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Abstract- Genetic evaluation was estimated for salt tolerance between the selected wheat genotype through plant breeding program and local cultivar by using random amplified polymorphic DNA (RAPD-PCR reaction) with two selected primers (OP1-01 and GB8). According to the amplification results, the genetic distance, Dendrogram illustrated genetic fingerprint and relationships between the selected genotype (Dijila) and local cultivar (Tamooze-2) were determent under non and saline condition. Also the chlorophyll content and organic compounds of the upper leaves were measured.

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Abstract- Genetic evaluation was estimated for salt tolerance between the selected wheat genotype through plant breeding program and local cultivar by using random amplified polymorphic DNA (RAPD-PCR reaction) with two selected primers (OP1-01 and GB8). According to the amplification results, the genetic distance, Dendrogram illustrated genetic fingerprint and relationships between the selected genotype (Dijila) and local cultivar (Tamooze-2) were determent under non and saline condition. Also the chlorophyll content and organic compounds of the upper leaves were measured.

The amplification results using two primers (OP1-01 and GB8) showed that there are no differences in banding patterns between the selected genotype and local cultivar under non-saline condition, this reflecting that there isn't genetic variation between them under such condition with the two primers which used. Whilst the result showed that there are differences between the selected genotype and local cultivar in their banding patterns with the two primers when grown under saline conditions. Also these results reflecting that there are large genetic variation and distance between the selected genotype and local cultivar in their salt tolerance only under saline condition. The chlorophyll content in the upper leaves of selected genotype increased under saline condition while it decreased in the upper leaves of the local cultivar under saline condition as compared with those under non-saline condition. Also the results indicated that the organic compound in upper leaves increased under saline condition in both genotype and cultivar as compared with those organic were much higher in the selected genotype than local cultivar.

Keywords: wheat, salt tolerance, RAPD-RCR reaction, chlorophyll, organic compounds.

I. Introduction

Salinity is a major factor limiting plant growth and production in the middle and south of Iraq, which caused reduction in agricultural land production. Hence induced salt tolerant cultivars or genotypes of crops are needed to sustain in increase in yield production in agricultural land under salinity condition, and also reducing the spread of secondary salinity (Munns, 2005). Increased salt tolerance in plant requires new genetic sources of this character. Powerful new molecular techniques for determining the genetic variation are useful for increasing this character in plants. But we need to the applications of the new technologies to introduce new genes for salt tolerance in to current local cultivars. Genetic diversity is very important factor for the development of many crop plants including wheat plants, because the breeders depend on the availability of genetic diversity during the selection to the developed cultivars. Genetic distance estimation among genotypes is useful to select the parents, which be used in a breeding program (Van Becelaere *et. al.,* 2005). The random amplified polymorphic DNA (RAPD) technique has been used for the identification of genetic diversity within a population of breeding materials, which important for genotypes improvement (Williams et. al., 1990; Welsh & McClell and, 1990; Mainifes to *et.al.,* 2001). Also they reported that the most distinct genotypes or cultivars will be used to increase the genetic diversity in wheat, which will be used in the plant breeding program. RAPDs have been used for identification of genotypes in crop plant, for determining the genetic variability within species and for showing the relationships among populations (Freitas *et. al*., 2000). Using RAPD for variability estimation canguide plant breeders to select genotypes with diverse genetic base, which can be used in their breeding programs. On the their hand, RAPD analysis also has been used to determine phylogenetic relationships among species, subspecies and cultivars (Landry *et.al*., 1994) and to measure genetic variation in populations and species (Nesbitt *et.al*., 1995), as well as identification of cultivars, breeding line and clones (Nybon, 1994).

Organic compounds would be essential to balance the osmotic pressure of the cell cytoplasm and to allow turgor maintenance of cells that would otherwise dehydrate (Wyn Jones *et.al.,* 1977). In addition, these compounds could stabilize membrane proteins and so maintain growth at high salinity levels. The benefit of these compounds can be measured only their effect on leaf injury and plant growth rate, so an increasing in any one of these compounds may not make a plant grow more quickly, but make it grow more slowly under a biotic stress (Manns, *et. al.,*1983).

