*Available online at* www.scholarsresearchlibrary.com

**Scholars Research Library**

**Annals of Biological Research, 2012, 3 (8):3898-3904**
*(http://scholarsresearchlibrary.com/archive.html)*

Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

# Selection for drought tolerance in durum wheat genotypes

**Pedram Mohammadi[1], Mohtasham Mohammadi[2] and Rahmatollah Karimizadeh[2]**

*[1]Islamic Azad University-Kermanshah branch, Iran*
*[2]Dryland Agricultural Research Institute, Gachsaran Station, Iran*

## ABSTRACT

*The variable nature of rainfall makes it difficult to select wheat genotypes for drought tolerance in most dry environments. This research was done to determine optimum drought tolerance indices from evaluations of 18 durum wheat (Triticum turgidum ssp. durum) genotypes to stress from drought and reduced water conditions. Genotypes selected for tests were planted in two experiments; one under dryland condition and another with supplemental irrigation (in anthesis and grain filling stages). The experiment was set up as a completely randomized block design with four replications at Gachsaran Agricultural Research Station in 2009-2010. On the basis of grain yield tested in conditions of dryland and supplemental irrigation five drought tolerance indices were assessed: mean productivity, geometric mean productivity, tolerance, stress susceptibility index and stress tolerance index. Drought stress significantly reduced the yield of some genotypes while others were tolerant to drought. These results provide information on genetic variability useful for breeding programs. Based on principal component analysis there was a high correlation between mean productivity, geometric mean productivity and stress tolerance index with grain yield in both conditions, these indices were identified as the more effective indices for durum wheat selection under drought and water limited environments. A bi-plot graph demonstrated that genotypes 18, G14, G4 and G11 were located within regions for potential yield and drought tolerance. Grouping in the cluster analysis confirmed the results of the bi-plot display. The same genotypes had the best ranking with low standard deviation among their ranks. So, they were recognized as tolerant genotypes that are high yielding in both dryland conditions and with supplemental irrigation. It was concluded that selection for yield under partial high water stress can identify superior cultivars, not only for dry environments, but also for those characterized by frequent mild and moderate water stress conditions; G18 is an example of such a genotype that was recently released for sowing in semitropical dryland regions in Iran.*

**Key words:** Bi-plot; Clustering; Drought tolerance indices; Principal component analysis; Supplemental irrigation

## INTRODUCTION

Durum wheat (*Triticum turgidum* ssp.durum) is the second most important wheat species and is cultivated in about 21 million hectares [2]. Durum production has been a part of people's diet, for a long time [7].

There is a planning strategy in Iran to have at least 6.7 million hectares; of a total of 14 million hectares of land area dedicated to the cultivation of wheat cultivars. The plan is that about one third of this area is to be sown under irrigation conditions and the other parts specified to dryland environments. Wheat production was between 1.9 and 3.9 million tons in dryland environments in different years [7].

Water deficiency is the main universal constraint to cause reduced yield in cereal crops and it is a problem that may intensify in the future [21]. The current global average yield of wheat is approximately 2.5 tons per hectare. By 2020 yield needs to increase to 4.2 in order to meet the global demand. This translates into an annual increase of 85 kilograms per hectare for the next 20 years [16].

At least 60 million hectares of wheat is grown in marginal rainfed environments in developing countries. National average yields range from 0.8 to 1.5 t/ha, approximately 10 to 50% of their theoretical irrigated potential [17]. Half the area sown to wheat in developing countries and up to 70% of that grown in developed countries suffers from periodic drought [24].

The annual gain in genetic yield potential in drought environments is only about half (0.3-0.5%) of that obtained in irrigated, optimum conditions. Many investigators have attempted to produce wheat adapted to semiarid environments but with limited success. The CIMMYT wheat program follows a system of breeding for drought tolerance in which yield responsiveness is combined with adaptation to drought conditions. Because semiarid environments differ significantly in terms of annual precipitation distribution and water availability across years in these environments, it is therefore, prudent to construct a genetic system in which plant responsiveness provides a bonus whenever higher rainfall improves a production environment [23].

