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Estimation of Genetic Diversity and Proline Content in Some Iraqi Bread Wheat Cultivars

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Abstract: The genetic diversity of six Iraqi bread wheat cultivars: (Dijlah, Furat, Ibaa-99, Inia-66, Uruq and Tamooz 2) were studied with three treatments of salt stress (0,5 and 10ds/m). The RAPD technique was used to estimate the genetic diversity. Salt stress pattern was used. RAPD data by using cluster analysis revealed that Dijlah and Furat cultivars are closed to each other and behave as one group, and Inia-66 and Uruq cultivars are closer to each other because they are sharing the same origin. Ibaa-99 cultivar was nearer to the second group (Inia-66 and Uruq). Under salt stress condition, differential response in term of leaves proline content was observed, and it is ranged between (15.16 to 6.55 M mole/g).
The results showed that there is a genetic diversity among mentioned cultivars with relation to the proline accumulation by salt stress condition.

Key words: Wheat, Proline, Diversity, Stress.

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Introduction

Stress is the result of abnormal physiological process that influenced by one or a combination of biological and environmental factors (1). Salinity and drought are the most important stresses which affect plant growth, so it is necessary to exploit the genetic diversity to find stress tolerant cultivars.
The bread wheat has a six fold genome makes it high diversity and can spreads in most parts of the globe. This diversity opens the way for plant breeders to produce convoy genotypes for climate changes and population growth (2).
It is widely accepted that information about germplasm diversity and genetic relatedness among elite breeding materials is a fundamental element in plant breeding (3).
The advance bread wheat varieties have been reported with narrow genetic base, hence breeding wheat genotypes with diverse genetic base is a vital factor to achieve the level of self-sufficiency, sustainability and entering the export market (4).
Free proline accumulates in response to imposition of a wide range of biotic and a biotic stresses. Under different a biotic stress conditions treatments, proline is accumulated in cells as an osmo protectant. Proline accumulation was found to be one of the common characteristics of many monocotyledons under saline conditions (5). It was first reported by Kemble and MacPherson (6). Other reports indicated that proline

accumulates in plants in response to salt stress.

Gupta and Srivastava (7), showed that the leaf area and proline concentration in tolerant wheat cv. Kharchia, under salt and drought stress, was higher than in a sensitive one. Route and Shaw (8) reported that the salinity is caused to oxidative stress. Ali *et al.* (9) indicated that stimulating of oxidative stress is the most important pathway applied by salinity for aquatic plants and may be applied for all plants. Maggio *et al* (10) showed that a significantly higher accumulation of proline, probably associated with osmotic adjustment and production of membrane.

Accumulation of proline might be used as an index in selection for salt tolerance and wheat cultivars.

This study was conducted to show the genetic diversity among some of local bread wheat cultivars on accumulation of proline under salt stress.

Materials and Methods

Plant Material and DNA Extraction

A RAPD study was conducted to estimate the genetic diversity among commercially grown cultivars (Dijlla , Ibaa-99, Furat, Inia-66, Uruq and Tamooz 2)(table2). The seeds were planted in pots in greenhouse with three salinity levels (0ds/m, 5ds/m, 10 ds/m) of Nacl solution. The treatments were distributed according to split – plot design. Each treatment was replicated three times. Leaves tissues were collected at 15 days after germination for proline estimation and DNA extraction. Genomic DNA was isolated using Genomic DNA purification mini kit (Geneaid Taiwan) according to the manufacturers instructions and DNA was quantified on spectrophotometer at absorbance of 260/280nm. The quality was further checked on 0.8 % agarose gel (11).

DNA Amplification

Twelve primers (table 1), the 10-base oligonucleotide primers obtained from (Operon Technologies, Alameda, CA, USA) were used for amplification of DNA. Using optimized PCR protocols and master mixes. The PCR reaction was carried out according to the program amplification condition were as follow temperature cycles for DNA amplification, the first denaturation step of 5 min at 94 °C followed by 40 cycles of 94 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min. 40 final extension at 72 °C was carried out for 7 min to ensure the completion of the primer extension reaction. Agarose gel electrophoresis stained with ethidium bromide (0.8%) was used for analysis of PCR products and the result visualized under UV trans illuminator (12).

Proline Content

Proline content was determined by the ninhydrin method described by Bates *et al.,* (13). Samples of 0.5 g of fresh weight of leaves from each treatment were homogenized in sulphosalicylic (3% w/v H2O), then centrifuged at 3000 rpm for 5 minutes. Samples of 2 ml from the supernatant were added to 2 ml of each of ninhydrin and glacial acetic acid and incubated at 100ºC for 30 min in water bath. The reaction was arrested in an iced bath and the chromophore was extracted with 5ml toluene and its absorbance at (520 nm) was determined in spectrophotometer (Varian Australia PTY LTD).

