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# Characterization Of Some Iraqi Wheat Cultivars According Their Capability For Callus Induce And Salinity Tolerance

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**Abstract**

Three experiments aimed to (1) classify sixteen Iraqi wheat varieties according to their ability to induce callus from the mature embryo under different concentrations of sodium chloride;(2) to investigate the effect of two medium types on the ability of wheat varieties to induce callus in-vitro; and (3) to study the performance of Iraqi wheat cultivars according to seedling characters under different concentrations of sodium chloride. These experiments were conducted at Crop Biotechnology Laboratory / Dept. of Agronomy and Horticulture/ University of Nebraska/ USA. Three Experiments have been conducted; the first experiment used two types of media were used for callus induction. Iraqi wheat varieties showed great differences in their ability toward callus induction, where Rabea variety own high performance according to callus induction, Media type II was superior to media type I. Second experiment included two treatments of sodium chloride (0, 400 mM) to induce callus using media type II for the sixteen genotypes. The results showed superiority of Iraq and Furat genotypes. The third experiment studied the effect of four concentration of sodium chloride (0, 100, 200, 400 mM) on seedling characters. The results showed supremacy of Iraq and Furat cultivars.

**Keywords:** Iraqi wheat cultivars, callus induce , salt tolerance.

## 1.Introduction

Bread wheat (Triticum aestivum L.) is an allohexaploid, has three distinguished but genetically related (homologous) copies of genomes, A, B, and D[1]. The yield and quality of wheat have been gradually raised during the past several decades by classical breeding methods. However, these methods have some limitations such as long time required and rather limited gene pool available for wheat breeders. For this purpose, a group of activities was focused on in vitro culture and regeneration as a tool of cereal breeding in recent years. Further, it is also well documented that the genetic engineering of cereals currently depends on the use of tissue culture and plant regeneration[2]. It provides approaches to study the physiological and genetic processes of the plant, also it offers the potential to support in the breeding programs through raising the genetic variability in cereal[3].

Salinity is a significant factor limiting the crop productivity in arid and semi-arid areas of the world, especially when precipitation can be insufficient for leaching[4] and low water quantity and the poor drainage systems[5]. Salinity a common issue around the world, where nearly half of the irrigated land and 20% of the world's cultivated land are currently affected by salinity and Over 800 million hectares of land throughout the world are salt affected[6] and it is a major obstacle to food production as long as it ultimately reduces the average yield of most crops[7] by decreasing the osmotic potential of soil solutes, thus making it difficult for roots to absorb water from their surrounding zone[8]. The reduction in osmotic potential of plants subjected to salt stress may be to either water loss or an increase in dissolved solutes or a combination of both[9] due to a lack of osmotic adjustment, resulting in the inhabitation of water uptake, and physiological drought has long been considered a major cause of salt damage to plants [10]. Therefore, the identification of new sources of salt tolerance would be important for developing saline agriculture.

The object of this study is to (1) examine the effect of two medium types on the ability of wheat cultivars to induce callus in vitro; and (2) distinguish between wheat cultivars according to their tolerance for salt stress throughout subjecting (a)the callus to two concentration of sodium chloride and (b) the seedlings to four concentration of sodium chloride.

## 2.Materials and Methods

This experiment was conducted at Crop Biotechnology Laboratory / Dept. of Agronomy and Horticulture/ University of Nebraska/ USA during 2013.

### 2.1.Plant materials:

Sixteen Iraqi wheat cultivars were obtained from Iraqi Ministry of Agriculture. The pedigree information on the Varieties was obtained from their sources or from website (http://genbank.vurv.cz/wheat/pedigree/).

### 2.2.Callus induction:

The sterilized seeds were kept in petri dishes contained filter paper for 24 h., then the mature embryos from a sample of 20-25 seeds from each cultivar were used to culture. They were excised from the caryopsis by a scalpel and forceps and placed on the two callus induction media (MS medium) [11] in sterile 90 mm petri dishes for three months and Induced calli were sub-cultured every 30 days on 25C.