Several indices have been used to evaluate genotypes for drought resistance based on grain yield such as mean productivity (MP) and tolerance (TOL) [19], stress susceptibility index (SSI) [5], geometric mean productivity (GMP) and stress tolerance index [4]. According to Richards [18], selection for yield automatically integrates all the known and unknown factors that contribute to drought resistance. These indices have been compared in other research [6, 8, 11, 22].

Nachit [14] maintains that drought, cold and heat are the most important constraints for durum wheat production in Mediterranean regions. The combination of abiotic and biotic stresses makes plant breeding in the Mediterranean dryland areas complex and very challenging. The ICARDA program's main objective is to develop genotypes and genetic stocks combining yield potential with resistance to drought and other abiotic and biotic stresses that also facilitate improved grain quality [7].

Most durum wheat produced in Iran is cultivated in semitropical dryland regions. Heat and drought stresses are the main constraints in this region. Various strategies can be employed or developed to improve the efficiency of germplasm development targeted specifically to dry environments. Identification of durum wheat varieties with high production values together with tolerance to environmental stresses is aim of an optimum breeding strategy.

The present study was undertaken to assess selection criteria for identifying drought tolerance and high yield production in durum wheat genotypes, so that suitable genotypes can be recommended for cultivation in drought prone areas of Iran under various climatic conditions in semi-warm regions.

## MATERIALS AND METHODS

Experiments were conducted in Gachsaran Agricultural Research Station (30º 20´N, 50º 50´E, with an elevation of about 710 m above sea level) during 2009-2010. Eighteen durum wheat genotypes were planted on 25 November in a randomized complete block design under dryland and supplemental irrigation conditions with four replications.

Each plot was 7.03 m long with six rows spaced 17.5 cm apart and sown by a small-plot planter (Wintersteiger) at a density of 300 seeds/m$^2$. The soil texture was silty-clay loam, with pH= 7.3-7.8, and less than 1% organic matter. Fertilizers were applied completely before sowing (90 kg N ha-1 and 75 kg P2O5 ha-1). Supplement irrigation was applied at the flowering and grain filling stages. The harvested plot size for grain yield was 6 m$^2$ and the grain yield of each individual plot was separately harvested and measured.

For estimating the tolerance and susceptibility of genotypes the following indices were used:
Stress Susceptibility Index (SSI) [5], Tolerance (TOL) [19], Mean Productivity (MP) [19], Geometric Mean Productivity (GMP) and Stress Tolerance Index (STI): [4].

$$SSI = [1 - (Ys / Yp)] / SI \qquad SI = 1 - \left( \bar{Ys} / \bar{Yp} \right) \qquad TOL = Yp - Ys$$

$$MP = (Yp + Ys) / 2 \qquad GMP = \sqrt{(Yp \times YS)} \qquad STI = [Ys \times Yp] / \left( \bar{Yp} \right)^2$$

Where: *Yp* =Mean yield of the genotype under non-stress conditions; *Ys* =Mean yield of the genotype under stress conditions, *Yp* =Mean yield of all genotypes under non-stress conditions and *Ys* = Mean yield of all genotypes under stress conditions.

To classify the indices as well as the genotypes, a bi-plot display was used based on principal component analysis (PCA). Furthermore, grouping of genotypes was performed using UPGMA based on Euclidean distance. Grain yield at two environments and values of different indices for each genotype were ranked as well as calculations done for standard deviation.

Data were analyzed using SAS and Genestat software for analysis of variance, PCA, genotype clustering and Duncan's multiple range test was used for means comparisons.

Total rainfall was 402.8 mm, and that was 28.2 less than that of the long-term data. Distribution of rainfall was 225, 111.4 and 66.4 mm in fall, winter and spring respectively. The mean temperature during the cropping season was 20 ˚C; that is 1.1˚C less than the long-term average.

## RESULTS

Maximum grain yield in dryland condition was recorded for genotypes G18 and G14 respectively, which showed significant preference compared to other genotypes. In supplemental irrigation condition, G5, G18 and G16 had significant preference to G12, G3, G10 and G2 (Table 1).

