Evaluation of the 3rd Generation of Backcrosses and its Parents of Two Bread Wheat (*Triticum aestivum* L.) Cultivars for Drought Tolerance

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Abstract

Although drought stress has been well documented as an effective parameter in decreasing crop productivity in arid and semi-arid regions, developing and releasing new cultivars which are adaptable to water deficit can be a constructive program to overcome unsuitable environmental conditions. The present study was carried out to evaluate the performance of bread wheat genotypes in relation for yield and its components, biological yield, water use efficiency WUE, harvest index HI and stress susceptibility index (SSI) fewer than three of water irrigation regimes. The experiment was laid out in split plot based on a complete randomized block design, with three replications at the Center of Plant Breeding and Improvement at Al-Tuwaitha Research Station (30km southeast of Baghdad). Irrigation treatments (200, 300 and 400mm as control) were considered as the main plots, whereas sub-plots were assigned of two bread wheat cultivars Rasheed and Baraka sensitive cultivars having good yield and quality and their two hybrids at fourth back cross hybridization; 643xRasheed, 649x Baraka).

Results showed that grain yield and its component, decreased as the water amount decreased. Whatever, the biological yield behaved the same trends but the reduction differ from genotype to another, and the two hybrids showed less reduction in all characters mentioned above. Results also reveal that all genotypes, cultivars in addition to their hybrids differed in water use efficiency and there was significant difference among them. The hybrids (643xRasheed) and (649xBaraka) exceeded in WUS of their parents and gave 1.837, 1.857kgm^-3, respectively under 200 mm of water irrigation. Interpretation may related to the high grain yield (3675, 3715kgm^-3), respectively. The highest HI (33.49%) founded in the treatment of 200mm than the other two water irrigation treatments. The increase of HI in the 200mm of water irrigation was related to the decreasing biological yield under water deficit condition. Under 200 and 300mm of water irrigation treatments, the genotypes 643, 649c and their hybrids showed the lowest SSI values (<1), whereas cultivars Rasheed and Baraka gave more than 1SSI. The two hybrids gave the lowest values of SSI, especially under the drought stress (200mm) by 0.835, 0.813 for (643xRasheed) and (649c Baraka), respectively. Significance differences for interaction between water irrigation supplies and genetic materials for WUE and SSI revealed the importance of diagnosing the genetic stability in further studies. Backcross (649c Baraka) gave the highest mean value for WUE (1.837 Kg m^-3) at 200mm in comparison with the other water supplies (1.367 and 1.111Kg m^-3 for 300 and 400mm), respectively. The lowest mean value for SSI under the drought stress of 200mm were 0.835 and 0.813 for (643xRasheed) and (649c Baraka), respectively.

Keywords: *Triticum aestivum* L.; Water irrigation supplies; Grain and biological yield; Water use efficiency; Harvest index

Introduction

Drought is a major abiotic stress affecting global crop production. Many genes are involved in plant responses to drought, and some can be used for the improvement of drought tolerance by genetic engineering [1]. Regulatory genes that activate or deactivate suites of drought responsive genes are of particular interest to biotechnologists [2-4]. Genes encoding dehydration-responsive element-binding (DREB) transcription factors (TFs) comprise one of the major groups of genes involved in drought response regulation [5]. According to some estimates, almost 50% of wheat cultivated in the developing world (50 million ha) is sown under rain-fed systems, which receive less than 600mm of precipitation per annum. This rainfall could be as low as less than 350mm per annum in areas inhabited by the poorest and most disadvantaged farmers of the developing countries [6].

Wheat crop in Iraq affected by different types of biological and non-biological stresses during the growing season in almost all agricultural areas, regardless the crop irrigation methods. The scarcity of water during the planting season has become
an obstacle to the cultivation of the first food security crop in Iraq and despite the presence of the Tigris and Euphrates rivers; planting of about 2 million hectares with wheat crop does not exceed 2.5 million tons. The decrease in grain yields due to drought conditions associated with a severe shortage of rainfall during season, and other abiotic and biotic constraints [7].

