Australian Journal of Crop Science AJCS

AJCS 7(3):407-413 (2013) ISSN:1835-2707

# Gene action for physiological parameters and use of relative water content (RWC) for selection of tolerant and high yield genotypes in $F_2$ population of wheat

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**Abstract**

In order to identify parents suitable for use in a breeding program for the development of high quality and high yield varieties of bread wheat with drought tolerant genotypes, the combining ability and gene action for certain physiological traits were investigated in half-diallel crossings among eight parental lines. The cultivars investigated (Irena/Babax//Pastor, S-78-11, Tajan, Chamran, Moghan3, Hamoon, Veery/Nacozari and Hirmand) possess different tolerance levels to drought stress. Eight parental genotypes, and their resulting 28 $F_2$ generations, were grown in a triplicate randomised complete block design. Drought stress and non-stress conditions were achieved through irrigation at 75% and 25% soil moisture depletion. Data were subjected to analysis of variance, combining abilities factor analysis and correlation analysis between drought tolerance indices and factor scores (according to Griffing's method 2, model 1). General combining ability and specific combining ability effects were significant for traits; however, non-additive gene effects were dominant over additive effects. The cultivar Chamran transmitted high relative water content (RWC) to its progeny, based on general combining ability. Broad-sense heritability was high and strict-sense heritability was low for the traits, confirming the importance of non-additive gene effects. The results of factor analysis revealed that three factors explained approximately 70% of total variation; these factors were strongly influenced by chlorophyll *a* and *b*, proline content, cell membrane stability, RWC and plant yield. Based on drought stress indices (STI and GMP), the cross Irena×Chamran was the most tolerant genotype. Correlation coefficients between two drought stress indices and the third factor from the factor analysis, which influenced RWC and plant yield, were positive and significant. Thus, RWC may be a good criterion for selection of tolerant genotypes with higher yields in breeding programmes.

**Keywords**: Combining ability, Gene action, Drought stress, Physiological parameters, Factor analysis.
**Abbreviations**: GCA - general combining ability; SCA - specific combining ability; Chl - chlorophyll; D - additive genetic variance; H1, H2 - dominance genetic variance and corrected dominance genetic variance respectively; E - environment variance; RCBD - randomised complete block design; $h^2$(bs) - heritability for diallel in a broad sense; $h^2$(ns) - heritability for diallel in a narrow sense; RWC - relative water content; GMP - geometric mean productivity; STI - stress tolerance index.

## Introduction

Drought stress inhibits the photosynthesis of plants by causing changes in chlorophyll content, affecting chlorophyll components and also damaging the photosynthetic apparatus and reducing relative water content (RWC) (Iturbe-Ormaetxe et al., 1998). Genetic improvement of crops for drought resistance requires investigation of possible physiological and yield attributes and the exploitation of their genetic variation (Brancourt et al., 2003). Identification of the gene, or genes, responsible for the desired characteristics of drought resistance at different stages of plant growth and development is of great importance (Dhanda et al., 2002). A contentious topic in the field of drought stress research is the determination of whether drought stress limits photosynthesis through stomatal closure or metabolic impairment (Sharkey, 1990). Physiological characteristics play an important role in grain yield. Stomata are involved in regulating plant water stress, and RWC varies between species, and is influenced by the environmental conditions under which a plant grows (Munir, 1997). Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988). Fernandez (1992) defined geometric mean productivity (GMP) and the stress tolerance index (STI), which can be used to identify genotypes that produce high yield under both stressed and non-stressed conditions. Naroui Rad et al. (2010) suggested that selection for drought tolerance in lentil (*Lens culinaris)* could be conducted for high GMP and STI under stressed and non-stressed conditions. The best approach for crop production, yield improvement and yield stability under soil water stress conditions is to develop drought tolerant varieties. A physiological approach is the most attractive way to develop new wheat varieties rapidly, but breeding specifically for water stress tolerance requires a deeper understanding of the yield determining traits (such as physiological characteristics), and this is where the knowledge of crop responses to water stress may be applied (Desalegn et al., 2001). One of the most complex, and extensively employed, designs for the genetic analysis of quantitative characteristics, such as drought stress tolerance, is the diallel cross. Diallel cross designs are frequently used in plant breeding research to obtain information about genetic properties of parental lines, or estimates of general and specific combining abilities and heritability (Iqbal et al., 2007). In general, breeding for drought tolerance involves combining good yield potential in the absence of stress, and the selection of highly heritable traits that provide drought stress tolerance (Jones, 2007). The success of any selection or hybridization breeding program for the development of drought-tolerant varieties depends on precise estimates of genetic variation components for traits of interest, consisting of additive, dominant and non-allelic interaction effects (Farshadfar et al., 2008). Hence, a diallel cross provides early information on the genetic behaviour of attributes in the generation (Griffing, 1956a). Several methods have been proposed for the genetic analysis of data from a diallel cross. The approaches of Griffing (1956b) and Hayman (1954) are statistically similar in their analyses of variance; Griffing's general and specific combining abilities are mathematically identical to Hayman's additive and dominance components. They differ, however, in their genetic assumptions, information and interpretation. Therefore, this study was conducted (1) to estimate the combining ability of selected lines and cultivars for tolerance to drought using physiological traits as indirect indicators; (2) to assess the type of gene action for these traits in an $F_2$ population of wheat; and (3) to assess the selection criteria for identifying drought tolerance with higher yield wheat genotypes, so that suitable genotypes can be recommended for plant breeding projects.

