Coping with salinity: HANAA new salt tolerant wheat (*Triticum aestivum* L.) cultivar selected by *In Vito* mutation breeding program

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*In vitro* mutation breeding program was conducted to induce salt tolerant trait in spring wheat (*Triticum aestivum* L.) Ajeeba cultivar. Immature embryos were cultured on modified MS medium. Calli were subjected to (0, 10, 20, or 30 Gy) of gamma rays. Irradiated calli were transferred to MS medium of 2.5, 9, 12 or 15 dsm\(^{-1}\) salt levels. The survival calli were transferred to regeneration medium and the regenerated plants were acclimatized and transferred to the field. Several hundred plants were obtained and tested for salt tolerance in a hydroponic culture. After two months in the hydroponic culture selected plants were transferred to the soil. Seeds of the survived plants were grown in salt affected land and screened for several generations. Plants were selected based on their morphological and production traits under salt stress in the field. Selected lines were subjected to several analyses such as total protein content, disease tolerant, elasticity, and other rheological properties. One line was registered and released by The Ministry of Agriculture, The National Committee for the Registration, Release and Protection of Iraqi Cultivars under the name HANAA which is new salt tolerant cultivar that can tolerate up to 18 dsm\(^{-1}\) in the field with large seeds, high 1000 seeds weight, high protein content and good elasticity suitable for Iraqi flat bread.

**Keywords:** gamma rays, immature embryos, salt tolerant, *In vitro*, hydroponic culture

**INTRODUCTION**

Food productivity is affected by salt stress worldwide. Searching for salt tolerant crops is of great concern to cope with salinity. However, salt tolerance is complicated trait and controlled by several genes (Mahjan and Tuteja, 2005, Colmer, et al., 2006). Therefore conventional techniques required long time and tedious work as well as large land with equal salinity level for the selection process. Tissue culture which is performed under controlled environmental conditions provides suitable method for the selection of such trait at the cell level in a short period of time and in a limited space. The addition of high salt level as a selection agent in an artificial medium has been used successfully to select salt tolerant cell lines and plants of different crop species (Bhaskaran et al., 1983; McCoy, 1987; Javed, 2002). Moreover, tissue culture proved to induce variations in cells and subsequently the plants regenerated from them. This phenomenon is known as Somaclonal Variation (Larkin and Scowcroft, 1981) which has been utilized by many researchers to select cell lines and plants with desirable traits during the last century (Cheng, et al., 1998; Hashim, 1990; Larkin et al., 1984). Although the amount of genetic variations coming from tissue culture is limited, those variations provide a new source for...
genetic improvement of many plant species such as barley, potato, rice, flax, blackberry, tomato and wheat (Larkin, 2004).

Genetic changes can be induced by physical mutagen agents such as gamma radiation. This technique has been used in plant breeding both in vitro and in vivo for long time (Sobieh, 2002; Al-Hattab, 2015). The occurred mutations are random and ranged from simple undetectable to drastic changes in the chromosomes and the DNA molecules (Al-Hattab et al., 1992, Al-Ouda et al., 2004). Application of ionizing radiation with tissue culture will increase the mutation frequency and provide a wide range of variability for selection (Wi, et al., 2007; Al-Naggar, et al., 2004).

Based on the above information, in this research combination of tissue culture and ionizing radiation were used to induce genetic changes in wheat calli for the selection of salt tolerant wheat plants. Moreover, all the steps of the selection and the registration of the selected line up to the release of the line as new salt tolerant cultivar will be discussed.