Statistical data indicate that more than 65% of wheat production comes from the northern region of Iraq. Although the high percentage of wheat production, it faces two problems, 1) delayed of rain fall season and 2) inadequate in most seasons. The residual quantity of production (35%) comes from the central and southern regions where irrigated agriculture suffers from scarcity of water irrigation and all cultivars cultivated in these areas are not drought-tolerant. Thus, each phase of crop growth will be negatively affected relatively by drought. For this purpose, elite and high yielding wheat cultivars that are sensitive to drought may be used as recipient parents in a backcrossing program involving drought-tolerant genotypes as the male donor parent. In this study, conventional hybridization and backcrossing were used to transfer-gene(s) from promising moderate tolerant genotypes (643 and 946c) for local released wheat cultivars (Baraka and Rasheed). The study includes the following: investigation of the inheritance of constitutive and inducible levels of transgenic expression in hybrid plants and progeny during three consecutive backcrosses, (ii) evaluation of the transgenic influence on development of the recipient cultivars in the absence of stress, and (iii) confirmation of the transgenic product functionality in backcross-derived lines by comparison of (1) drought tolerance and (2) grain yields of control and trans gene-containing plants under well-watered conditions and under moderate drought stress at six growth stages.

Materials and Methods

Table 1 reveals that the soil texture of the field experiment was silt- clay-loam with low organic matter content, 2.1 and 7.6 of Ec and pH, respectively. After preparing the field by plowing, disking and properly leveled it was divided into plots of (1.0×1.0m) dimension which separated by a distance of 1/2m to prevent the movement of water laterally. Two back crosses at BC3 seeds with their parents (643, 649c, Rasheed and Baraka) were planted on 20 - 12 - 2015 for each water irrigation supply (400, 300 and 200 mm) under rainout shelter. The genotypes called 643 and 649c were obtained from previous breeding program which practiced hybridization among saber beg, the local cultivar, Mexipak and the mutant R24 [8]. However, about 70 experimental genotypes were evaluated for drought tolerance and resulted the exceeding the two indicated genotypes [9]. Nitrogen fertilizer was applied by the recommended dose of N (200Kg ha⁻¹) during planting and tillering (45 days after planting). Phosphorus fertilizer was applied at the rate of 70kg of P₂O₅ ha⁻¹ as the form of superphosphate (16% P₂O₅) at planting [10] (Figure 1).

![Figure 1: Backcrossing cycles practiced among the bread wheat genotypes and cultivars (according to Yousif and Al-Kafaje, 2000).](image)
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Table 1: Physical and chemical properties of 0-40 cm of soil profile.

| Properties       | Values   | Units   |
|------------------|----------|---------|
| Sand             | 140      | g kg⁻¹  |
| Silt             | 310      | g kg⁻¹  |
| Clay             | 550      | g kg⁻¹  |
| Soil texture     | slit-clay loam |       |
| EC 1:1           | 2.1      | dsm⁻¹   |
| pH 1:1           | 7.6      |         |
| O.M              | 4.2      | g kg⁻¹  |
| Bluck density    | 1.27     | g cm⁻³  |

Table 2: Water irrigation supplies during wheat crop growth and development (mm) according to each growth stages.

| Growth Stages and Dates | Irrigation Amount (mm) |
|-------------------------|------------------------|
| Sowing(20-Dec. 2015)    | 70                     |
| Tillers (15-Jan. 2016)  | 60                     |
| Stem elongation (10-Feb. 2016) | 65                |
| Booting (1-March 2016)  | 65                     |
| Anthesis (16-March 2016) | 75                  |
| Physiological maturity (15-April 2016) | 75                |
| Total of water irrigation supplies | 400                  |

WUE = (Grain yield / total water used) …….. (Ehdaie [12])

SSI = [(1-YS /YP) / (1-ȲS/ȲP)] ………… (Fisher & Maurer [13])

YS, YP = Yield for genotypes under stress and non-stress conditions.

Water irrigation supplies

Spikes m⁻²: Table 3 reveals that water deficit conditions during the different growth and development stages were decreased the number of spikes m⁻². Water scarce decreased spikes number by 11%, 19% in the 200 and 300 mm compared to the 400mm (control). This result may due to the death of some tillers carrying spikes. The highest negative effect of water limitation was observed during the floral initiation (Booting and anthesis stages). Previous similar results were reported by Adel et al. [14]; Nazeri [15]; Robertson & Guinta [16] for wheat and triticale.

Number of grain per spike

The number of the grain per spike is an important quantitative trait as an essential grain yield component under drought and/or good environment. Current results indicated that there was significant difference among water irrigation supplies. Water deficit caused decreasing of number of grain per spike by 10%, 11% for 200 and 300mm in comparison with control (400mm). The booting to anthesis was the most susceptible period for grain number per spike under water-limited condition, and could be considered the most crucial growth and developmental stage for the final grain yield. Results agreed with Al-Maeeny [17].