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The aims of the present study are investigation of genetic diversity and identification between selected genotypes and local cultivar of wheat for salt tolerance by using RAPD technique and genetic distance analysis. Also some organic compounds in upper leaves of these genotypes were measured.

II. Materials and Methods

a) Salt tolerance test

The growth of the wheat genotypes which were selected for salt tolerance through plant breeding programs after 6 cycles of exposure and selection, as compared with the local cultivar (Tamooze-2) were examined in salinized soils at two levels (0, 16, ds/m). The experiment was carried out in pots, set up in glasshouse. 7 seeds were sown in each pot. Seeds and plants were watered with tap water (200 mill/ pot) according to the filed capacity. Leaves samples were taken after 6 weeks from the sowing date, which will be used for the molecular studies and for organic compounds measurement. Also the chlorophyll content in the upper leaves was measured by using chlorophyll meter (Japan).

b) RNA isolation and cDNA synthesis

Total RNA were isolated from the upper leaves according to the manufacturer's instructions, by using Gene aid total RNA purification mini kit (Taiwan). Isolated RNA was treated with RNase-free DNase-I (Biobasic, Canada) for 20 min at 37°C, DNase-I was in activated at 65°C for10min. The RNA integrity was verifiedafter separation by electrophoresis on a 1.5% agarose gelcontaining 0.5% (v/v) ethidium bromide. First-strand cDNA wassynthesized from 500ng of total RNA using Reverse Transcriptionsystem (Bioneer, Korea) with an oligo-dT_{15} primer. The reaction solutionwas used as templates for reverse transcriptase polymerase chainreaction (RT-PCR).

c) cDNA-RAPD

Two primers (table1) were used for amplification of cDNA using optimized PCR protocols and master mixes. Polymerase chain reaction was initiated with hot start method using the single strand cDNA template on LabnetThermocycler (USA). The PCR reaction was carried out according to the program of 35 amplification cycles (94°C for 30 s, 61°C for 45 s and 72°C for 90 s). Ethidium bromide agarose gel electrophoresis (1%) used for analysis of PCR products. The generated bands were compared, the differential amplified bands were recorded and the sequences of these bands aligned to related sequences in NCBI blast database (Chen *et.al.*, 2003).

Table (1) : RAPD primers used

Primers	Sequence
OPI-01	5'-AACCTGGCA-3'
GB8	5'-GTCCACACGG -3'

d) Estimation of genetic distance

Data generated from the detection of polymorphic fragment were analyzed. The amplification profile of all the used isolates for any given were compared with each other, the presence scored as (1) and the absence of the same band of the same size in other isolate scored as (0). Only clear and reproducible amplified fragments were considered for genetic relationship analysis. Estimates of genetic distance (G.D) were calculated between the selected genotype and local cultivar according to (Neiand Li.,1979) Based on following formula: G.D=1-{2Nab/ (Na + Nb)}.

Where Na= the total number of fragments detected in individual (a); Nb= the total number of fragments shown by individual (b) and Nab= the number of fragments shared by individuals (a) and (b). Cluster analysis was performed to construct genetic relationship tree diagrams among studied. Among Triticum *aestivum L.*cultivars using an Unweighted Pair-Group Method with Arithmetic Average (UPGMA). All computations were carried out using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc), Version 1.7 package (Rohlf., 1993). The percentage of polymorphic bands was defined as ratio of the number of polymorphic bands amplified by a single primer to that of the total number of bands produced by the same primer.

e) Estimation of organic compounds

i. *Ash (carbon) percentage*

Ash percentage was estimated according to the (Aoac, 1980) 5g of the plant were taken and burn in the oven (Muffle Furance) at 500 C^0 until the plant simple changed to the white Ash, then the sample was weighted and the percentage of the ash was calculated.

ii. *Total lipids percentage*

This percentage was estimated according to the (AACC, 1984) by using soxhlet. 200ml of the pertroleum ether were added the soxhlet and the thumble which contained 10g the dried plant material was put in the soxhlet. After 8h from the extraction, the solvent was evaporated by using Rotary evaporator at 45C^0. Then the total lipids were weighted and the total lipids percentage was calculated.

iii. *Crude protein percentage*

Nitrogen percentage in the plant sample was measured by using microkjeldahl (Heilenz *et.al.,* 1972). The percentage of crude protein was calculated as following:-

% crude protein = % Nitrogen X 6.25

f) Chlorophyll content

The Chlorophyll content in the upper leaves was measured by using Chlorophyll meter.