MATERIALS AND METHODS

Spring wheat, Triticum aestivum L. Ajeeba cultivar plants were grown in the field and used as an explants source. Wheat spicks were covered with bags before anthesis and they were collected 10-12 days after anthesis. Caryopses were surface sterilized and immature embryos were removed aseptically from the caryopses and cultured on callus induction medium consisted of double the amount of (MS) salts medium (Murashige and Skoog, 1962) with some modifications as described in (Al Hattab, et al., 2002). Induced calli were subjected to 10, 20, 30 Gy of gamma- rays (Co 60) and transferred immediately to fresh callus induction medium. Survived calli from the radiation treatments are transferred to salt stress media of 2.5 (control), 9, 12 or 15 dsm⁻¹ which were adjusted by adding drain water to the callus induction medium with one fold of MS inorganic salts. Shoots that were forming during this period were rooted on regeneration medium which consisted of one fold of MS inorganic salts with corresponding salt level and without growth regulators and transferred to pots for seed production.

Seeds of the regenerated plants were subjected to fast selection in saline hydroponic culture under controlled conditions. Seeds were germinated on filter paper saturated with water of 10 dsm⁻¹. Seedlings were transferred to test tubes size (2 X 15 cm) filled with half strength Hoagland solution (Hoagland and Arnon, 1938) with the addition of drain water to adjust the EC to 10 dsm⁻¹ (Al Hattab, et al., 2002). Well growing plants were transferred to the field for seed production. Seeds of the survived plants were grown the next season in the field with soil of 20 dsm⁻¹ salinity level or above at Al Lattifia Research station of The Ministry of Science and Technology, Iraq to evaluate their salt tolerance ability. Several lines were selected based on their morphological characteristics and production parameters. The selected lines were subjected to several analyses such as total protein content, disease tolerant, and rheological properties. One promising line was then selected and submitted to the Iraqi Ministry of Agriculture, The National Committee for the Registration, Release and Protection of Iraqi Cultivars for the registration as a new salt tolerant cultivar.

RESULTS AND DISCUSSION

Calli were formed on the upper surface of the scutellum during the first week in culture. Fast growing embryogenic calli with green sectors were formed which indicated that Ajeeba cultivar has a good response to callus induction and plant regeneration (Figure 1- A). Many factors affect callus induction from different plant species such as medium components, type and the amount of growth regulators, type and age of the explants and some environmental parameters. Cultivars of the same plant species varied in their response to tissue culture under the same conditions have been reported as in barley (Al Qaudhy et al., 1998) and wheat (Sears and Decckard, 1982). Using substitution analysis technique in some wheat cultivars, it has been found that genes located on chromosome 1B control callus induction (Galiba et al., 1986) which confirmed that tissue culture response is genetically controlled. Moreover, researchers found that in Arabidopsis callus is induced through the genetic pathway mediating lateral root initiation not as dedifferentiated as previously reported (Atta et al., 2009; Sugimoto et al., 2010; Ikeuchi et al., 2013).

Well growing calli were selected and subjected to different radiation treatments and transferred to fresh callus induction medium to eliminate radiation effect on the old medium. This step is very important to protect callus cells from the changes in the medium component by the radiation treatment. Radiolysis products in the medium will impose negative effects on both control and irradiated callus cells (Reinert and Bajaj, 1977).
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**Figure 1:** (A) Callus induce from immature embryos of Ajeeba wheat cultivar grown on double MS medium (B) Calli types after treatment with different levels of salinity and different doses of gamma-rays

Callus from all radiation treatments subjected to salt selection agent on media with different salinity levels. The calli were evaluated according to their type (Figure1-B) and the results showed that salinity had drastic effect on callus development (Figure 2). As the salinity increased callus differentiation and regeneration decreased in the control radiation treatment. This reduction could be due to the inhibition of DNA and amino acids synthesis in the from the control salinity level 55% which was decreased as the salinity level increased except at 12dsm⁻¹ the percentage of calli with shoots was 7 compared with 19% at 15dsm⁻¹ salinity level. This might be attributed to the genetic variations which might occur in some cell lines increasing their salt tolerance and don't loss their regeneration ability. These variations are known as somaclonal variations are source for the selection of plants with desirable characteristics (Cheng, et al., 1998; Nwauzoma and Jaja , 2013).