## Results

### *Combining ability and genetic analysis*

Analysis of variance showed a significant difference in studied traits (Table 2). Phenotypic values of chlorophyll *a* and *b*, cell membrane stability index (MSI), proline content, (RWC), stomatal conductance and plant grain yield, differed significantly among the eight parental lines and 28 $F_2$ hybrids ($P \leq 0.01$). Both GCA and SCA were highly significant for chlorophyll *a* and *b*, stomatal conductance, proline content, RWC and plant grain yield ($P \leq 0.01$), but GCA variance was not significant for the MSI (Table 3). Mean square values were higher for GCA than for SCA of chlorophyll *a*, stomatal conductance and proline content; however, for chlorophyll *b*, RWC, MSI and plant grain yield, the mean square of SCA was higher than the mean square of GCA, indicating the importance of both additive and non-additive gene effects.

Among the parents the highest values for different variables were observed in the following lines: line Veery/Nacozari for chlorophyll *a* and *b* , line Irena/Babax//Pastor for MSI, Hirmand for proline content, Chamran for RWC, Veery/Nacozari, S-78-11 for plant grain yield and Chamran for stomatal conductance (Table 4). Thus, for general combining ability these lines can be considered as the most photosynthetically efficient cultivars based on their performance, and also for their specific combining ability. Crosses Irena×Tajan, Chamran×Moghan3, S-78-11×Moghan3 and S-78-11×Chamran had high values for chlorophyll *a* and *b*, MSI, RWC and plant grain yield; however, the crosses Irena×Moghan3 and Irena×S-78-11 had a high negative value for proline content and stomatal conductance under drought stress conditions (Table 5).

For all studied characteristics, dominance gene effects (H1, H2) were higher than additive gene effects (Table 6), indicating dominance control. Based on the uv value, it was evident that the positive and negative alleles at these loci were not in equal proportions in the parental genotypes for all traits. The positive F values for all traits indicate an excess of dominant genes for these characteristics in the parents. The values of average degree of dominance $(H1/D)^{1/2}$ for all traits were greater than one, suggesting the presence of overdominance in this set of diallel crosses. The ratio (KD/KD+KR) for the traits showed an unequal presence of dominant and recessive genes; there was a slight tendency toward dominant genes. Expected environmental variance (E) was significant for all traits, indicating that they were strongly influenced by the environment. The proportion of positive and negative genes (uv) was unequal, showing different distributions of genes among parents. The uv component ranged from 0.16 for stomatal conductance to 0.24 for RWC and proline content. High broad sense heritability was obtained for almost all of the traits studied, ranging from 72% for MSI to 98% for stomatal conductance. However, for narrow sense heritability the values were reduced, and ranged from 0.11 to 0.30. The low values of narrow sense heritability are due to a greater non-additive proportion of genes than additive.

