Evaluation of Random Amplified Polymorphic DNA as a Genetic Indicator of Salt Tolerance in Iraqi Wheat

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Abstract:-

Random amplified polymorphic DNA (RAPD) was utilized in order to generate information on polymorphism, genetic relatedness and diversity in two local salt tolerant wheat varieties Dijlah and Furat collected from different locations in Iraq(Latyfia, Twaitha and Hasoa) compared to three local non-salt tolerant varieties Tahdi, Tamose2 and Nakhoa for breeding purposes. Out of the 23 random decamer primers used, 15 primers produced monomorphic and polymorphic amplification patterns. Primers A11 and N16b showed the highest efficiency of 0.069, primer A11also showed the highest discriminatory power of 24.3%. Data obtained were used find the genetic distance and construct the dendogram. There was 26% polymorphism between the studied cultivars. In addition to that we were able to fingerprint seven cultivars.

Key words: RAPD-PCR, genetic diversity, dendogram, wheat, salt-tolerance.

Introduction:-

Agricultural productivity in arid semi-arid regions of the world is very low this is due to the fact that crops in these areas are naturally subjected to a multitude of abiotic stress such as soil salinity. The genetic improvements of salt tolerance in genotypes of crops such as wheat have been suggested to allow exploitation of salt affected soils. Molecular studies are a fundamental aspect in plant breeding and breeding programs depend on the availability of genetic variance to develop salt tolerant varieties and to increase productivity [1]. In the past few years, plant breeders have made significant achievements improving salinity tolerance in a number of agriculturally important crops through artificial selection and conventional breeding techniques [2]. Salt tolerance in plants represent genetic, environmental effects and integration of physiological mechanisms combined, it depends on the genetic variation in Ma^+ accumulation, K^+/Ma^+ discrimination and osmoregulation [3].

productivity, increased salt tolerant crops and horticultural species is needed to maintain the growing food demand in the world as natural or primary salinity is more widespread than it was first realized and secondary salinity resulting from irrigation continues on growing [9]. The objective of the present study is to generate useful information on polymorphism, genetic relatedness and diversity in two local salt tolerant wheat varieties collected from different locations in Iraq and compare it to three local non-salt tolerant varieties for breeding purposes.

Materials and Methods

Plant material and DNA extraction:-

Latyfia, Twaitha and Hasoa south and south-east suburbs of Baghdad/ Iraq known for their natural salinity were selected to grow two salt tolerant genotypes namely Dijlah and Furat (these two cultivars were induced through improvement and breeding programs and were registered by the National Committee for Registration and Release of Agricultural Varieties/ Ministry of Agriculture/Iraq). Fields within the locations were divided into 5x5m squares; soil salinity of each square was tested before planting and at each growth stage. NP 27:27 (200kg/h) was added to the prepared soil and seeds were sown in lines with 0.25m distance. Plants were irrigated with drainage water except the first irrigation. Urea was added to the plants in two batches, after 45 of planting and 90 days with 80kg/h (in the beginning of the elongation stage). At the end of the maturity stage, plants were harvested and spikes from two local salt tolerant wheat varieties collected from different locations including Dijlah and Furat. Dijlah was collected from locations Latifia 1, Latifia 2 and Twaitha and were given the names L1D, L2D and TD respectively. Furat was collected from 4 different locations these are Latifia1, Latifia 2, Twaitha and Hasoa and were given the names L1F, L2F, TF, and HF. Three local non-salt tolerant varieties were also included; Tahdi, Tamose2 and Nakhoa given names are L○Th, L○Tm and L○N respectively. Grains from ten different samples were grinded in a cold mortar and a pestle, 0.5gm for each sample was weighed and used to extract DNA. Two different protocols were used for DNA extraction in addition to High Pure GMO DNA sample preparation kit from Roche. Purity and concentration of DNA was measured at 260nm in a spectrophotometer and integrity was checked on 0.7% agarose gel prepared in 0.5X Tris borate EDTA buffer from Promega [10]. Only DNA samples extracted using the kit were further used in RAPD-PCR since purity, concentration and integrity were best produced by the kit. Purity ranged between 1.2 and 1.4 while concentration ranged between 0.86 to 1.5µg/µl.