1000 grain weight

Although the number of grain spike⁻¹ has a predominant importance over grain weight with regard to grain yield, grain weight is well documented to be a major yield component determining final yield in Mediterranean environments [15]. Results in Table 3 indicated that water deficit in the 200 and 300mm water supplies were decreased the 1000 grain weight by 9.8, 23.5% respectively compared with the control. The most susceptible growth and developmental stage with regard to 1000 grain weight is the anthesis to grain filling stage. Researchers have reported that water limitation during grain filling significantly decreased grain weight Nazeri [15], Slafer & White-church [18].

Effect of water irrigation on grain and biological yield

The results of the present study indicated that different of water irrigation supplies during growth and development stages had different considerable effects on grain yield (Table 3). There was significant difference among the water amount on grain yield (4405Kg ha⁻¹) which produced under optimum water irrigation supply (control) whilst the lowest (3525Kg h⁻¹)
was observed in the lowest water irrigation supply (200mm). Grain yield decreased by 10.5, 19.9 % for 300 and 200 mm water irrigation supply, respectively compared with the control (400mm). Reduction of grain yield may due to the drastically decrease for one or more of yield component.

Table 3 showed that the biological yield decreased as the water irrigation supplies decreased in 300, 200 mm by 16.6, 27.6%, respectively in comparison with control. There were significant differences among the water irrigation supplies. The highest biological yield (14350Kg ha⁻¹) was produced under optimum irrigation treatment (400mm) whereas, the lowest (10524Kg ha⁻¹) was investigated in 200mm. Result may interpreted to the decrease the number of tillers per area unit. Results agreed with Saleem [19], García Del Moral et al. [20], and Krigwi et al. [21] for bread and durum wheat.

### Table 3: Effect of water irrigation supplies on grain and biological yield, harvest index and water use efficiency of wheat backcrosses and its parents.

| Irrigation amount (mm) | No. of spike m⁻² | No. of grain spike⁻¹ | 1000 grain weight (g) | GY Kg ha⁻¹ | BY Kg ha⁻¹ | HI % | WUE Kg m⁻³ |
|-----------------------|------------------|----------------------|-----------------------|------------|------------|------|-------------|
| 400                   | 553.4            | 44.78                | 31.52                 | 4405       | 14530      | 30.32| 1.101       |
| 300                   | 491.3            | 40.26                | 28.43                 | 3942       | 12121      | 32.52| 1.314       |
| 200                   | 443              | 35.52                | 24.12                 | 3525       | 10524      | 33.49| 1.762       |
| LSD P ≤ 0.05          | 45.26            | 3.51                 | 2.93                  | 389.7      | 987.8      | 0.85 | 0.21        |

Data presents the average of all genotypes over three replications.

**Effect of water irrigation on Harvest Index (HI)**

Harvest index is the proportion of grain yield to biological yield and it shows the ability of the plants to translocation physiological matters to grains. Table 3 showed that there was significant difference among the three water irrigation supplies on harvest index. The highest HI (33.49%) founded in 200 mm. The increase of the HI in the lowest irrigation supply was due to the decreasing of biological yield under water deficit conditions. These results are concurrent with the findings of Al-Maeeny [17].

\[ \bar{YP} = \text{Average yield of genotypes in the drought stress and non-drought condition.} \]

The experiment was laid out in randomized completely block with split-plot design. Water irrigation supplies presents the main plots and genotypes as sub plots with three replications. Data were subjected to Analysis of Variance, and means were separated by LSD at P≤0.01 using Gen Stat Statistical software (version 2013).

**Water Use Efficiency (WUE)**

Results in Table 4 reflect the significant differences among water irrigation supplies. Since, the highest water use efficiency (1.762 Kg m⁻³) was observed at 200mm in comparison with the other water supplies (1.314 and 1.101 Kg m⁻³ for 300 and 400mm). The low water use efficiency for 400 mm may be attributed to higher irrigation water use during growing season and on the other hand, the increasable amounts of the evapotranspiration and decreasing the net assimilation rate for all genetic materials under investigation. Previous studies emphasized that moisture stress affects water use efficiency for wheat [21]. Oweis et al. [22] for instance reported that water use efficiency varied from 0.66 to 1.34 Kg m⁻³ among different irrigation regimes. Table 4 showed that all genetic materials under investigation differed in water use efficiency and there was significant difference observed. The backcrosses (643xRasheed) and (649cx Baraka) were exceeded in WUE in comparison with their parents and gave 1.837 and 1.857Kg m⁻³, respectively under drought stress of 200mm. The high grain yield for wheat backcrosses (643xRasheed) and (649cx Baraka) (3675 and 3715Kg m⁻³), respectively caused to increase the WUE and may due to the high productivity of its recurrent parents cultivars. Results statistical analysis showed that there was significance interaction between water irrigation and genotypes in WUE, wheat backcrosses (649cx Baraka) gave highest mean value WUE1.837 Kg m⁻³ at 200 mm in comparison with the other water supplies (1.367 and 1.111Kg m⁻³ for 300 and 400mm).