### *Evaluation of drought tolerant genotypes*

Two selection indices of drought tolerance (Fernandez, 1992), the STI and GMP, were calculated. Based on the results of the STI and grain yield, the crosses Irena×Chamran and S-78-11×Chamran proved to be the most drought tolerant with a high STI under drought stress; other crosses were identified as semi-tolerant or semi-sensitive to drought stress. Additionally, the crosses Irena×Chamran and S-78-11×Chamran displayed a high GMP and approved STI index for these two genotypes.

### *Factor analysis*

Factor analysis is a multivariate analysis method which aims to explain the correlation between a large set of variables in terms of a small number of underlying independent factors. It is assumed that each of the variables measured depends upon these underlying factors, but it is also subject to random errors. The principal factor analysis method (explained by Harman, 1976) was followed in the extraction of the factor loadings. The KMO value, which measures the sampling adequacy, was 0.516 and thus satisfactory to proceed with factor analysis (results are presented in Table 7). The analysis identified seven factors, of which only three were extracted and together explained 69% of the variance among the entries. The first factor, with an Eigen value of 1.69, accounted for only 25.74% of the variance and was primarily related to chlorophyll *a* and *b*; this factor was named 'chlorophyll performance'. The second factor accounted for 23.81 % of the total variance, and was mainly loaded by MSI and proline content, but with opposite signs; this factor was named 'membrane stability'. The third factor accounted for 19.25 % of the total variance, and was primarily related to plant grain yield and RWC; thus, this factor was named 'tolerance index'. The communality values ranged from 0.89 for chlorophyll *b* to 0.53 for RWC. Drought tolerance indices and factor analysis scores are presented in Table 8.

**Table 1.** Genotype name and pedigree.

| No | Pedigree/Name | Tolerance status |
|---|---|---|
| 1 | Irena/Babax//Pastor | Tolerant |
| 2 | S-78-11 | Tolerant |
| 3 | Tajan | Susceptible |
| 4 | Chamran | Tolerant |
| 5 | Hamoon | Semi-tolerant |
| 6 | Moghan3 | Susceptible |
| 7 | Veery/Nacozari | Tolerant |
| 8 | Hirmand | Semi-tolerant |

**Table 2.** Analysis of variance for traits (Means squares) under drought stress.

| Source of variation | DF | Chl (a) | Chl (b) | Cell.M.S | Proline content | RWC | Stomatal conductance | Plant grain yield |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 0.11 | 0.04 | 114.3 | 0.002 | 4.52 | 66.08 | 0.56 |
| Genotype | 35 | 0.68** | 0.087** | 173.17** | 0.088** | 132.6** | 6855** | 7.77** |
| Error | 70 | 0.08 | 0.021 | 49.9 | 0.001 | 5.86 | 106.6 | 0.83 |

** Significant at 1% statistical level, Chl(a):chlorophyll (a), Chl (b): chlorophyll (b), Cell.M.S:cell membrane stability;RWC;relative water content

**Table 3.** Mean squares obtained from analysis of variance.

| Source of variation | DF | Chl (a) | Chl (b) | Cell.M.S | Proline content | RWC | Stomatal conductance | Plant grain yield |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 0.20 | 0.03 | 200** | 0.0007 | 13.2 | 115.5 | 0.15 |
| GCA | 7 | 1.04** | 0.05 | 96.16 | 0.09** | 814.2** | 7112** | 6.30** |
| SCA | 28 | 0.59** | 0.09** | 112.5** | 0.08** | 3106.2** | 63808** | 6.98** |
| Error | 70 | 0.09 | 0.02 | 54.2 | 0.002 | 286 | 6181 | 0.80 |

GCA, general combining ability; SCA, specific combining ability, ** significant at 1% statistical level, Chl(a):chlorophyll (a), Chl (b): chlorophyll (b), Cell.M.S:cell membrane stability;RWC;relative water content.