RAPD-PCR reactions:-

A dilution series of 25ng/µl for each sample was prepared and used in RAPD-PCR reactions consisting of 1X GoTaq Green Master Mix from Promega, MgCl2 was added to a final concentration of 2.5mM and DNA concentration of 75ng/25µl and 10pmols of each primer was used. Primers were provided by Operon Technologies except for P6b, P7b and N16b

which were synthesized by Alpha DNA. Amplification reactions were performed in a thermal cycler (Eppendorff Master Cycler/Personal), PCR profile was 94°C for 5 min, 45 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension at 72°C for 10 min[4].

Elution Experiment:-

DNA bands were recovered from low melting temperature agarose by organic extraction according to the protocol in Sambrook *et al*. (2001) [10].

Analysis of RAPD profiles:-

PCR amplification products were separated on 1.2% agarose gel in 0.5X Tris borate EDTA buffer and visualized by staining with 0.5µg/ml ethidium bromide. A gel documentation system was used to store gel images on computer and Photo-CaptMwt version 10.01 computer software was used to estimate molecular weights of amplified fragments. Amplification profiles of 10 individuals were compared with each other; bands were scored from the top of the lanes and ordered in tables as present 1 or absent 0. Genetic similarity was estimated as the number of shared amplification products using the formula: No. of shared amplification products= 2 x (No. of common bands between any two lanes) / (Total No. of bands in the same two lanes) [11]. Genetic relationship among the studied individuals was verified with the dendogram through NT-SYS PC software.

Results and Discussion:-

At each station Latyfia, Twaitha and Hasoa, salt tolerant cultivars Dijlah and Furat showed significant production in comparison to local cultivars which are non-salt tolerant. Salt tolerant cultivars had more than twice to fourth the seed yield/m^2 obtained from the local cultivars at both stations latyfia and Twaitha due to large differences in number of spike/ m^2, number of seed/spike and 1000 grain weight. At high salinity levels, salt tolerant cultivars showed higher number of plants/ m^2 as determined by the percentage of germination. These results are in agreement with results reported by Al-Mishhdany and Mohamed (1999). Number of tillers/ plant was also higher in Dijlah and Furat, there appears to be some stimulation by salinity in this character [12] and so a significant positive correlation between yield and number of tillers/plant and number of plant/m^2 especially at higher levels of salinity was present.

The study was aimed at finding the genetic polymorphisms between salt-tolerant and non salt-tolerant cultivars i.e. finding diagnostic markers for Dijlah and Furat in the presence of local varieties for purposes of selection and breeding programs at one level and to detect if genetic variations exist in Dijlah and Furat varieties according to different locations.

RAPD is a sensitive technique i.e. all factors involved in RAPD-PCR may affect the reproducibility of results including reaction chemicals, instruments used and programming conditions, thus adherence to standardized protocols was followed and careful optimization of $MgCl_2$ and template concentrations was necessary as faint irreproducible banding patterns resulted due to qualitative inhibitors naturally occurring in plant DNA extracts. 2.5mM concentration of $MgCl_2$ produced the best results; Williams *et al.* (1990), (1993) reported that a change in $MgCl_2$ concentration can result in the alteration of RAPD banding patterns [4, 13]. Iqbal *et al*. (2007) found that 3mM $MgCl_2$ produced optimum amplifications in the assessment of genetic diversity in wheat [1]. Serial dilutions of DNA samples of 25ng/µl produced better results than 50ng/µl. All reactions were run with a negative control.

A total of 23 decamer primers were used for the characterization of 10 wheat samples. 8 primers did not produce amplification patterns A16, A18, C19, E2, F13, N19, R2, and R3 while 15 primers produced monomorphic and polymorphic banding patterns those are listed in table 1. Total number of fragments generated by the 15 primers was 1281, 37 of which were polymorphic fragments. The highest molecular weight 3.279Kb was produced by primer A13 while the lowest was produced by primer N16b of 0.318Kb. Primer efficiency ranged between 0.008 to 0.069 with primers A11 and N16b showing the highest efficiency. Discriminatory power ranged between 2.7 and 24.3% with primer A11 having the highest discriminatory power. There was 26% polymorphism in the wheat cultivars.