Medea Type I composed of: MS salt 4.3 g ,Sucrose 30 g , 2,4-D 3mg and Kin 0.5 mg ). This media was prepared as following: These materials were solved in 800 ml of distilled water pH was adjusted 5.8 and after adjusting the volume to 1L, 7 gm of agar was added and a hot plate magnetic stirrer was used to solve them.The media then, was poured in vials and autoclaved at 121°C for 15 min. Finally the vials were kept under room temperature with slop angel 45°.

Medea type II composed of : MS salt 4.3 g Glucose 20 g , Mannitol 15 g , Sorbitole 15 g , mgCl2 0.75 g L-Csteine 40 mg , **52 ,** Ascorbic acid 100 mg , Casein hydrolysate 500 mg , Aspargine 5mg and Dicamba 2 mg (4.1 µL). Also prepared as the following procedure: 600 mL of distilled water was poured in a beaker having a magnetic stirrer and placed on electric stirrer plate and the materials above were added. More distilled water was added to reach the final volume (1L). pH of the solution was adjusted to 5.8, then this solution was divided into two bottles (each with 500 ml) and after adjusting the volume, 2.5 gm of phytagel were added to each bottle. The solution was autoclaved at Liquid cycle 20, and then it was cooled off. 500 µL of B5 vitamins were added to each 500ml (it has been already sterilized by filtration) Silver Nitrate at 100 µL/500 ml = 4 mg/L (autoclaved previously) were added They were mixed and poured into plates.

### 2.3.Effect of salt stress on callus induction:

100 mg of each wheat cultivar callus, induced on media Type II, were taken to investigate their tolerance to salt stress. The composition of media was used as control, and the same media containing 400 mM of sodium chloride was used for salt stress.

### 2.4.The experiment of plant salt tolerance

The experiment involved planting 25 seeds in each pot, and kept in the greenhouse then thinned to ten plants per boat after emergence. These treatments were done by adding saline water with four concentrations (0, 100, 200, 400 mM) of sodium chloride. A week later, they were thinned to five plants per pot. When plants were two months old, data of shoot length, shoot weight, leaflets number, root length, root weight and Salt Tolerant Trait Index (STTI) were calculated.

$$STTI = \frac{Valu\ of\ trait\ under\ stress\ condition}{Valu\ of\ trait\ under\ controled\ condition} X100$$

**2.5. Statistical Analysis**

Data obtained from experiments of media type, salt stress effect on callus induction and salt stress effect on plant were collected and analyzed according to Complete Randomized Design (CRD) included five replicates for each treatments, by using software program Genstat 5 version 3.2.

**3.Results**

**3.1. Experiment of Media Type**

Bread wheat cultivars were significantly affected by media composition. D cultivar was the best one in its ability of callus induction and recorded the highest weight of induced callus in both types of media (table 1). B and S genotypes showed contradictory performance towards the type of media on callus induction. The genotypes performance are vary according to the media type because of the variation in the nutrients and vitamins, therefore [13] found superiority of G cultivar as compare with Iraq excellence when they used one type of media. On the other hand, some excellence didn't respond to the variation in the media type, i.e. I and P, where they showed similar performance in both media types. Media type II was superior to media type I; therefore it is a good idea to recommend for using it in the next studies.

Table 1 Effect of media type on induced callus fresh weight (g) of wheat varieties after 90 days