***Correlation analysis between factor scores and drought stress indices***

The factor three score exhibited a significant positive correlation with GMP and STI ($0.69^{**}$, $0.71^{**}$; Table 9); RWC and yield were hidden with high positive scores. These results suggest that any positive increase in RWC and yield will improve plant tolerance in drought stress conditions. We recommend that breeders perform preliminary evaluations under drought stress conditions before plant maturity, to identify the genotypes with high yield and those more tolerant to drought stress. This would reduce the cost of field evaluation until maturity and harvest of seed yield.

**Discussion**

The concept of combining ability is important in designing plant breeding programmes; in particular, it is useful in testing procedures for the study and comparison of the performance of lines in hybrid combinations. Chlorophyll concentration is known to be an index for evaluation of source (Herzog, 1986), therefore a decrease in chlorophyll concentration can be considered as a non-stomata limiting factor under drought stress conditions. In this study, the variation exhibited by the seven characteristics under consideration indicated that selection for some of these drought-related characteristics could be effective in developing drought-tolerant cultivars; however, the selection efficiency is related to heritability. For the purpose of crop production, yield improvement and yield stability under water stress conditions, the development of drought tolerant varieties is the best approach (Siddique et al., 2000). Therefore, physiological and biochemical approaches are of great importance for a deeper understanding of the complex responses of plants to water deficiency, and the rapid development of new varieties. Water availability predominantly affects the accumulation of some organic compatible solutes (e.g. sugars, betaines and proline), which adjusts the intercellular osmotic potential, and is an early reaction of plants to water stress. The dominant values (H1, H2) were greater than the additive values indicating dominance gene control for this trait. Kuar et al. (2010) reported that GCA and SCA were significant for proline content, suggesting additive and non-additive gene control, and they found one specific cross that had a negative effect on this parameter. Some researchers have supported a decrease of chlorophyll in drought stress conditions (Majumdar et al., 1991, Naroui Rad et al., 2012). Combining ability analysis for physiological traits exhibited significant differences for both GCA and SCA indicating the involvement of additive and non-additive types of gene action in the control of these traits. Additive gene action for specific traits will increase the selection success in a breeding programme (Topal et al., 2004). Naroui Rad et al. (2012) found genetic gain per cycle of selection in preliminary generations to be less for chlorophyll due to a low narrow sense heritability and dominance effect for traits studied. Schonfeld et al. (1988) reported additive dominance and additive x additive genetics effects for RWC in wheat. Gene action for the MSI was dominant for the second generation of wheat. Farshadfar et al. (2012) showed that cell membrane stability was mainly controlled by the dominance type of gene action in the first generation. In this study, stomatal conductance was notably reduced with water deficit; which accords with the results of Sikuku et al. (2010). The results of these studies confirm a large contribution of non-additive gene action in the inheritance of stomatal conductance; we obtained a result of 0.30 narrow sense heritability for this trait. Wajid et al. (2012) reported negative SCA effects for stomatal conductance under stress conditions in $F_1$ hybrids of the crosses TD-1 × TJ-83, Sarsabz × Moomal and Kiran × Sarsabz, suggesting they are more suitable crosses for