III. Result

a) Genetic variation and Distances

The genetic variations between the selected wheat genotype and local cultivar were evaluated using RAPD markers amplified from two primers, each primer varied greatly in their ability to resolve variability between the selected genotype and cultivar. Fig.1 shows the amplification results using two primer OPI-01 and GB8) of the two cultivars grown under non-saline condition. The results showed there are no differences between the selected genotype and local cultivar in their banding patterns with the two primers, this reflecting that there are not genetic differences between the selected genotype and cultivar under non-saline condition with the two primers which used to identify genetic variation in their salt tolerance. Whilst the result in Fig.2 showed that there are differences between the selected genotype and local cultivar in one – bands (300 bp) with OPI-01 that found only in selected genotype (Dijila) when grown in saline conditions (16 ds/m). Also the results showed that the selected genotype and cultivar differed in four bands with GB8 which only found in selected genotype (Dijila) under the same saline conditions (16 ds/m), two of them with 300 bp and the other two, one with 200 bp and the other with 150 bp (fig.2). These results reflecting that there are large genetic differences between the selected genotype and cultivarin their salt tolerance with GB8 primer under salinity condition as compared with the OPI-01 primer.

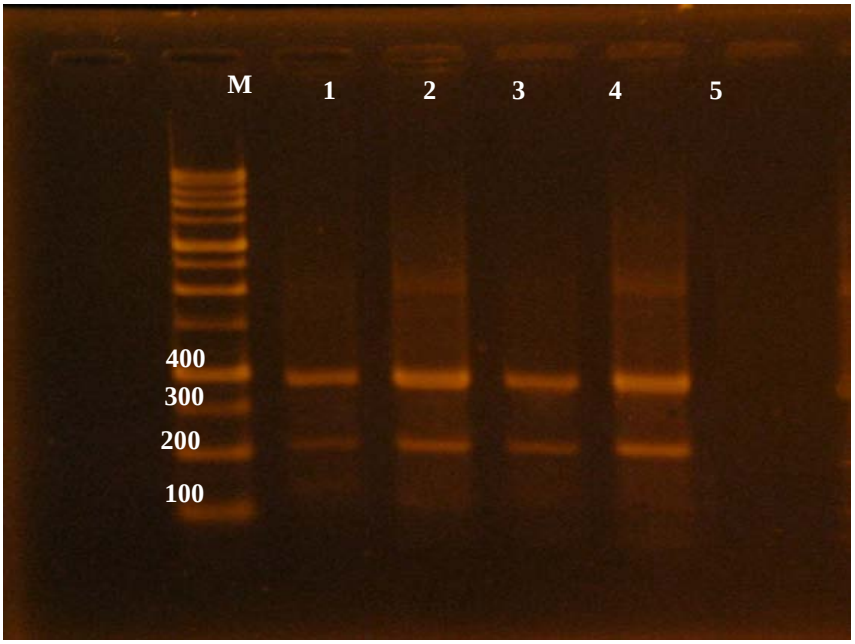

Figure 1 : Agarose gel electrophoresis of RAPD-PCR reaction two primers, cDNA samples of the selected genotype and cultivar(Dijila & Tomooze-2) under non-saline condition. Bands were fractionated by electrophoresis on a 1.2% agarose gel (2hr, 5V/cm, 1X Tris-borate buffer) and visualized under U.V. light after staining with ethidium bromide. M: 100bp DNA ladder. Lane 1: Dijila, Lane 2: Tomooze-2 (primer OPI-01). Lane 3: Dijila, Lane 4: Tomooze-2 (primer GB8). Lane 5: negative control.