Table 1: Primers used in the study, their sequences, amplification products, primer efficiency and discriminatory power

No	Primer	Sequence 5¯-3¯	Highest Mwt Kb	Lowest Mwt Kb	Total no. of bands	Monomrphic or Polymorphic (no. of polymorphic bands)	Primer Efficiency	Primer Discriminatory power %
1	A2	TGC CGA GCT G	1.525	0.500	69	P(1)	0.015	2.7
2	A3	AGT CAG CCA C	1.257	0.390	78	P(2)	0.026	5.4
3	A7	GAA ACG GGT G	2.471	0.500	94	P(4)	0.043	10.8
4	A8	GTG ACG TAG G	1.517	0.413	64	P(3)	0.047	8.10
5	A11	CAA TCG CCG T	2.157	0.500	131	P(9)	0.069	24.3
6	A12	TCG GCG ATA G	1.479	0.690	43	P(1)	0.023	2.7
7	A13	CAG CAC CCA C	3.279	0.500	100	M	0	0
8	B5	TGC GCC CTT C	1.350	1.350	10	M	0	0
9	B10	CTG CTG GGA C	1.275	0.368	90	M	0	0
10	C5	GAT GAC CGC C	2.382	0.441	129	P(1)	0.008	2.7
11	D20	ACC CGG TCA C	2.139	0.373	141	P(8)	0.057	21.62
12	H4	GGA AGT CGC C	1.250	0.370	70	M	0	0
13	P6b	TCG GCG GTT C	2.149	0.801	60	M	0	0
14	P7b	CTG CAT CGT G	2.000	0.500	101	P(1)	0.009	2.7
15	N16b	CAA GGT GGG T	2.179	0.318	101	P(7)	0.069	18.9

Shared or common bands indicate the annealing of primers to similar DNA regions in the different varieties studied that might be conserved regions in wheat DNA, identification of such fragments is beneficial to detect dominant sequences in the wheat genome in order to design species specific markers [14]. Bands that appear common in two or more varieties may also prove important as they can be used by linking it to traits that are shared by these varieties such as a specific phenotype, resistance to pathogens or environmental stress [15]. The data obtained from this study was analyzed to find the genetic distance and construct the dendogram, genetic distance between different cultivars of wheat was estimated based on the shared amplification products. Values of genetic distances range was 0.01913-0.07807 (Table 2). The lowest value appeared between L1F and L2F indicating the high genetic similarity between these cultivars, both cultivars are of Furat variety in this case no evidence of genetic variation according to location is suggestive. The highest value was shown between TD and L◦Th an indicative of high polymorphism between Dijlah and the local non salt tolerant variety Tahdy.

Table 2: Similarity matrix of ten wheat cultivars obtained from RAPD markers

	L1D	L2D	TD	L1F	L2F	TF	HF	L◦Th	L◦Tm	L◦N
L1D	0.00000									
L2D	0.02407	0.00000								
TD	0.06712	0.07506.	0.00000							
L1F	0.03905	0.03099	0.07239	0.00000						
L2F	0.01961	0.02755	0.06098	0.01913	0.00000					
TF	0.05713	0.04840	0.06640	0.03895	0.04368	0.00000				
HF	0.04337	0.03518	0.06861	0.03455	0.03098	0.05131	0.00000			
L◦Th	0.04390	0.06837	0.07807	0.04266	0.03922	0.06007	0.06298	0.00000		
L◦Tm	0.03223	0.05670	0.07506.	0.03895	0.02755	0.06506	0.05947	0.01958.	0.00000	
L◦N	0.02378	0.03982	0.05712	0.03880	0.01949.	0.04798	0.04299	0.03536	0.02369	0.00000