**Table 4.** Values of general combining ability (GCA) of physiological parameters in $F_2$ generation.

| Cultivar/line | Chl (*a*) | Chl (*b*) | Cell.M.S | Proline content | RWC | Stomatal conductance | Plant grain yield |
|---|---|---|---|---|---|---|---|
| Irena/Babax//Pastor | -0.08 | -0.04 | 4.54* | -0.03** | 1.89** | 38.75** | 0.49 |
| S-78-11 | -0.24** | -0.01 | 1.88 | -0.02* | 2.17** | -14.9** | 0.60* |
| Tajan | 0.28** | 0.05 | -0.52 | 0.11** | -3.33** | 16.14** | -0.68** |
| Chamran | -0.28** | -0.10** | -2.75 | 0.03** | 4** | -26.42** | 0.32 |
| Hamoon | -0.15 | -0.01 | -0.85 | -0.01 | -1.83** | -7.31* | -0.07 |
| Moghan3 | 0.23** | -0.02 | -2.07 | 0.01 | 0.44 | -2.64 | 0.37 |
| Veery/Nacozari | 0.32** | 0.05 | 0.32 | 0.04** | -2.28** | -26.86** | 0.04 |
| Hirmand | -0.09 | 0.07* | -0.55 | -0.13** | -1.06 | 22.53** | -1.07** |
| SE(gi) | 0.08 | 0.04 | 1.8 | 0.01 | 0.67 | 3.15 | 0.26 |

** , *Significant at 1% and 5% statistical levels respectively, Chl(*a*):chlorophyll (*a*), Chl (*b*): chlorophyll (*b*), Cell.M.S:cell membrane stability;RWC;relative water content.

**Table 5.** Values of specific combining ability (SCA) in $F_2$ generation.

| Hybrids | Chl (*a*) | Chl (*b*) | Cell.M.S | Proline | RWC | Stomatal conductance | Plant grain yield |
|---|---|---|---|---|---|---|---|
| Irena ×S-78-11 | 0.21 | 0.12 | -9.25 | 0.49** | -9.67** | -39.9** | -0.24 |
| Irena×Tajan | 0.57* | 0.29* | 1.82 | -0.21** | 0.49 | 9.94 | -0.29 |
| Irena×Chamran | 0.14 | 0.03 | 1.05 | -0.06 | 2.16 | -30.1** | 2.04* |
| Irena×Hamoon | -0.32 | -0.17 | 2.49 | -0.09* | -5.67** | 57.7** | 0.10 |
| Irena×Moghan3 | 0.38 | 0.17 | 9.71 | -0.22** | 6.38** | -11.2 | -1.35 |
| Irena×Veery | -0.59* | -0.32** | -4.01 | 0.12** | 9.44** | -18.3 | -0.02 |
| Irena×Hirmand | -0.39 | -0.12 | -1.81 | -0.03 | -3.12 | 31.8** | -0.24 |
| S-78-11×Tajan | -0.38 | -0.22 | -0.52 | -0.03 | 2.21 | -13.7 | -2.74** |
| S-78-11×Chamran | -0.26 | -0.02 | -2.36 | -0.01 | -3.79 | 11.44 | 2.26** |
| S-78-11×Hamoon | 0.10 | -0.09 | 7.81 | -0.09* | 5.71** | -1 | 0.98 |
| S-78-11×Moghan3 | -0.71 | -0.15 | -5.97 | -0.04 | 14.7** | -1.33 | 0.21 |
| S-78-11×Veery | 0.52* | 0.15 | 6.98 | -0.21** | -6.17** | 55.5** | -1.13 |
| S-78-11×Hirmand | 0.52* | 0.21 | 3.31 | -0.10** | -3.06 | -11.1 | 0.65 |
| Tajan×Chamran | -0.82** | -0.27* | 3.44 | 0.15** | -3.62 | -31.56** | 1.21 |
| Tajan×Hamoon | 0.02 | 0.10 | 0.54 | 0.21** | -2.79 | 28** | 0.60 |
| Tajan×Moghan3 | 0.29 | -0.02 | -7.57 | -0.08* | -4.40* | -4.33 | -0.18 |
| Tajan×Veery | -0.11 | -0.01 | 2.71 | -0.02 | -3.67 | -30.7** | 0.48 |
| Tajan×Hirmand | 0.45 | 0.12 | -0.42 | -0.02 | 11.77** | 42.5** | 0.93 |
| Chamran×Hamoon | 0.29 | 0.13 | -13.22* | -0.02 | 6.88** | 4.89 | -0.74 |
| Chamran×Moghan3 | 0.42 | 0.17 | 12.33* | 0.01 | 0.27 | 25.8** | -0.52 |
| Chamran×Veery | 0.20 | 0.05 | -5.06 | -0.07* | -5.01* | 25.7** | -0.52 |
| Chamran×Hirmand | 0.03 | -0.08 | 2.81 | 0.02 | 3.10 | -6.28 | -3.74** |
| Hamoon×Moghan3 | 0.01 | -0.08 | -2.90 | 0.03 | -5.23* | -29.2** | 0.21 |
| Hamoon×Veery | 0.27 | 0.10 | -0.96 | -0.02 | 5.83** | -10.3 | -0.13 |
| Hamoon×Hirmand | -0.37 | 0.02 | 3.24 | -0.01 | -4.73* | -35.06** | -1.02 |
| Moghan3×Veery | -0.23 | 0.04 | 1.93 | 0.17** | -4.12 | 2.67 | -0.24 |
| Moghan3×Hirmand | -0.17 | -0.14 | -5.53 | 0.13** | -7.67** | 17.61 | 1.87* |
| Veery×Hirmand | -0.07 | 0.02 | -1.59 | 0.03 | 3.71 | -24.5** | 1.54* |
| SE.sij | 0.27 | 0.12 | 5.7 | 0.04 | 2.08 | 9.66 | 0.8 |