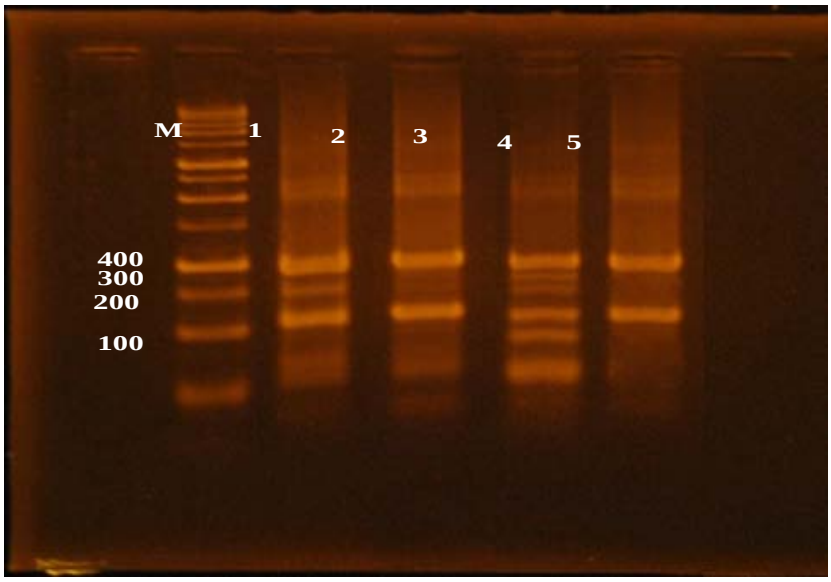

Figure 2 : Agarose gel electrophoresis of RAPD-PCR reaction two primers, cDNA samples of the selected genotype and cultivar(Dijila & Tomooze-2) under saline condition. Bands were fractionated by electrophoresis on a 1.2% agarose gel (2hr, 5V/cm, 1X Tris-borate buffer) and visualized under U.V. light after staining with ethidium bromide. M: 100bp DNA ladder. Lane 1: Dijila, Lane 2: Tomooze-2 (primer OPI-01). Lane 3: Dijila, Lane 4: Tomooze-2 (primer GB8). Lane 5: negative control.

Genetic distance value and dendrogram illustrated genetic distanced were summarized in fig.3. The value of the genetic distance was 0.64404, this reflecting there are large genetic variations between the selected genotype and local cultivar in their salt tolerance. The genetic distance between the selected genotype and cultivar depends on this value;therefore,this value (0.64404) revealed there is high distance between the selected genotype and cultivar in their genetic variation for salt tolerance. Also the result of the dendrogram appeared there are two groups 1 & 2 and the distance between them reflecting the genetic distance between the selected genotype (Dijila) and local cultivar (Tomooze-2) in their salt tolerance (Fig.3).

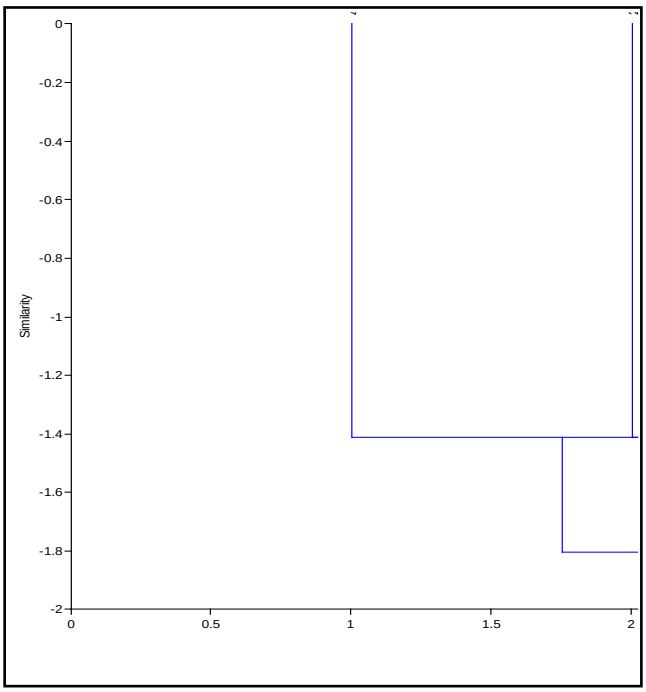

Figure 3 : Dendrogram illustrated genetic fingerprint and relationships between selected genotype and cultivar(Dijila & Tomooze-2) developed from RAPD data