According to the TOL, records showed that G12, G17 and G8 had the most tolerance and G5, G9 and G15 had the least tolerance. In terms of SSI, G18, G17 and G12 showed the least susceptibility. Based on the MP index, G18, G16 and G5 were identified as the most tolerant genotypes and G9, G10 and G3 with lower values on this index were the most susceptible genotypes. Using STI and GMP indices it can be deduced that G18, G14 and G16 were the most tolerant genotypes: in contrast genotypes G9, G10 and G3 showed high sensitivity. It seems that G18, G14 and G16 had better performance in dryland and supplemental irrigation conditions. Ranking of grain yield for genotype in two environments and different indices for each genotype showed that genotypes G18, G14 and G11 had the best ranking with low standard deviation of rank.

Grain yield in the two environments that were tested had positive significant correlation, in addition to high significant correlations with MP, GMP and STI indices. Generally, those indices having high correlation with performance in different conditions, were introduced as the best indices because they separated and identified genotypes with high production in diverse environments. So, MP, GMP and STI indices were identified as the best indices for screening and identification of superior genotypes in various environments with different levels of stress.

Principal component analysis on grain yield and different indices formed five components. The first two components justified more than 99 percent of existing data variation (Table 3).

The first component explained 68.1 percent of variation between existing data and depicted positive coordination with grain yield in dryland and supplemental irrigation conditions and Mp, GMP and STI indices. So this component identified high yield and tolerance. The first component separates high production and tolerant genotypes from low yield and sensitive genotypes. Maximum value of this component belonged to genotypes G18 and G14.

**Table 1. Values of stress tolerance indices from the potential yield and the stress yield data for18 bread wheat genotypes**

| Gen | Ys (kg.ha$^{-1}$) | Yp (kg.ha$^{-1}$) | TOL | MP | STI | GMP | SSI | R | SDR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2108de[17] | 4793abcdef[11] | 2685[14] | 3450[13] | 0.426[14] | 3178[14] | 1.15[15] | 15 | 1.826 |
| 2 | 2138cde[15] | 4461cdef[15] | 2323[10] | 3300[15] | 0.402[15] | 3089[15] | 1.06[14] | 16 | 1.864 |
| 3 | 2231bcde[13] | 4250ef[17] | 2019[4] | 3241[16] | 0.400[16] | 3080[16] | 0.97[8] | 13 | 4.981 |
| 4 | 2684abcd[6] | 4967abcdef[9] | 2283[8] | 3825[8] | 0.562[5] | 3651[5] | 0.94[6] | 5 | 1.604 |
| 5 | 2401bcde[12] | 5492a[1] | 3092[18] | 3947[3] | 0.556[7] | 3631[7] | 1.15[15] | 10 | 6.245 |
| 6 | 2520bcd[10] | 5067abcd[6] | 2547[12] | 3793[9] | 0.539[11] | 3573[11] | 1.03[10] | 11 | 1.952 |
| 7 | 2562bcd[9] | 5145abc[4] | 2583[13] | 3853[7] | 0.556[7] | 3631[7] | 1.03[10] | 9 | 2.854 |
| 8 | 2732abcd[4] | 4723abcdef[12] | 1991[3] | 3728[11] | 0.545[10] | 3593[10] | 0.86[4] | 8 | 3.861 |
| 9 | 1813e[18] | 4633bcdef[14] | 2820[17] | 3223[17] | 0.354[18] | 2898[18] | 1.24[18] | 18 | 1.464 |
| 10 | 2115de[16] | 4300def[16] | 2185[6] | 3207[18] | 0.384[17] | 3015[17] | 1.04[12] | 17 | 4.237 |
| 11 | 2717abcd[5] | 5032abcde[7] | 2315[9] | 3874[5] | 0.557[4] | 3698[4] | 0.94[6] | 3 | 1.799 |
| 12 | 2512bcd[11] | 4181f[18] | 1670[1] | 3346[14] | 0.443[13] | 3241[13] | 0.82[2] | 12 | 6.370 |
| 13 | 2593bcd[8] | 5125abc[5] | 2532[11] | 3859[6] | 0.561[6] | 3646[6] | 1.01[9] | 7 | 2.138 |
| 14 | 2786ab[2] | 4983abcde[8] | 2196[7] | 3884[4] | 0.586[2] | 3726[2] | 0.90[5] | 2 | 2.498 |
| 15 | 2150bcde[14] | 4958abcdef[10] | 2808[16] | 3554[12] | 0.450[12] | 3265[12] | 1.16[17] | 14 | 2.498 |
| 16 | 2595bcd[7] | 5317ab[3] | 2722[15] | 3956[2] | 0.582[3] | 3714[3] | 1.05[13] | 4 | 5.350 |
| 17 | 2780abc[3] | 4718abcdef[13] | 1938[2] | 3749[10] | 0.553[9] | 3621[9] | 0.84[3] | 6 | 4.282 |
| 18 | 3329a[1] | 5491a[2] | 2162[5] | 4410[1] | 0.771[1] | 4275[1] | 0.81[1] | 1 | 1.496 |