The radiation treatment of 20 Gy increased the percentage of callus developing roots with the control salinity level and it reached 45% (Figure 2-B) compared with 13% from the control radiation. The callus development was inhibited when treated with 30 Gy gamma-rays and grown on media supplemented with the salt levels 12 and 15dsm⁻¹ (Figure 2-C). The results showed that the interaction between salinity and ionizing radiation had negative effect on callus differentiation. No shoots were regenerated from 30 Gy treatments with all salinity levels as well as 20 Gy treatment with 12 and 15 dsm⁻¹. However, several plants were regenerated from 20 Gy treatments with 9 dsm⁻¹. Moreover, many plants were regenerated from the calli that grown on the highest salinity level 15dsm⁻¹ without radiation treatment. The formation of shoots from this treatment might be due to genetic changes in few cells that were able to tolerate the salt selection condition. This result indicated the random effect of radiation on the callus cells. Generally the number of the regenerated plants is reduced as the radiation dose increased. Therefore, the maximum recommended dose is 20 Gy of gamma-rays (Co⁶⁰). The selected line in the current investigation was originally regenerated from calli treated with 20 Gy of gamma-rays (Co⁶⁰) and grown on medium of 9 dsm⁻¹ salinity level.

Seeds of the regenerated plants were subjected to10 dsm⁻¹ salinity level in hydroponic culture along with other regenerated plants from other cultivars in a comparison experiment and the results are published in Al Hattab, et al., (2002). Hydroponic culture is the best for fast plant screening under salt stress. Salinity level is controlled and adjusted by measuring the electric conductivity (EC) of aqueous culture periodically during the growth period of the plants. Moreover, the selecting factor is in direct contact with the roots which prevent the falls selection process. Also large number of plants could be tested in limited space, short time and less men labor.
Figure 2: Radiation and salinity effect on callus development induced from immature embryos of Ajeeba wheat cultivar.
Hundreds of plants could be eliminated in this step at the seedling stage. Researchers found that seed germination and seedling growth are more sensitive than the plant grown under salt stress (Ali et al., 2017; Alwan et al., 2015). From our work 10 EC is the recommended level for wheat seedling selection in hydroponic culture.

Only 14 seedlings were selected from the hydroponic experiment and transferred to the field to be tested in the saline land for further selection at advanced growth stage.

The line under investigation was coded 81 and it was among the selected plants from the hydroponic culture which were transferred to the field with high salinity level (20 – 30 EC) for seed production (Figure 3). Researchers used different techniques to screen large number of wheat genotypes. Most of those experiments were conducted in the glasshouse and for a short time. Some of the techniques are summarized by (Munns and James 2003). Other researchers used the biomass of the plants growing in high saline level relative to the biomass in normal soil as a screening criterion (Kingsbury and Epstein, 1984; Martin et al., 1994; Sobieh, 2015). In the current experiment the selection was based on the performance in the field under high salinity stress.

After several compression field experiments with the local cultivars and salt tolerant introduced cultivars (Data not shown), line 81 was the best under salt stress in the production parameters such as spick length, number of seeds per spick, weight of 1000 seeds. The morphological characteristics of line 81 (Hanaa cultivar) plants grown under salt stress are summarized in (Table 1) as shown in (Figure 4).

Table 1. Characteristics of line 81 (Hanaa wheat cultivar) grown under salt stress

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Measurements*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Average plant height</td>
<td>95 cm</td>
</tr>
<tr>
<td>2 Average stem width</td>
<td>6 mm</td>
</tr>
<tr>
<td>3 Leaves color</td>
<td>Dark green</td>
</tr>
<tr>
<td>4 Average flag leaf length</td>
<td>28 cm</td>
</tr>
<tr>
<td>5 Average flag leaf width</td>
<td>2.5 cm</td>
</tr>
<tr>
<td>6 Average awn length</td>
<td>8.5 cm</td>
</tr>
<tr>
<td>7 Average spick length</td>
<td>17 cm</td>
</tr>
<tr>
<td>8 Average number of tillers</td>
<td>7</td>
</tr>
<tr>
<td>9 Average 1000 seeds weight</td>
<td>35 g</td>
</tr>
<tr>
<td>10 Spick color at maturity</td>
<td>Dark beige</td>
</tr>
<tr>
<td>11 Seed texture</td>
<td>Chalky</td>
</tr>
<tr>
<td>12 Total seed protein</td>
<td>14</td>
</tr>
</tbody>
</table>