b) Chlorophyll content

The results in Fig.4 showed the Chlorophyll contentrelation in the upper leaves of the selected genotype and local cultivars grown under saline condition (16 ds/m) and non saline conditions (0 ds/m). These results indicated that the Chlorophyll concentration in the upper leaves was similar in the both the selected genotype and cultivar under non saline condition, but this concentration was differed between the selected genotype and cultivar under saline condition (16 ds/m). By contrast, under saline condition the Chlorophyll concentration was increased in the upper leaves of the selected genotype, while it decrease in the leaves of the local cultivar (fig.4) as compared with those when grown under non-saline condition.At 16 ds/m the difference between the selected genotype and cultivar in their Chlorophyll content washighly significant ($p \leq 0.01$).

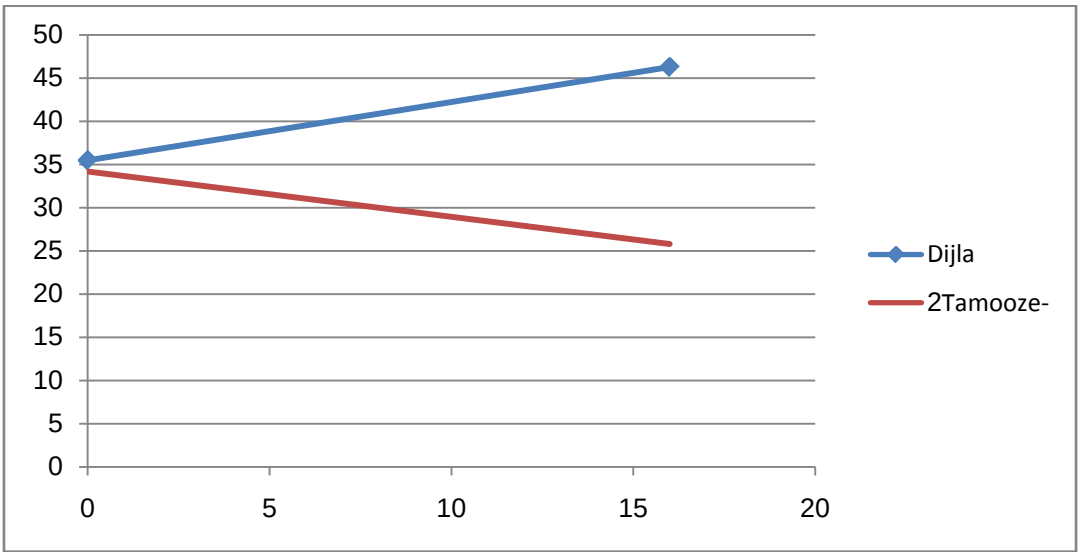

Fig 4 : Chlorophyll concentration in the upper leaves of the selected genotype and local cultivar under salinity conditions

c) Organic compounds

Carbon (Ash), total lipids, and crude protein contents in the upper leaves of the selected genotype and cultivarwere grown under saline and non-saline conditions were summarized in table 2. It showed that the Carbone content in the leaves of selected genotype was higher than this of local cultivar at both salt levels, but it was much higher under saline condition (16 ds/m). At 16 ds/m, the carbon content increased in both the selected genotype and cultivaras compared with this at non-saline condition, but was higher in the selected genotype than local cultivar. Also the result showed that the total lipids content increased in both the selected genotype and cultivarwere grown in the saline condition as compared with those grown under normal condition, but increasing was much higher in the Dilila genotype than Tamooze-2cultivar (Table-2). However, the leaves of selected genotype (Dijila) had total lipid much higher than those of the local cultivar (Tamooze -2). The results of crude protein showed that the leaves of selected genotype also had much higher crude protein percentage than the leaves of the local cultivar at both salinity levels. At both the selected genotype and cultivar, the crude protein percentage was higher at salinity level than the normal condition (non-saline condition) (table.2)

Table 2

	0 ds/m			16 ds/m		
genotypes	Organic compounds			Organic compounds		
	Carbone	Lipid	protein	Carbone	Lipid	protein
Dijila	9.33%	0.98 %	1.6 %	11.5 %	5.75 %	2.2 %
Tomooze-2	8.1 %	0.22 %	1.1 %	8.6 %	1.2 %	1.53 %