The second component justified 31.7 of variation and showed negative correlation with grain yield in the dryland environment and positive correlation with grain yield under supplemental irrigation, SSI and TOL indices. This component separates those genotypes with moderate yield and low stability. In consideration of this component, the most value related to G5 and G15 (Table 4).

**Table 2. The correlation coefficients between Yp, Ys and drought tolerance indices.**

| | Ys | Yp | TOL | MP | STI | GMP | SSI |
|---|---|---|---|---|---|---|---|
| Ys | 1 | | | | | | |
| Yp | 0.521* | 1 | | | | | |
| TOL | -0.396$^{ns}$ | 0.577* | 1 | | | | |
| MP | 0.856** | 0.888** | 0.136$^{ns}$ | 1 | | | |
| STI | 0.937** | 0.782** | -0.056$^{ns}$ | 0.980** | 1 | | |
| GMP | 0.937** | 0.786** | -0.051$^{ns}$ | 0.982** | 0.998** | 1 | |
| SSI | -0.802** | 0.087$^{ns}$ | 0.861** | -0.380$^{ns}$ | -0.545* | -0.545* | 1 |

**Table 3. The results of stepwise regression analysis on grain yield and different indices**

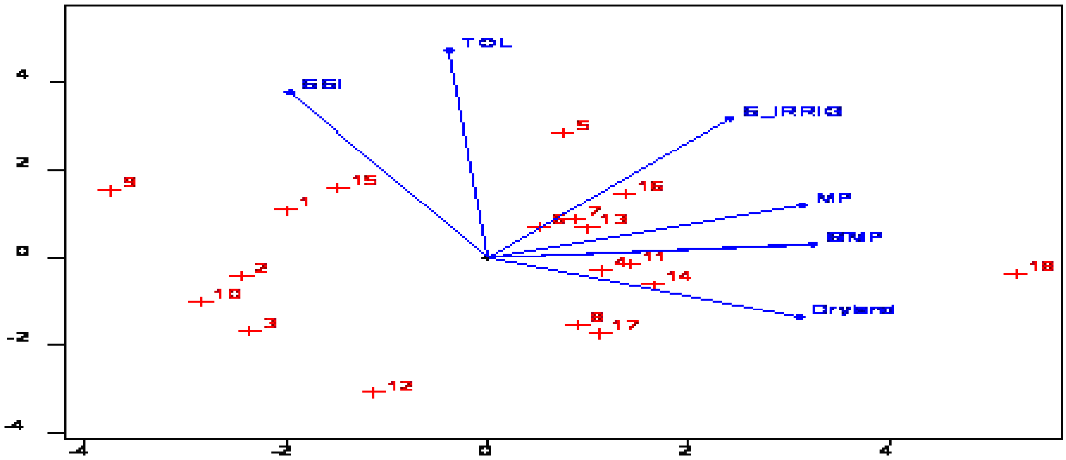
| Component | %Var. | Cumulative Var. | Ys | Yp | TOL | MP | STI | GMP | SSI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.6814 | 0.68141 | 0.4387 | 0.3404 | -0.0537 | 0.4431 | 0.4563 | 0.4567 | -0.2745 |
| 2 | 0.3174 | 0.99888 | -0.1916 | 0.4483 | 0.6658 | 0.1684 | 0.0421 | 0.0445 | 0.5355 |
| 3 | 0.0009 | 0.99987 | 0.1093 | -0.2323 | -0.3605 | -0.0830 | 0.5867 | -0.0782 | 0.6684 |
| 4 | 0.0000 | 0.99997 | 0.2035 | -0.0403 | -0.2655 | 0.0648 | -0.6014 | 0.5904 | 0.4145 |
| 5 | 0.0000 | 1 | 0.4640 | 0.2484 | -0.1812 | 0.3907 | -0.2894 | -0.6591 | 0.1381 |