*All numbers are the average of 10 samples
Figure 4. Hanaa cultivar growing in the field during the selection process (A), Spicks of Hanaa cultivar

Table 2. Physical test of Hanaa cultivar seed sample

<table>
<thead>
<tr>
<th>Contamination (weed &amp; dirt) %</th>
<th>% of wrinkled seeds</th>
<th>% Seed moisture (Momo)</th>
<th>% Seed moisture (field unit)</th>
<th>1000 seeds weight</th>
<th>Hectoliter weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.2</td>
<td>7.7</td>
<td>7.7</td>
<td>34.2 gm</td>
<td>76 Kg/ hectoliter</td>
</tr>
</tbody>
</table>

Table 3. Milling test of Hanaa cultivar seeds

<table>
<thead>
<tr>
<th>Sample Seeds weight (gm)</th>
<th>Flour weight (gm)</th>
<th>Fine bran (gm)</th>
<th>Coarse bran (gm)</th>
<th>Extraction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1135</td>
<td>659</td>
<td>134.7</td>
<td>78.1</td>
<td>75</td>
</tr>
</tbody>
</table>

The quality tests were performed by The Iraqi Ministry of Trade, Grain Company, Quality Control Department. The results of seeds physical tests are shown in (Table 2) which indicated that the seeds are clean of any contaminations, small seeds 0.2%, moisture 7.7%, 1000 seeds weight 34.2 g and the Hectoliter weight 76 kg/ hectoliter. Moreover, milling results (Table 3) showed that out of 1135 g the amount of fine bran was 134.7 g, coarse bran was 78.1 g and extraction percentage was 75%. After water addition flour weight was 1300 g, the moisture was 15.3% (by Motomco moisture model 919). Moreover, bread quality evaluation showed that bread color is light brown, test and smell were good. It is good for Iraqi bread type.

This line was then submitted to the Iraqi Ministry of Agriculture, The National Committee for the Registration, Release and Protection of Iraqi Cultivars. Twenty kilo grams of pure seeds were given to the office which were tested in salty land and compared with other local cultivars according to their standards. The National Committee confirmed our results and accepted the line as a new salt tolerant wheat cultivar.

CONCLUSION
Combination of tissue culture, physical mutation, hydroponic culture and field test were applied for the selection of salt tolerant wheat plants. The result of all these steps is the registration and the adaptation of line 81 as a new salt tolerant bread wheat Iraqi cultivar which was released under the name HANAA cultivar.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT
The authors warmly thank the technical staff of the tissue culture laboratory of Biotechnology Center at The Ministry of Science and Technology Mr. Jabar Naseer and Mr. Shukor K Ibrahim for the great efforts in the plant nursery management. Especial thanks to the staff of Al Lattifa Research
station, Quality Control Department of Grain Company, Iraqi Ministry of Trade and The National Committee for the Registration, Release and Protection of Iraqi Cultivars for their efforts, without them the fruits of this research would never be achieved.

AUTHOR CONTRIBUTIONS
The breeding program was suggested by Dr. Zahra N. Al Hattab who supervised all the research steps and wrote the manuscript. Mr. Mahmood A. Al Ani did the tissue culture part, collected and analyzed all the data. Mr. Raad J. Al Gburi Mr. Salih Kh. Al Raheem and Mr. Falah N. Hussain conducted the hydroponic and the field experiments and collected the data. All authors reviewed and approved the final manuscript.

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REFERENCES
Hoagland, D.R. and Arnon, D.I. 1938 The water
culture method for growing plants without soil. California Agricultural Experiment Station Circulation, 347, 32.