| Media type / Cultivars | 1 | Rank | 2 | Rank | Mean |
|---|---|---|---|---|---|
| Tamooz2(A) | 0.394 | 3 | 1.447 | 6 | 0.921 |
| Iraq (B) | 0.158 | 16 | 2.429 | 2 | 1.294 |
| Iba 99 (C) | 0.272 | 8 | 1.566 | 5 | 0.919 |
| Rabea(D) | 0.596 | 1 | 2.799 | 1 | 1.698 |
| Um Rabee(E) | 0.331 | 5 | 1.050 | 10 | 0.691 |
| Tamooz 3(G) | 0.296 | 7 | 0.774 | 13 | 0.535 |
| Iba 95(H) | 0.354 | 4 | 1.065 | 8 | 0.710 |
| Dore 29(I) | 0.453 | 2 | 0.829 | 11 | 0.641 |
| Latifia(J) | 0.317 | 6 | 1.689 | 4 | 1.003 |
| Dijla(M) | 0.258 | 9 | 1.252 | 7 | 0.755 |
| Furat(N) | 0.232 | 11 | 1.056 | 9 | 0.644 |
| Fatih(O) | 0.258 | 10 | 0.748 | 14 | 0.503 |
| Abu Graib(P) | 0.167 | 15 | 0.625 | 16 | 0.396 |
| Hashimia(S) | 0.178 | 14 | 1.919 | 3 | 1.048 |
| Buhooth (X) | 0.209 | 12 | 0.739 | 15 | 0.474 |

| | | | | | |
|---|---|---|---|---|---|
| Dore 85(Y) | 0.181 | 13 | 0.812 | 12 | 0.496 |
| LSD | 0.2887 | | 0.2041 | | |
| Mean | 0.291 | | 1.300 | | 0.795 |
| LSD | 0.0722 | | | | |

**3.2.Experiment of Salt stress**

**3.2.1.Callus Weight**

Adding salt (sodium chloride) to the media affected callus growth significantly and the Cultivars responses toward salt stress and all excellence showed lower callus growth rate in the media contained salt in compression to callus grown on free media(Table 2). Cultivar A was more tolerant to salt stress than others in spite of own lowering callus weight. These results confirm the role of genetic variances of wheat crop in their ability to callus induction from mature embryo. Cultivar N was excellent in both treatments and also in STTI value. while Cultivar B possesses highest callus weight with moderate STTI value, and this refers to the efficiency of B cultivar in salt tolerance.

Table 2 effect of salt stress on callus induction weight (g) of wheat varietie

| Variety | Control | Salt stress(400 mM) | Mean | % Reduction | STTI |
|---|---|---|---|---|---|
| A | 0.141 | 0.122 | 0.131 | 13 | 86.525 |
| B | 0.374 | 0.254 | 0.314 | 32 | 67.995 |
| C | 0.203 | 0.119 | 0.161 | 41 | 58.559 |
| D | 0.253 | 0.168 | 0.210 | 34 | 66.482 |
| E | 0.159 | 0.141 | 0.150 | 11 | 88.324 |
| G | 0.254 | 0.204 | 0.229 | 20 | 80.338 |
| H | 0.187 | 0.133 | 0.160 | 29 | 71.169 |
| I | 0.191 | 0.166 | 0.179 | 13 | 87.205 |
| J | 0.230 | 0.194 | 0.212 | 16 | 84.368 |
| M | 0.236 | 0.207 | 0.222 | 12 | 87.474 |
| N | 0.254 | 0.204 | 0.229 | 20 | 80.102 |
| O | 0.180 | 0.106 | 0.143 | 41 | 58.791 |
| P | 0.246 | 0.198 | 0.222 | 20 | 80.333 |
| S | 0.206 | 0.128 | 0.167 | 38 | 61.900 |
| X | 0.200 | 0.182 | 0.191 | 9 | 91.287 |
| Y | 0.191 | 0.162 | 0.177 | 15 | 84.684 |
| LSD | 0.058 | | 0.041 | | |
| Mean | 0.219 | 0.168 | 0.194 | | 77.221 |
| LSD | 0.015 | | | | |

**STTI: salt total tolerance index**

### 3.2.2.Shoot Length

Cultivars B and D were the superior among the others at the control treatment (0 mM) (table 3). They surpassed the less ones, E and Y, by 52% and these superiority can be observed at other significant salt levels. Y recorded the highest value of STTI (85.25%) although its shoot length was only 18.2 cm ai high rate of salt. Although B was superior to other cultivars in shoot length under 400mM NaCl stress, it was more affected than other by salt stress. Cultivar D gave high level of shoot length and at the same time it was moderate affected by salt stress. Shoot length significantly decreased with increasing level of salt stress depending on salts concentrations[14].