Regarding the bi-plot display based on the first two components, G18, G14, G4 and G11, in the vicinity of MP, GMP and STI indices were identified as stable high yielding genotypes. This was mainly due to yield potential and drought tolerance region (Fig 1: right). Genotypes No. 9, G1, and G15 were identified as drought sensitive due to location in regions sensitive to drought stress and low yield (Fig 1: left).

Genotype grouping by cluster analysis (UPGMA method), using MP, GMP and STI indices and yield in dryland and supplemental irrigation conditions are shown in Figure 2. The Dendrogram showed that only G18 was placed in the first group. This genotype, in terms of yield in supplementary irrigation and dryland conditions was superior compared to other genotypes, according to MP, GMP and STI indices. The other genotypes were separately classified in the second group (Figure 2).

**Table 4. Results of principal component analysis for Yp, Ys and drought tolerance indices on 18 Durum wheat genotypes**

| Gen. | First component | Second component | Third component | Fourth component | Fifth component |
|---|---|---|---|---|---|
| 1 | -2.00456 | 1.11471 | 0.04515 | 0.028428 | 0.013806 |
| 2 | -2.43338 | -0.41392 | 0.04614 | 0.003322 | -0.015661 |
| 3 | -2.35944 | -1.66845 | 0.02440 | -0.013854 | -0.022239 |
| 4 | 1.12777 | -0.31052 | -0.01773 | 0.026613 | 0.001904 |
| 5 | 0.76289 | 2.85208 | -0.15247 | -0.057275 | -0.007852 |
| 6 | 0.52431 | 0.71600 | -0.01495 | 0.022922 | -0.005544 |
| 7 | 0.87335 | 0.89212 | -0.01336 | 0.024509 | -0.007049 |
| 8 | 0.90963 | -1.54589 | -0.05844 | 0.007866 | 0.011792 |
| 9 | -3.72936 | 1.53763 | 0.09917 | -0.028119 | 0.028487 |
| 10 | -2.84222 | -0.98341 | 0.09917 | 0.000715 | -0.018824 |
| 11 | 1.41647 | -0.15939 | -0.01362 | 0.017916 | -0.003975 |
| 12 | -1.14108 | -3.07569 | -0.11758 | -0.042833 | 0.005718 |
| 13 | 0.99635 | 0.68243 | -0.02431 | 0.012847 | -0.011474 |
| 14 | 1.65607 | -0.62523 | -0.01621 | 0.013889 | 0.003413 |
| 15 | -1.48547 | 1.61860 | -0.01387 | 0.008588 | 0.012025 |
| 16 | 1.36540 | 1.48008 | -0.02917 | 0.001226 | -0.012399 |
| 17 | 1.11812 | -1.74080 | -0.06042 | 0.013558 | 0.024583 |
| 18 | 5.24514 | -0.37033 | 0.21810 | -0.040319 | 0.003289 |