Proline standard curve was plotted by different concentration of proline (0, 1, 5, 10, 25, 50, 75 and 100 ppm) (fig.3), then 2ml from each concentration was added to the dry and clean test tube, then (2 ml) of ninhydrin and 2 ml of glacial acetic acid were add to each proline concentration, and incubated at 100˚C for 30 min, the samples were rigorously mixed with 4ml toluene, light absorption of toluene phase was estimated at 520 nm using spectrophotometer.

Table 1: Twelve random primers obtained from (Operon Technologies, Alameda, CA, USA)

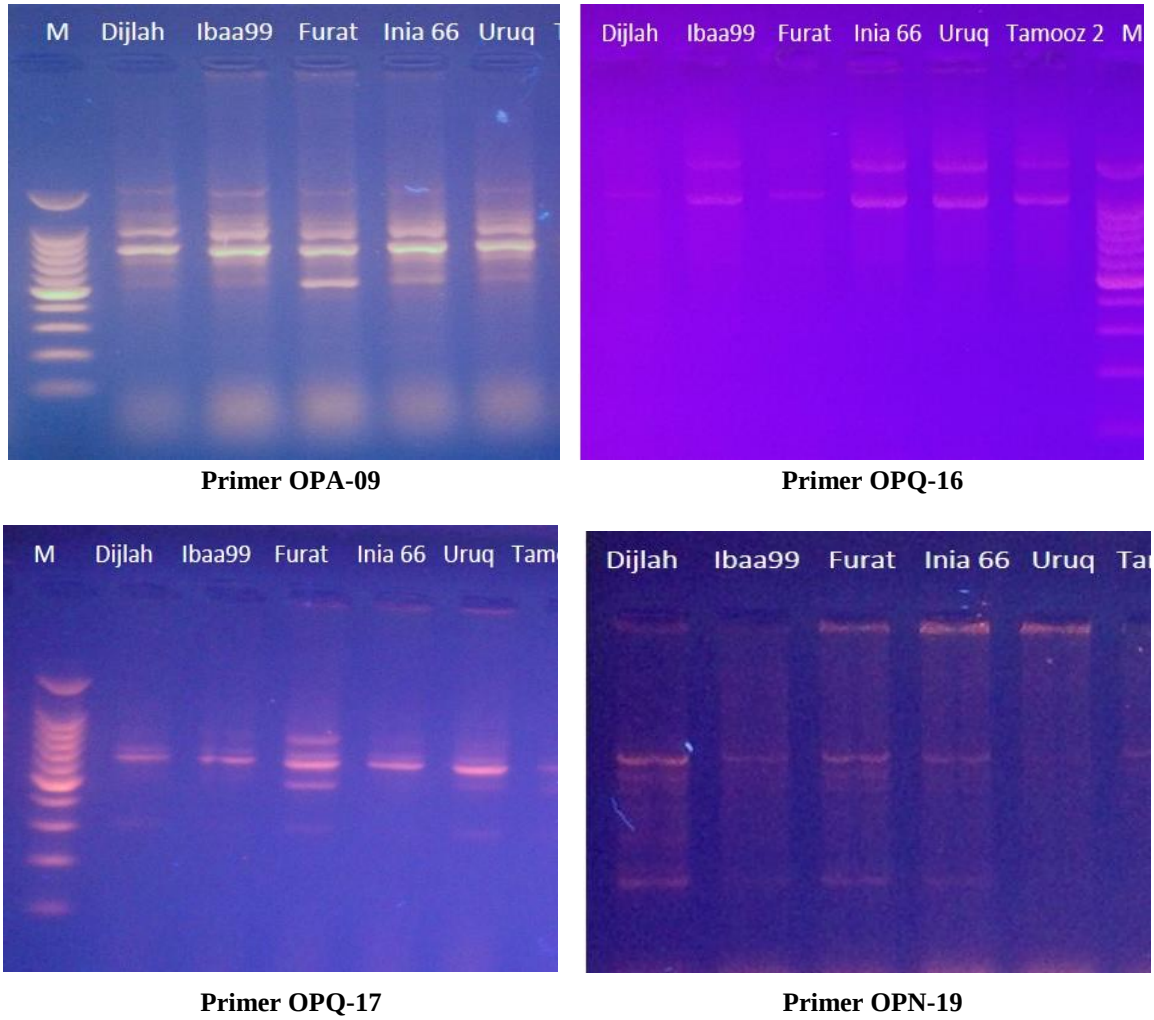
Sequence	Primer
GGGTAACGCC	OPA-09
GTGATCGCAG	OPA-10
CAGCACCCAC	OPA_13
TTCCGAACCC	OPA-15
CCTTGACGCA	OPB_12
AGTGCAGCCA	OPQ_16
GAAGCCCTTG	OPQ_17
GGAGGAGAGG	OPI_02
TGACGGCGGT	OPI-14
GTCCGTACTG	OPN_19
ACGTAGCGTC	OPO_02
AGATGCAGCC	OPE_7