Table 3 Effect of salt concentration on shoot length (cm) of wheat varieties

| Salt con. / Var | 0 mM | 100 mM | 200 mM | 400 Mm | Mean | STTI |
|---|---|---|---|---|---|---|
| A | 25 | 25 | 23.83 | 18.5 | 23.08 | 74.00 |
| B | 35 | 30.17 | 22.57 | 21.67 | 27.35 | 61.91 |
| C | 22.67 | 23 | 19.13 | 18.17 | 20.74 | 80.15 |
| D | 30.67 | 29 | 28.17 | 19.37 | 26.8 | 63.16 |
| E | 22.25 | 23.03 | 17.83 | 17.23 | 20.09 | 77.44 |
| G | 25 | 24.5 | 18.83 | 17.93 | 21.57 | 71.72 |
| H | 25 | 24.5 | 22.5 | 17.67 | 22.42 | 70.68 |
| I | 23.83 | 22.67 | 20.83 | 17.9 | 21.31 | 75.12 |
| J | 23.83 | 23.93 | 23.17 | 19.67 | 22.65 | 82.54 |
| M | 28.83 | 28.33 | 20.83 | 16.67 | 23.67 | 57.82 |
| N | 29 | 29.17 | 21.17 | 19.67 | 24.75 | 67.83 |
| O | 22 | 22.87 | 19.17 | 14.97 | 19.75 | 68.05 |
| P | 26.67 | 25.83 | 21.57 | 19.1 | 23.29 | 71.62 |
| S | 22.1 | 22.73 | 22 | 15.33 | 20.54 | 69.37 |
| X | 23 | 23.67 | 23.5 | 17.83 | 22 | 77.52 |
| Y | 21.35 | 22.51 | 22.33 | 18.2 | 21.1 | 85.25 |
| LSD | 3.417 | | | | 1.709 | |
| Mean | 25.39 | 25.06 | 21.71 | 18.12 | 22.57 | 72.14 |
| LSD | 0.854 | | | | | |

*STTI calculated for 100mM & 400mM

### 3.2.4.Shoot Fresh Weight

The effect of salt stress on shoot weight of wheat plant was clear through the highly significant differences among treatments and among cultivars. (Table 4). At 400 mM NaCL, the weight of fresh shoot declined in all varieties. D was higher than the lowest one, O, by 237 % at control treatment. The average of shoot fresh weight at concentration 400mM decreased by 42.3% as compare to control. The difference may be ruled by their genetic differences. The highest fresh weight at concentration 400 mm NaCL was recorded by cultivar S (259.7 mg) followed by J (224.0mg), which was less influenced by salt stress than S, as it’s value of STTI was higher than this value in S cultivar.

Table 4 Effect of salt concentration on fresh leaf weight (mg) of wheat varieties