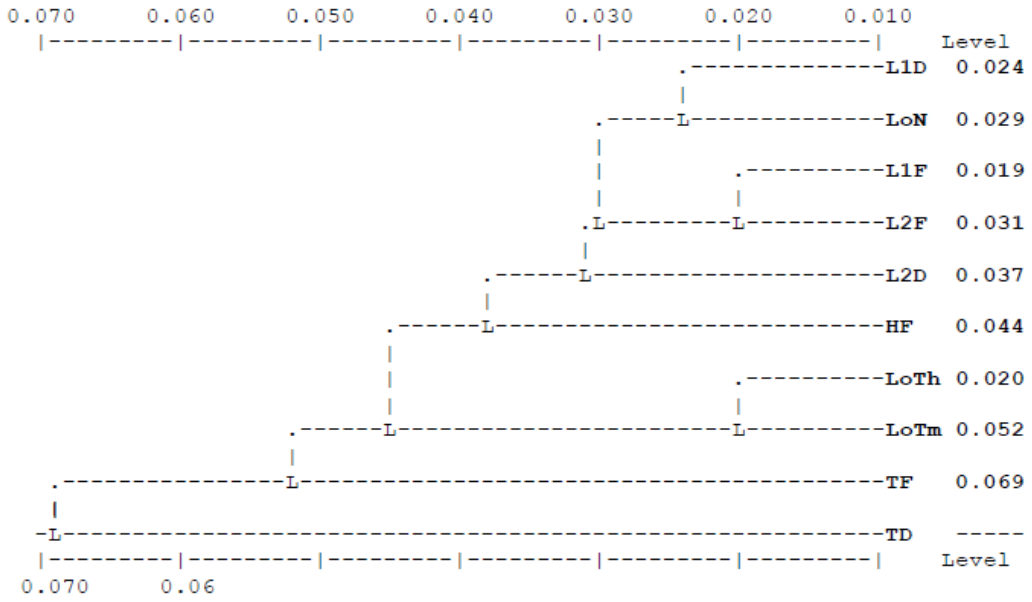

Figure 1: Dendogram of ten wheat cultivars developed from RAPD DNA data using NTSYS-PC software

Cultivar L2F appeared closest in similarity with L1F and to a lesser degree with cultivar L◦N and L1D respectively. While TD showed high genetic distance with L◦Tm, L2D, L1F with values of 0.07506 for L◦Tm and L2D and 0.07239 for L1F see table 2. It is worth mentioning that cultivar TD continuously produced distinct patterns whether in banding patterns or in the intensity of some of the bands. A further indication is the fact that, in comparison to other cultivars TD produced unique fingerprints with four different primers namely A7, 11, D20 and N16b some of which gave more than one fingerprint (Figures 2, 3, 4, 5). Genetic fingerprinting is determined through the presence of amplification products in all samples and absence in one sample or the absence of amplification product and presence in one sample meaning the unique band. The data obtained were also helpful in fingerprinting seven wheat cultivars. Primer A11 showed the highest discriminatory power in addition to producing fingerprints for cultivars L2D, TD, TF and HF which is in agreement with results of Mahenthiralingam (1996) where he mentioned that high efficiency and discriminatory power of primers is important in obtaining fingerprints [16]. Also primer A7, although its discriminatory power was relatively lower than A11, it produced fingerprints with TD, TF and L◦Th providing useful information as these cultivars represent different salt and non-salt tolerant wheat varieties see table 3.

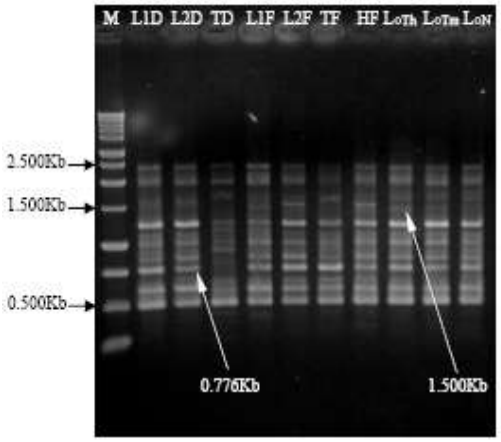

Figure 2: RAPD-PCR products of primer A7 resolved on 1.2% agarose gel. M:1Kb DNA ladder, L1D, L2D, TD, L1F, L2F, TF,HF, L○Th,L○Tm, L○N: salt and non-

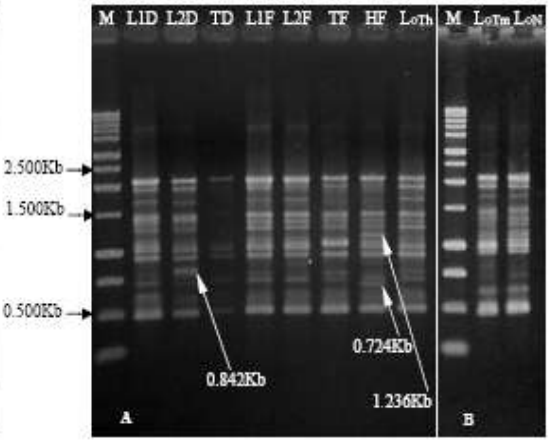

Figure 3: RAPD-PCR products of primer A11 resolved on 1.2% agarose gel. M: 1Kb DNA ladder, A: L1D, L2D, TD, L1F, L2F, TF, HF, L○Th, ,B:M: 1Kb ladder L○Tm,