*and ** Significant at 5% and 1% statistical levels, Chl(*a*):chlorophyll (*a*), Chl (*b*): chlorophyll (*b*), Cell.M.S:cell membrane stability;RWC;relative water content.

**Table 6.** Genetics parameters of Hayman type analysis for Physiological traits in $F_2$.

| Genetic Parameters | Chl (*a*) | Chl (*b*) | Cell.M.S | Proline content | RWC | Stomatal conductance | Plant grain yield |
|---|---|---|---|---|---|---|---|
| D | 0.19 | 0.02 | 99.9 | 0.03 | 27.10 | 3197.6 | 1.87 |
| H1 | 0.89 | 0.10 | 194.4 | 0.10 | 179.01 | 9392.7 | 8.38 |
| H2 | 0.67 | 0.09 | 139.2 | 0.08 | 160.3 | 6144.03 | 7.80 |
| F | 0.27 | 0.02 | 137.2 | 0.03 | 26.3 | 5047.2 | 1.19 |
| E | 0.02 | 0.007 | 16.28 | 0.007 | 1.85 | 36.08 | 0.26 |
| $(H1/D)^{1/2}$ | 2.17 | 2.26 | 1.39 | 1.67 | 2.57 | 1.71 | 2.11 |
| KD/KD+KR | 0.66 | 0.63 | 0.74 | 0.63 | 0.59 | 0.73 | 0.57 |
| uv | 0.18 | 0.22 | 0.17 | 0.24 | 0.22 | 0.16 | 0.23 |
| $h^2$(bs) | 0.89 | 0.78 | 0.72 | 0.97 | 0.96 | 0.98 | 0.90 |
| $h^2$(ns) | 0.26 | 0.11 | 0.14 | 0.29 | 0.18 | 0.30 | 0.21 |

D: additive genetic variance, H1 and H2: dominance genetic variance and: corrected dominance genetic variance, F: product of additive by dominance, hh: square of difference P vs. All, E: Expected environmental variance, whole, $(H1/D)^{1/2}$: average of degree dominance, KD/KD+KR: proportion of dominance genes, uv: balance of positive and negative alleles,$h^2$(bs): heritability for diallel in a broad sense, $h^2$(ns): heritability for diallel in a narrow sense Chl(*a*):chlorophyll (*a*), Chl (*b*): chlorophyll (*b*), Cell.M.S:cell membrane stability;RWC;relative water content.