| Salt con. / Var | 0 Mm | 100 mM | 200 mM | 400 Mm | Mean | STTI |
|---|---|---|---|---|---|---|
| A | 317.0 | 248.0 | 247.0 | 130.7 | 235.7 | 41.2 |
| B | 568.0 | 450.7 | 293.3 | 211.7 | 380.9 | 37.3 |
| C | 273.7 | 285.7 | 251.7 | 143.0 | 238.5 | 52.2 |
| D | 467.0 | 458.0 | 380.0 | 218.3 | 380.8 | 46.7 |
| E | 281.3 | 339.3 | 157.3 | 150.0 | 232.0 | 53.3 |
| G | 293.3 | 385.7 | 247.3 | 169.7 | 274.0 | 57.9 |
| H | 307.0 | 265.3 | 255.0 | 165.7 | 248.3 | 54.0 |
| I | 255.3 | 245.7 | 227.7 | 179.0 | 226.9 | 70.1 |
| J | 269.7 | 274.0 | 226.0 | 224.0 | 248.4 | 83.1 |
| M | 204.7 | 218.3 | 220.3 | 128.0 | 192.8 | 62.5 |
| N | 211.3 | 194.7 | 146.7 | 139.0 | 172.9 | 65.8 |
| O | 196.7 | 211.7 | 193.3 | 136.3 | 184.5 | 69.3 |
| P | 227.3 | 284.7 | 207.0 | 154.3 | 218.3 | 67.9 |
| S | 359.7 | 368.0 | 355.0 | 259.7 | 335.6 | 72.2 |
| X | 315.0 | 318.0 | 312.7 | 194.7 | 285.1 | 61.8 |
| Y | 290.7 | 307.0 | 267.0 | 196.0 | 265.2 | 67.4 |
| LSD | | 83.3 | | | 41.7 | |
| Mean | 302.4 | 303.4 | 249.2 | 175.0 | 257.5 | 60.2 |
| LSD | | 20.8 | | | | |

### 3.2.5. Root Fresh Weight

Table 5 reveals the treatments of salt concentrations were steadily affected by increasing salt concentrations. Most cultivars in this study did not be affected by salt stress at 100mM. D and B cultivars showed highest root weight at all treatments. STTI of Y was the highest among other varieties (72.63). Also B was more affected by salt stress than others (STTI =28.71).

Table 5 effect of salt concentration on root weight (g)

| Salt con. / Var | 0 mM | 100 mM | 200 mM | 400 mM | Mean | STTI |
|---|---|---|---|---|---|---|
| A | 0.804 | 0.808 | 0.624 | 0.574 | 0.703 | 71.46 |
| B | 1.800 | 1.808 | 0.603 | 0.517 | 1.182 | 28.71 |
| C | 0.803 | 0.682 | 0.564 | 0.512 | 0.640 | 63.79 |

| | | | | | | |
|---|---|---|---|---|---|---|
| D | 1.194 | 1.194 | 0.823 | 0.697 | 0.977 | 58.42 |
| E | 1.007 | 1.075 | 0.600 | 0.528 | 0.802 | 52.45 |
| G | 0.831 | 0.802 | 0.631 | 0.484 | 0.687 | 58.22 |
| H | 1.046 | 0.875 | 0.980 | 0.371 | 0.818 | 35.50 |
| I | 1.019 | 0.908 | 0.526 | 0.487 | 0.735 | 47.82 |
| J | 1.060 | 0.775 | 0.452 | 0.419 | 0.676 | 39.49 |
| M | 1.082 | 0.926 | 0.585 | 0.455 | 0.762 | 42.09 |
| N | 1.073 | 0.690 | 0.450 | 0.311 | 0.631 | 29.03 |
| O | 0.870 | 0.875 | 0.507 | 0.432 | 0.671 | 49.66 |
| P | 0.767 | 0.785 | 0.662 | 0.419 | 0.658 | 54.67 |
| S | 0.809 | 0.794 | 0.764 | 0.419 | 0.696 | 51.85 |
| X | 0.947 | 0.553 | 0.543 | 0.242 | 0.571 | 25.58 |
| Y | 0.707 | 0.664 | 0.550 | 0.513 | 0.608 | 72.63 |
| LSD | 0.088 | | | | 0.044 | |
| MEAN | 0.989 | 0.888 | 0.616 | 0.461 | | 48.83 |
| LSD | 0.022 | | | | | |

### 3.2.6. Root length

The highest value of root length was recorded by Y cultivar (24.8 cm) (Table 6). The effect of salt stress on cultivars was also significant. Y and X were the best among other varieties at the concentration of 100mM, however, at 400mM the difference between them became highly significant. G cultivar recorded second highest value of root length under salt stress of 400mM (22.8 cm) and highest STTI (98.3) followed by N and I which recorded high values of length (21.4 and 21.2 cm respectively) which means un affected by salt stress.