**Stress Susceptibility Index (SSI)**

Stress susceptibility index (SSI) were calculated on the basis of grain yield for all genetic material under investigation the three water irrigation supplies of irrigation. The backcross (649cxBaraka) exceeded significantly with all genetic material except the backcross 643xRasheed and gave the lowest SSI. With the 200 and 300mm water supplies, the genotypes 643, 649 had higher SSI and revealed values more than 1 (Table 4). The two BC gave the lowest mean value for SSI, especially under the drought stress (300 and 200m) which was 0.785 and 0.785 for (643xRasheed) and (649cxBaraka), respectively. These results agreed with Khan & Naqvi [23] which they found that cultivars with SSI was more than 1 which considered as water susceptible and vice versa for the backcrosses that emphasize its tolerant for drought.

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Table 4: Interaction between irrigation and genotypes affecting water use efficiency for grain yield and Stress Susceptibility Index.

| Genotypes | Water Irrigation supplies (mm) | WUE (Kg m⁻³) | Stress Susceptibility Index (SSI) |
|-----------|-------------------------------|--------------|---------------------------------|
|           | GY (Kg h⁻¹)                   | mean         |                                   |
| 400       | 300                           | 200          | 400                             | 300                           | 200                       |                             |
| 643       | 4305                          | 3940         | 3515                             | 3920                          | 1.076                     | 1.313                     | 1.757                     | 1.382                     | 0.807                     | 0.919                     | 0.863                     |
| 649c      | 4352                          | 3992         | 3570                             | 3971                          | 1.088                     | 1.331                     | 1.765                     | 1.401                     | 0.787                     | 0.899                     | 0.843                     |
| 643 x Rasheed | 4411          | 4070         | 3675                             | 4052                          | 1.103                     | 1.365                     | 1.837                     | 1.432                     | 0.735                     | 0.835                     | 0.785                     |
| 649c x Baraka | 4435         | 4100         | 3715                             | 4083                          | 1.111                     | 1.376                     | 1.857                     | 1.439                     | 0.719                     | 0.813                     | 0.766                     |
| Rasheed   | 4454                          | 3750         | 3310                             | 3838                          | 1.113                     | 1.250                     | 1.655                     | 1.339                     | 1.503                     | 1.286                     | 1.394                     |
| Baraka    | 4473                          | 3800         | 3365                             | 3879                          | 1.118                     | 1.267                     | 1.662                     | 1.356                     | 1.431                     | 1.239                     | 1.335                     |
| Means     | 4405                          | 3942         | 3525                             |                               | 1.101                     | 1.314                     | 1.762                     |                               | 0.997                     | 0.998                     |                            |
| LSD P ≤ 0.05 |               |              |                                  |                               |                          |                          |                          |                          |                            |
| Genotypes | 46.7                         | 49.3         | 40                               |                               | 0.012                     | 0.115                     | 0.163                     |                               | 0.115                     | 0.047                     | 0.081                     |
| Interaction | 17.2                      | 27.2         | 9.31                             |                               | 0.002                     | 0.010                     | 0.004                     |                               | 0.021                     | 0.022                     | 0.116                     |

There was significance interaction between water irrigation supplies and the genetic materials under investigation. The two BC₃ gave the lowest mean value for SSI under drought stress of 200mm (0.835 and 0.813) for (643xRasheed) and (649cx Baraka), respectively [24-29].

Conclusion

Although drought stress has been well documented as an effective parameter in decreasing crop productivity in arid and semi-arid regions, developing and releasing new cultivars which are adaptable to water deficit can be a constructive program to overcome unsuitable environmental conditions. The present study indicated that it is possible to improve the drought tolerance character in bread wheat by conventional backcrossing and transferring genes which are responsible for drought tolerance from moderate tolerance genotypes or cultivars to other having high yields and good quality but sensitive to drought. Results reflected the success in obtaining new genotypes with good grain yield and tolerate drought in the targeted region. Bread wheat cultivation in stressed environments which water irrigation supplies not more than 200mm may present the relative solution and solve the drought problem in Iraq. On the other hand, the measurement of WUE and SSI were efficient parameters for studying drought tolerance in wheat cultivars.

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