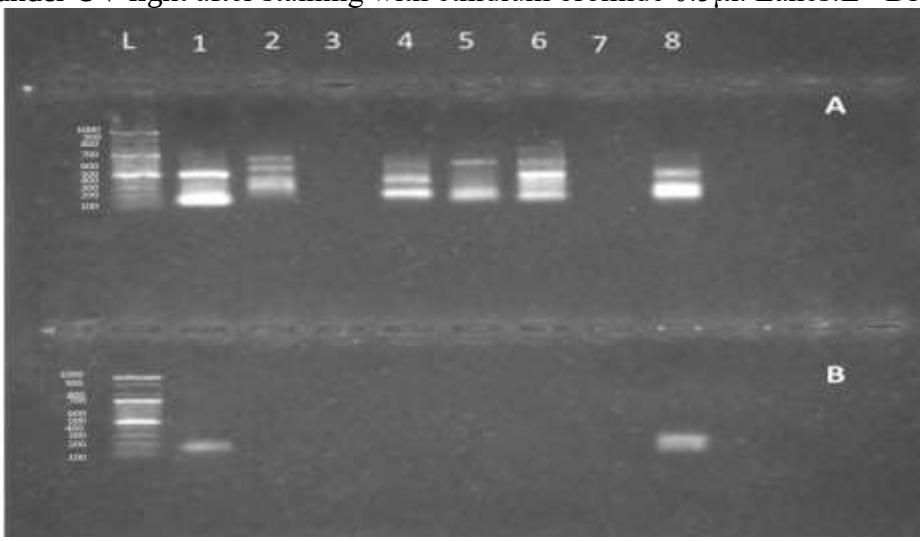

Figure 3: Agarose (1%) gel electrophoresis of PCR products generated by RAPD-PCR primers (OPB-01, OPB-06, OPC-08, OPE-13, OPE-16, OPF-20, OPG-09, OPH-01) respectively for wheat genotypes by 1% agarose gel (1X TBE, 75 v/cm, 1.15 hr) and visualized under UV light after staining with ethidium bromide 0.5µl. Lane:L= DNA ladder; 1H; 2H).

3.2 Genetic distance

The results of (Figure 4) illustrate the RAPD's phylogenetic analysis and the evolutionary tree and genetic relationships between the five wheat genotypes in the form of an evolutionary figure drawn based on RAPD data to determine the nature of genetic relationships. The results of the genetic similarity were used to isolate the genetically similar genotypes in the (cluster) groups, according to the Jaccard coefficient.

The dendrogram constructed based on similarity and distance matrix showed that the genotypes and cultivars of wheat studied could be divided into two main clusters, the first of which consisted of two minor clusters, the first group included the cultivar I and genotype 1H with a genetic distance of 85%, while the second group included the cultivar (A= Ibaa99) and genotype 2H with a genetic distance of 85%.

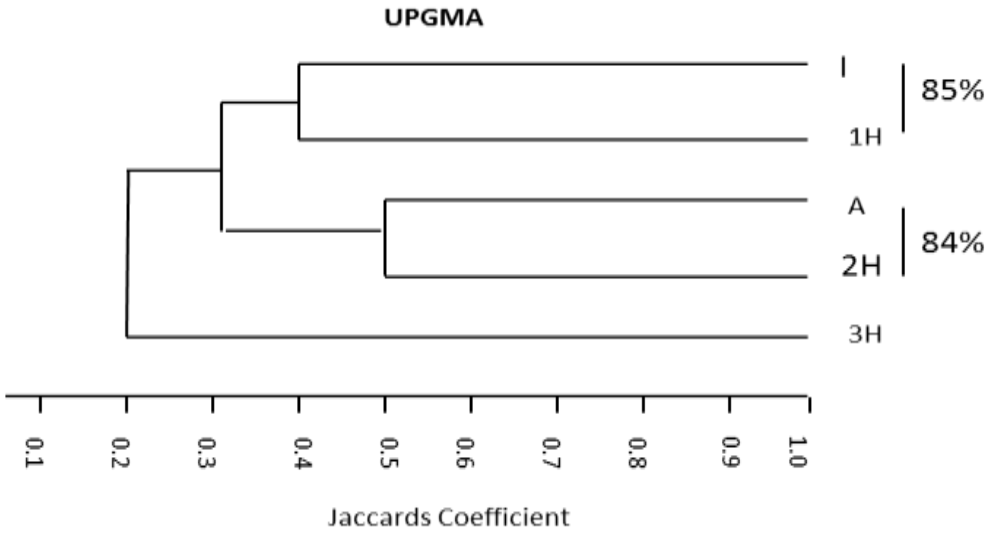

Figure 4: RAPD's phylogenetic analysis based on Jaccard's similarity coefficients using UPGMA between the three genotypes (1H, 2H, 3H) and (Ibaa99, Iraq) cultivars

The results based on RAPD data reflected (Table 3) showed the presence of genetic distance between the genotypes and local cultivars under a high level of salinity, the highest genetic distance was 0.428571 between the selected genotypes (1H and 2H), and 0.411765 between the selected genotype of (1H) and the local cultivar of (I), this indicated that there is big genetic variation in their salt tolerance under a high level of salinity. On the other hand, the lowest value was 0.066667 between the selected genotype (1H and 3H). Also, the values of genetic distance indicated that there are distances between the local cultivars and also among the selected genotypes.

Table 3: Genetic similarity matrices computed according to Dice coefficient based on RAPD data shows the relationship between the selected genotypes for salt tolerance and local cultivars

	3H	I	A	1H	2H
3H	0	-	-	-	-
I	0.238095	0	-	-	-
A	0.090909	0.176471	0	-	-
1H	0.066667	0.411765	0.25	0	-
2H	0.090909	0.176471	0.5	0.428571	0

The results of RAPD marker amplified to identify the genetic variation between the salt tolerant genotypes and sensitive cultivars by using 8 primers (Figures 1,2,3) showed that there are differences in specific bands with all primers which used except one (OPG-09) did not appear any band with the selected genotypes and local cultivars. At each primer, the selected genotypes divided into two or three bands with the local cultivars under high salinity condition. These bands showed high variation between the selected genotypes and local cultivars, this variation maybe concern with the differences between them in their salt tolerance, because in the previous studies these selected genotypes considered as high tolerance to salinity, while the local cultivars were sensitive to salinity [4, 5].

4. CONCLUSION

The conclusion of this study is the selected genotypes (1H, 2H and 3H) were improved for salt tolerance through plant breeding programs and could be beneficial as genetic resources for future breeding programs. The same determination of genetic variation in wheat genotypes and cultivars were reported by BiBi *et al*. (2012) [17] by using RAPD marker.

Salt tolerance in plants, according to [18, 19, 20], is more closely linked to the mechanisms of salinity stress tolerance which controlled by segregated salt tolerant genes through cycles of exposure and selection. These genes may be non-existent in the local salt-sensitive cultivars. The results confirmed the efficiency of RAPD based cDNA technique of those genotypes that are studied for determination of genetic diversity and distance among them, in addition, most of the studied primers associated with salinity tolerance in genotypes.

Ethics Approval and Consent to Participate

Only plant samples were used to carried out this research. No humans and/or animals participated in it.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding this work.

Availability of Data and Material

All data and materials mentioned in the manuscript, containg all relevant raw data, it will be freely available to any researcher who uses it for non-commercial purposes.

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