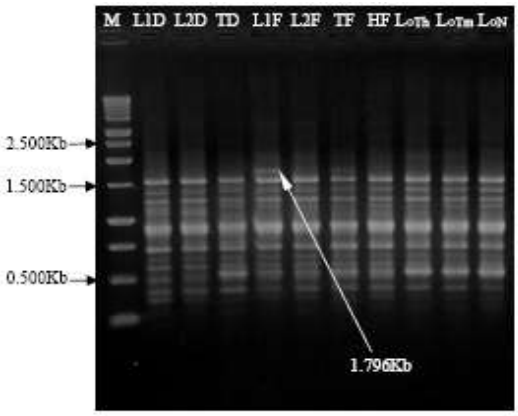

Figure 4: RAPD-PCR products of primer D20 resolved on 1.2% agarose gel. M:1Kb DNA ladder,L1D,L2D,TD,L1F,L2F,TF,HF,L○Th,

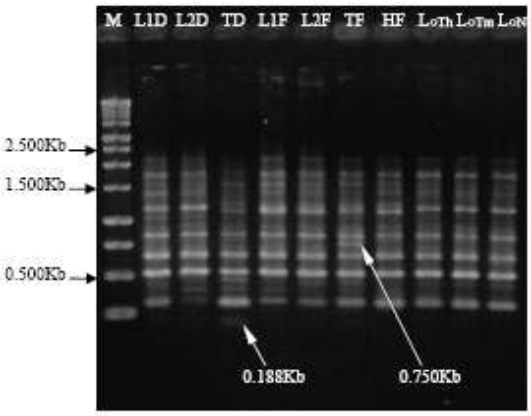

Figure 5: RAPD-PCR products of primer N16b resolved on 1.2% agarose gel. M:1Kb DNA ladder,L1D,L2D,TD,L1F,L2F,TF,HF,L○Th,L

Table 3: Fingerprints of seven wheat cultivars

Cultivars	Fingerprint	
	Primer	Band Mwt
L2D	A11	+0.842
TD	A7	-0.776
	A11	-1.957 -1.157 -0.627
	D20	-0.373
	N16b	-2.179 -1.774 -1.402 +0.188
TF	A7	-1.471
	A11	-1.708
	C5	-2.382
HF	A11	+1.236 +0.724
	N16b	+0.750
L○Th	A7	+1.500
	A8	-0.485
L○Tm	A2	-0.500
L○N	P7b	+0.500

+ band gain, - band loss

What is worth mentioning is that primer A3 produced a controversial 0.390Kb fragment which was relatively distinct in non-salt tolerant varieties and was absent in salt tolerant varieties. This result was reproducible, repeating the PCR reaction with the same primer produced in all times the same controversial band. Demeke *et al*. (1992), demonstrated that using both faint and bright bands gave a closer taxonomic relationship [17]. To verify whether this band is actually present in salt tolerant varieties but in low non detectable level in agarose gels, a further experiment was performed by elution of the 0.390Kb band and re-amplification with the same primer. In the second PCR round the band appeared in all of the cultivars (results not shown) .A possible explanation is that the band maybe associated with a specific protein that is possibly more expressed in non-salt tolerant local varieties.

In this study the search for diagnostic markers to distinguish between salt and non-salt tolerant varieties namely Dijlah and Furat cultivated in different locations was moderately met, the samples used in this study were highly homozygous revealing relatively low polymorphism. Further research is needed perhaps using a larger number of RAPD markers because the wheat genome size is large approximately 17.000 Mb and probably need more markers to cover all possible regions of genetic variations.

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تقييم التضاعف العشوائي متعدد الإشكال لسلسله الدنا للاستدلال على أصناف الحنطة المتحملة للملوحة في العراق

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الملخص

استخدمت تقنيه التضاعف العشوائي متعدد الاشكال لسلسله الدنا للكشف عن التباينات الوراثيه و التقارب الوراثي و التنوع في صنفين متحملين للملوحه(دجله والفرات) والتي جمعت من مواقع مختلفه من جنوب وجنوب شرق بغداد (اللطيفيه،التويثه والحصوه) وقورنت بثلاثه اصناف محليه غير متحمله للملوحه (تحدي، تموز ٢و نخوه) و ذلك لاغراض التربيه. تم استخدام ٢٣ بادئا عشوائيا مختلفا، ١٥منها انتجت انماط تكثير متجانسه و متغايره . البادئات A١١،N١٦b اظهرت اعلى كفاءة تكثير كما وان البادئ A١١ أظهر اعلى قوه تمييزيه بلغت ٢٤,٣% . المعلومات التي تم الحصول عليها استخدمت لايجاد التباعد الوراثي وتركيب مخطط التحليل التجميعي حيث وجد ان هناك ٢٦% تغاير وراثي بين الاصناف المدروسه كما وتم تحديد البصمه الوراثيه لسبعه اصناف من قبل عده بادئات.