IV. Discussion

The selected genotype (Dijilla) was induced through plant breeding programs for salt tolerance; this selected genotype was derived from F2-F7 generations after exposure to the 30 ds/m drainage water. Some experiments were done to exam the salt tolerance of the selected genotype (Dijilla) at different techniques and salinity level, they reported that the selected wheat genotype (Dijilla) is high salt tolerance genotypes and

grow and product very well under high salinity condition (AL- Mishhadani, 2012: AL- Mishhadani *et. al*., 2014). Also ismail, 2013 showed that Dijilla genotype has salt tolerant gene with high expression under salinity condition (20 ds/m). The improve of salt tolerance in this genotype may due to that this genotype was selected from F2-F7 generation generally contain high genetic variation in salt tolerance and also the seeds and seedling plants were exposed to high salinity concentration (30 ds/m) which allow only high salt tolerant genes segregated in very few plants still survived after this exposure. These genes exhibited high gene expression when grown in saline conditions. In contrast, these genes may not exist in the local cultivar which was sensitive to salinity. Munns (2005) reported that salt tolerance in plant is correlated with the salt tolerance mechanisms which controlled by segregated salt tolerant genes. Unless salt tolerance is controlled by major genes which seem unlikely to such a complex character (Ashraf and McNeilly, 1988). It is clear that salt tolerance is inherited and the degree of inheritance depends on the genetic variation range and salinity level (Azhar and McNeilly, 1989; AL- Mishhadani *et.al*., 2003). The difference in the salt tolerance between the selected genotype (Dijilla) and local cultivar may due to large genetic distance between them under high salinity condition as shown in fig.3. This genetic distance value (o.64404) reflects the large difference between them in their salt tolerance this conclusion have been supported by Van Becelaere *et.al*., 2005 which they reported that genetic distance estimation among genotypes is used to selected the parents, which be used in a breeding program to create high genetic variation in F2 materials.

One the other hand that reported by ismail 2013, showed that selected genotype (Dijilla) has salt tolerant gene (*TaGSK1*) with high gene expression under salinity condition, while this gene absence in the local cultivar. Also she reported that the salt tolerance degree in genotype was associated to this result, she concluded that Dijilla genotype more salt tolerance that local cultivar therefore, the results that reported by AL-Mishhadani, 2012: AL- Mishhadani *et.al.,* 2014 confirmed this conclusion. The results of RAPD marker amplified (figs. 1, 2) showed that there are not difference between the selected genotype and local cultivar in specific bands, but the difference between them in these bands appeared in salinity condition only. These bands reflect the genetic variation between them in their salt tolerance, because these bands appeared in salinity condition and in salt tolerant genotype only. The same genetic variation in wheat by using RAPD marker was reported by (sajida BiBi *et.al.,* 2009). Also the same RAPD technique has been used for the identification of improved genotypes (Manifeston *et.al.,* 2001) to screen the genetic similarity and difference between wheat germplasm. The superiority of selected genotype (Dijilla) in salt tolerance of the local cultivar may associated with high chlorophyll content in the upper leaves (Fig .4), that is important factor in photosynthetic and then increase the dry matter (Ashraf, 1994: munns ,1993). Increasing dry matter is very important in plant growth, tissues extantion, and in yield and its components production under salinity conditions (Al-mishhadani, 2010). Increasing chlorophyll content in upper leaves of the selected genotype may due to the modification in morphological characters as physiological mechanisms conferring salinity tolerance character. Also the difference between the selected genotypes and local cultivar in their salt tolerance may refer to their difference in organic compounds of the upper leaves (table 2). These organic compounds that much higher in the upper leaves of the selected genotypes (Dijilla) ascompared with those of local cultivar one very important in osmo regulation in cells plant, which is one the salt tolerance mechanisms to overcome the osmotic pressure of the soil affected salt (Al- mishhadani *et.al*., 2003: munns, 1993).

Generally, from the above results, the conclusions are there significant improvement in salt tolerance of the selected genotype (Dijilla) through plant breeding program and there is large genetic variation and genetic distance between selected genotype and local cultivar under salinity condition only. Also the high salt tolerance in selected genotype more correlated with high contents of chlorophyll and organic compounds in their upper leaves under high salinity level.

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