Table 2: Genotypes and origins of used cultivars

Genotype	Pedigree
Ibaa-99	Ures / Bow s / 3 / Jup / Biy s / ures
Furat	F_2 materials derived from a commercial wheat breeding program, USA
Inia-66	Crossing between Lerma Rokho 64 with Snora 64
Uruq	Induced mutations in Inia-66 cultivar
Tamooz 2	Maxipak x Saberbag (hybridization) → (Radiation)
Dijlah	F_2 materials derived from a commercial wheat breeding program, USA

Results and Discussion

Amplification profiles of the six genotypes were compared with each other, and bands of DNA fragments were scored as present or absent (figure1). The data of the primers were used to estimate genetic similarity based on Jacard Index depending on number of shared amplification products (14). Statistical Analysis System-(15) was used in different factors in studied parameters. Least significant difference-LSD test was used for significant comparison among means in this study.



Primer OPA-09 **Primer OPQ-16**

Primer OPQ-17 **Primer OPN-19**

Figure 1: the RAPD banding pattern obtained from four primers: OPA-09, OPQ-16, OPQ-17 and OPN-19 on 0.8% agarose gel, 5V/cm at 1hr. for 6 genotypes of wheat, Dijlla , Ibaa-99, Furat, Inia-66, Uruq and Tamooz 2 and lane M represented the molecular marker M = 100bp DNA Ladder Promega

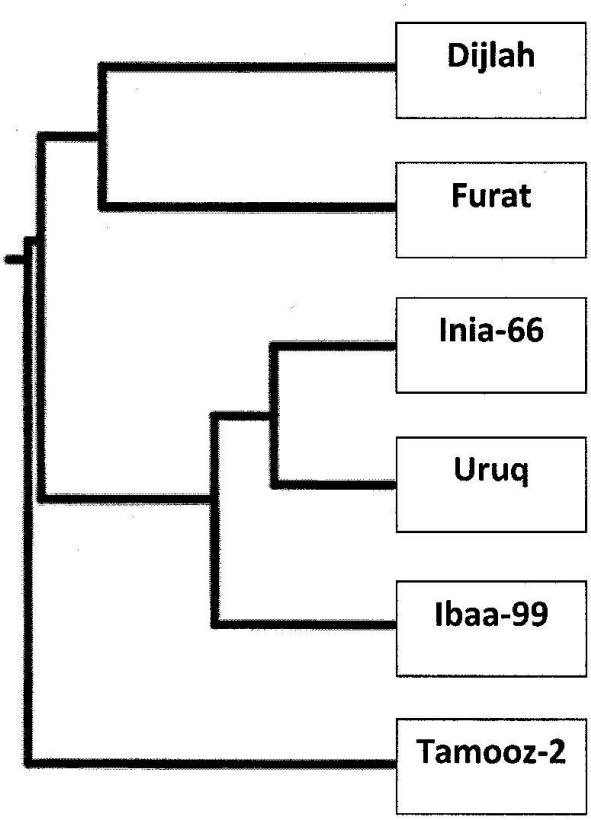


Figure 2: Dendrogram of six wheat cultivars showing the relationship between cultivars based on RAPD data by using UPGMA cluster analysis