**First component**

**Fig 1. Drawing bi-plot based on first and second components for 18 durum wheat genotypes**

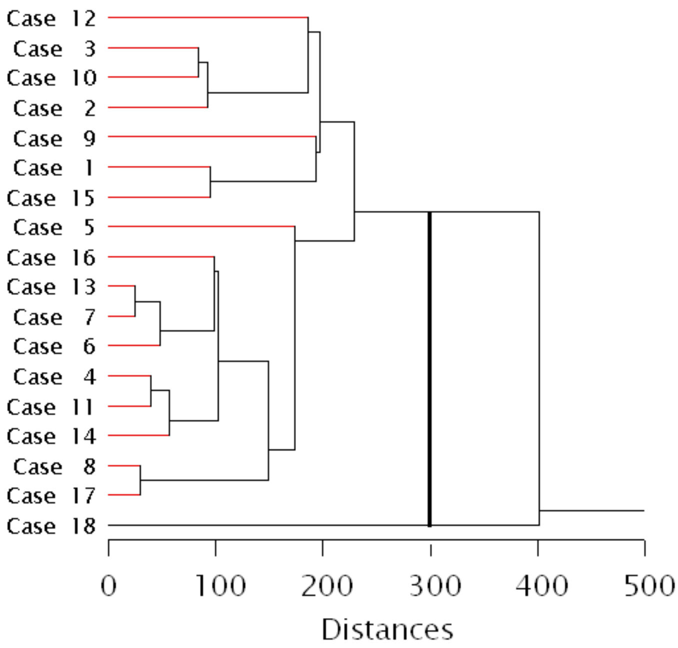



**Fig 2. Dendrogram of measured traits mean for 18 wheat genotypes by using of the UPGMA method**

## DISCUSSION

Significant difference between grain yield of genotypes in dryland and supplemental irrigation conditions indicated the existence of genetic variation and the possibility of selection for favorable genotypes in both environments (Table 1).

The significant and positive correlation of Yp, Ys and MP, GMP and STI showed that these criteria indices were more effective in identifying high yielding cultivars under different moisture conditions (G18, G4, G14 and G11). Similar results were reported by Fernandez [4], Sanjari pirevatlou et al., [20], Nouri et al., [15], Mohammadi et al., [12] and Karimizadeh and Mohammadi [9]. These studies all determined that these parameters were suitable for identifying the best genotypes under stress and irrigated conditions. However, it seems that the effectiveness of selection indices depends on the stress severity supporting the idea that only under moderate stress conditions, potential yield greatly influences yield under stress.

The genetics of drought tolerance in wheat is poorly understood and the highly variable nature of rainfall in most rainfed environments makes genetic progress extremely difficult. The spread of modern cultivars in drier areas has been much slower and their impact on yields far weaker than for favorable areas [3]. Wheat yield gains over traditional cultivars have usually been below 20 %, and often less than 10 %, and have even been negligible in extremely harsh environments. Nevertheless, considerable improvement in the adaptation of wheat to dry areas has been made by plant breeders over the last 50 years. The adoption of modern varieties, however, has lagged behind that of irrigated areas and the percentage yield advance has been considerably lower [24]. Crop selection performed in nurseries with good growing conditions is frequently translated to cultivars with increased productivity in a wide range of growing conditions, from non-limiting (e.g. with yields over 7´0 Mg ha±1), to mild (approx. 4´5±7´0 Mg ha±1) and moderate stress (approx. 2´0±4´5 Mg ha±1) environments. However, in environments subject to more stress the situation may reverse, with genotypes selected in good environments performing less well than those already selected under the poor conditions of a target environment [1].

In developing countries, farmers have traditionally grown landrace cultivars, which are well adapted to serious moisture stress conditions. However, these traditional cultivars are generally poor yielding in “good years” when rainfall is more plentiful. Some new cultivars now yield the same as or even more than traditional cultivars in dry years, yet will respond to more favorable moisture and nutrient conditions such as G18**.** The average for thousand-kernel weight of this cultivar was 8 gram more than the local check. New variety has spring type, earliness, resistance to lodging and shattering. Reaction of this variety to leaf and stem rust was semi-resistant and semi-sensitive respectively using artificial inoculation, but it did not show any susceptibility in natural conditions [13].

## CONCLUSION

Based on the results of this research, G18, G14, G11 and G4 maintained preference in both environments tested in the experiment. They also had high values for STI, GMP and MP indices. These genotypes had the best ranking with low standard deviation. These cultivars are capable of producing high yields when water is in adequate supply, and only suffer a minimum loss during droughts.