Table 6 Effect of salt concentration on root length (cm)

| Salt con. / Var | 0 mM | 100 mM | 200 mM | 400 Mm | mean | STTI |
|---|---|---|---|---|---|---|
| A | 23.7 | 22.9 | 22.0 | 19.0 | 21.9 | 80.1 |
| B | 22.4 | 22.5 | 21.9 | 18.7 | 21.4 | 83.3 |
| C | 20.0 | 20.9 | 17.1 | 15.8 | 18.5 | 79.0 |
| D | 24.9 | 24.1 | 23.5 | 18.0 | 22.6 | 72.4 |
| E | 26.1 | 26.0 | 23.9 | 21.3 | 24.3 | 81.9 |
| G | 23.2 | 23.2 | 23.0 | 22.8 | 23.1 | 98.3 |
| H | 28.0 | 15.3 | 15.0 | 14.7 | 18.3 | 52.6 |
| I | 30.0 | 25.0 | 22.0 | 21.2 | 24.5 | 70.7 |
| J | 30.0 | 20.0 | 19.1 | 18.6 | 21.9 | 62.1 |
| M | 22.0 | 22.0 | 20.6 | 15.1 | 19.9 | 68.6 |
| N | 23.0 | 22.6 | 22.0 | 21.4 | 22.3 | 93.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| O | 17.0 | 16.9 | 14.6 | 13.6 | 15.5 | 79.7 |
| P | 37.7 | 22.0 | 20.9 | 14.7 | 23.8 | 38.9 |
| S | 19.8 | 19.8 | 18.7 | 14.2 | 18.1 | 71.6 |
| X | 26.4 | 26.3 | 25.5 | 18.0 | 24.0 | 68.1 |
| Y | 26.5 | 25.5 | 23.9 | 23.3 | 24.8 | 87.8 |
| LSD | 1.5 | | | | 0.8 | |
| Mean | 25.0 | 22.2 | 20.9 | 18.1 | 21.6 | 74.3 |
| LSD | 0.4 | | | | | |

## 4.Discussion

D cultivar was excellent in both media while B cultivar was premium in media type II which state diversity of genetic ability in callus formation. Although cultivars A, E, X appeared the most tolerant cultivars according to STTI values, but they own the lower averages of callus weight. It appears that cultivar B had a high callus weight under both normal and saline treatment, but the STTI index was medium, and this indicates that the percentage of reduction is high between the two treatments.

Salinity affects many morphological, physiological and biochemical processes, including seed germination, plant growth, and water and nutrient uptake [15] and seedling stage is considered to be more sensitive in most plant species[16]. According to STTI index, cultivar Y was outstanding, but it had low averages for the studied characters. Cultivars N and B showed good averages for the studied charact**ers** with moderate values for STTI. It seems that the STTI index cannot be relied upon without looking at the rate of the characters under the salinization, however, the STTI index was the highest and reached 91 (Table 5). The inefficiency of the STTI index is due to the fact that it is related to the reduction between the character averages for both treatments.

Most of the cultivars with high trait averages under levels of salt stress had STTI moderate values. Therefore judging the cultivar's efficacy in tolerance salinization may be somewhat confusing, and STTI index would mislead for determining the suitable cultivar. Therefore, using traits average under salt stress with STTI index would be more accurate.

## 5.Conclusion

The mature embryo of Iraqi cultivars can be used efficiently to induce high weighted of callus depending on the cultivar itself and media type. Variety D was better than other Iraqi wheat cultivars in its ability to induce callus from the mature embryo followed by B cultivar.

The performance differed under salt stresses, where Y cultivar was the most tolerant one in most characters, but their characters averages were low. Cultivar B and N own the highest average of the studied characters with moderate STTI values. The information resulted from this study can be used to contribute to putting the core stone of the database for Iraqi wheat cultivars to be used in the future as an indicator for breeding programs.

**Conflict of Interest: None**

**Ethical Clearance:** Not required

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