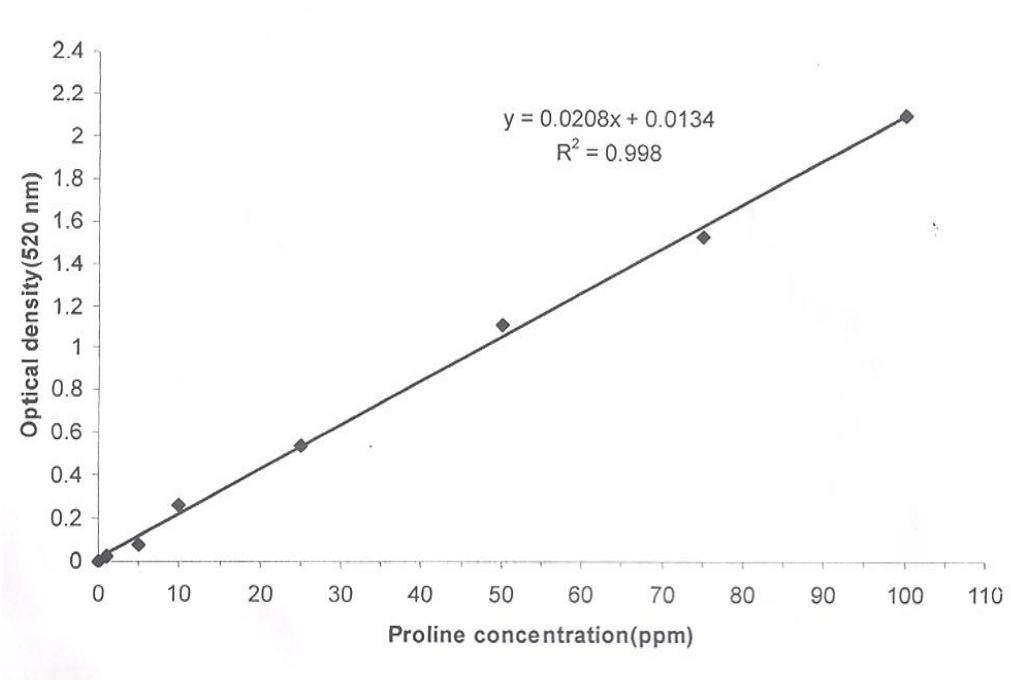


Figure (3): standard curve of proline

Table 3: The effect of salt concentration on proline content for five wheat cultivars

Salinity	Cultivars					Mean ± MSE
	Dijlah	Ibaa-99	Furat	Inia-66	Uruq	
0 EC	4.58	8.15	5.65	6.70	7.65	6.69
5 EC	7.13	12.86	6.83	8.58	8.93	9.06
10 EC	6.55	15.16	8.23	10.68	9.96	10.01
Mean ± MSE	6.24	11.67	7.09	8.94	9.01	----
LSD: salinity: 1.284 *, cultivar: 1.656*, Interaction(TxV): 2.922*						

Note: Tamooz 2 cultivar was used only for comparison at the DNA level.

Results presented in Table (3) exhibited that average of proline content was significantly increased by water salinity stress. Exposure of plants to 5 and 10 ds/m salt stress led to increase proline content of leaves. Average of proline content was significantly different among the test cultivars. The highest proline content (11.67µmole/g) was recorded by Ibaa99, while Dijlah and Furat recorded the lowest proline content (6.24 and 7.09 µmole/g, respectively). The interaction between water salinity treatments and wheat cultivars significantly affected proline content of leaves. At normal salinity (control treatment), Ibaa 99 cultivar had statistically higher leaves proline

content (12.86 µmole/g) than the other cultivars except Uruq (8.93 µmole/g). However, under salt stress condition, differential response in terms of leaves proline content has been observed. Maximum proline content (15.16µmole/g) was found in Ibaa99, Inia66 (10.68 µmole/g) and Uruq (9.96 µmole/g) by the higher salt stress, while Dijlah and Furat remained lower in their proline content.

It was reported that the net accumulation of proline as an organic compatible solute contributed to osmotic adjustment in water stress environments (16). Proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures, and protecting cellular functions by scavenging reactive oxygen species, it may reduce stress-induced cellular acidification or prime oxidative respiration to provide energy needed for recovery, even a small increase in proline biosynthesis might have a large impact on the level of reduction of the cellular NADP pool (17).

Under stress, the generation of MDA (malondialdehyde) takes place in plants due to lipid peroxidation in membrane; it is a mean of assessing oxidative stress induced membrane damage (18). Cell membrane stability has been used to differentiate among crop cultivars with respect to grade of salt tolerance (19). There is strong evidence that glycine, betaine and proline shield the sub cellular structures and mediate osmotic adjustment in stress. (20)

According to the RAPD data by using cluster test analysis (Figure 2), the relationship among cultivars showed that Dijlah and Furat cultivars might be close in genetically resource and behave as one group as long as the salt tolerance is concerned. These two cultivars were induced by using plant breeding programs from F2 materials derived from a commercial wheat breeding program, USA (21), while Inia-66 and Uruq cultivars behave as close related because they were originated from one genetic resource (Inia-66 produced from crossing between Lerma Rokho 64 with Snora 64, and Uruq cultivar produced by induced mutations in Inia-66 cultivar.

Ibaa 99 revealed that its origin were nearer to the genetic resource of Inia-66 and Uruq. That means the genotypes of the three mentioned cultivars (Uruq, Inia-66, and Ibaa99) have similar allele locations for proline production, this may related to the ability of these three cultivars to produce a higher proline ratio as compare with second group cultivars (Dijlah and Furat). While Tamooz 2 cultivar was used only for comparison at the DNA level. In such cultivars diversity, the effectiveness of crop improvement program depends on the extent of genetic diversity either existing or created (22).

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