Due to yield stability, optimum grain yield and agronomic traits, G18 was recently released in Iran by the Agricultural Research, Education and Extension Organization (AREEO) under the name of "Dehdasht". This new cultivar showed remarkable preference to Seimareh cultivar as check.

## REFERENCES

[1] Ceccarelli S., Grando S., *Plant Growth Regulation*, **1996,** 20: 149-155.
[2] CIMMYT, In: Wheat in developing world, CIMMYT. Mexico. **2010**,1, 342.
[3] Evans L.T., Feeding the ten billion: plants and population growth, Cambridge: Cambridge University Press, **1998,** 1, 247.
[4] Fernandez G.C., In: Kuo C.G. (ed) Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in temperature and water stress, Tainan Publication, Taiwan. **1992,** 13-16.
[5] Fisher R.A., Maurer R., *Australian Journal of Agricultural Res*earch, **1978,** .29: 897-912.
[6] Golabadi M., Arzani A., Mirmohamadi S., Maibody A.M., *African Journal of Agricultural Research*, **2006**, 1 (5): 162-171.
[7] ICARDA, In: Cereal program, annual report for 2010, **2011**, ICARDA, Aleppo, Syria. P. 369.
[8] Karami A.A., Ghanadha M.R., Naghavi M.R., Mardi M., *Irainian Journal of Crop Science*, **2006**, 37(2): 371-3799. (In Persian with English abstract).
[9] Karimizadeh R., Mohammadi M., *Australian Journal of Crop Science*, **2011**, 5:138-146.
[10] Keshavarz A., Kamali M.A., Dehghani B., Hamidnejad M., Sadri B., Heidari A., Summary plan to increase yield and produce of water and dryland wheat country (2002-2011). Ministry of Agriculture publications, Iran. Tehran, **2002**, 1, 256.
[11] Maleki A., Babaei F., Cheharsooghi H., Ahmadi J., *Research Journal of Agriculture and Biological Sciences*, **2008**, 3(8): 841-844.
[12] Mohammadi M., Karimizadeh R., Abdipour M., *Australian Journal of Crop Science*, **2011**, 5(4):487-493.
[13] Mohammadi M., Hosseini S.K., Roustaii M., Jafarby J., Hosseinpour T., Khalilzadeh K., Poorsiahbidi M.M., Hasanpour Hosni M., Torabi M., *Seed and Plant*, **2009**, 75(3):505-507.
[14] Nachit M.M., *Caravan*, **2002**, 17, 14-15.
[15] Nouri A., Etminan A., Jaime A., Teixeira S., Mohammadi R., *Australian Journal of Crop Science***, 2011**, 5: 8-16.
[16] Rajaram S., *Turkish Journal of Agricultural and Forestry*, **2005**, 29: 105-111.
[17] Reynolds M., Skovmand B., Trethowan R., Pfeiffer W., Evaluating conceptual model for drought tolerance, CIMMYT. Mexico, **2004**, WWW. CIMMYT.ORG.
[18] Richard R.A., *Plant Growth Regulation*, **1996**, 20: 157-166.
[19] Rosille A.A., Hambilin J., *Crop Science*, **1981**, 21: 43-46.
[20] Sanjari Pireivatlou A., Yazdansepas A., *Journal of Agriculture Science and Technology*, **2008**, 10: 109-121.
[21] Slafer G.A., J..L. Araus, C. Royo, L.G. Morol, *Annals of .Biology*, **2005**, 146: 61-70.
[22] Talebi R., F. Fayaz, A.M. Naji, *General Applied Plant Physiology*, **2009**, 35(1-2): 64-74.
[23] Timothy G., S. Rajaram, Ginkel M.V., Trethow R., Braun H.J., Cassaday K., New wheats, for a secure. Sustainble future, **2005**, http://www.CIMMYT.org/rainfed programe/ Publication.
[24] Trethowan R., Pfeiffer W.H., In: Ribaut, JM; Poland D., (eds.). Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments, Mexico, DF (Mexico), **2000**, 1, 45-48.