**Table 7.** Rotated Component Matrix.

| Trait | Component | | | Communalities |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Chlorophyll (*a*) | 0.905 | -0.073 | -0.210 | 0.86 |
| Chlorophyll (*b*) | 0.944 | 0.028 | -0.018 | 0.89 |
| Proline content | -0.165 | -0.858 | -0.113 | 0.77 |
| RWC | -0.145 | 0.321 | 0.639 | 0.53 |
| Stomatal conductance | -0.050 | 0.477 | -0.589 | 0.57 |
| Cell.membrane stability | -0.170 | 0.769 | -0.051 | 0.62 |
| Plant grain yield | -0.109 | -0.048 | 0.730 | 0.54 |
| % Comulative Variance | 28.3 | 52.12 | 69.5 | |

drought conditions. The differences among genotypes for grain yield per plant were highly significant. Naroui Rad et al. (2010) identified three main factors (which accounted for 77.09% of the total variability) in the dependent structure; the first factor (group) included chlorophyll *a*, chlorophyll *b* and proline content, which accounted for 40.78% of the total variability in the dependent structure. Toker and Cagirgan (2004) reported three factors that explained 92.9% of the total variance seen in the characteristics. In the present study, factors one, two and three explained 51.3%, 24.8% and 16.8% of total variance respectively. Correlation analysis between factor scores and the drought tolerance indices (STI, GMP) displayed a strong relationship with factor three, which had large scores for RWC and yield. Based on these results, in breeding programmes, to reduce the cost of evaluation in the field until seed harvest, and to reduce heritability for RWC, breeders can identify tolerant genotypes with high yield based on RWC.

## Materials and methods

### *Plant materials*

Eight bread wheat cultivars were used as parents (Table 1). Parental genotypes were derived from the Seed and Plant Improvement Institute, Karaj-Iran, where preliminary and advance experiments were performed to determine tolerance status. Crosses for a half-diallel among these wheat cultivars were made in the Agriculture and Natural Resources Research Center of Sistan-Iran. $F_2$ hybrids and their parents were planted under shelter in pots. The 28 $F_2$ hybrids and their eight parents were sown in plastic pots filled with a soil mixture containing soil/sand/organic matter in a ratio of 1:1:1 in the Experiments Farm of the University Putra Malaysia. Four seeds were sown in each pot for two experiments (non-stress and stress treatments).

### *Irrigation treatments*

The pots were irrigated after 25% and 75% depletion of the soil water for normal and stress conditions respectively. Each pot was filled with 3 kg of air-dried soil and soil field capacity was calculated on the basis of soil dry weight. In treatments for water stress, pots were subjected to 75% moisture depletion of field capacity by weight. The pots were weighed at two day intervals to compensate for the water loss by evapotranspiration, and irrigation was performed after 75% depletion of field capacity of soil. Genotypes were arranged as a completely randomised block design.

### *Trait measurements*

The chlorophyll content was measured three times. Measurements were made on the flag leaf, on two seedlings per pot, with a chlorophyll meter (SPAD-502, Soil Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan). Three readings were taken along the middle section of the leaf; the mean was used for analysis, and values were expressed as SPAD units. Stomatal conductance was measured on the abaxial surface of the mid-portion of the flag leaf using an IRGA (Infra Red Gas Analyzer, LCA-4, Analytical Development Corporation, UK), between 10:00 and 14:00 hours at the grain filling stage. Leaf membrane stability index (MSI) was determined according to the method of Premchandra et al. (1990), modified by Sairam (1994). Leaf discs (100 mg) were thoroughly washed in running tap water, followed by washing with double distilled water. Subsequently, the discs were heated in 10 ml of double distilled water at 40 $^{o}$C for 30 min. Electrical conductivity (C1) was recorded by EC (Electrical Conductivity) meter. The same samples were then placed in a boiling water bath (100 $^{o}$C) for 10 min and electrical conductivity recorded again (C2). The MSI was calculated as: MSI = [1 - (C1/ C2)] × 100. Leaf relative water content (RWC) was estimated according to the method of Ekanayake et al. (1993). Leaf material was weighed (two leaves) to determine fresh weight, and placed in distilled water at +4 °C for 19 h; thereafter, turgid weight was recorded. Finally, the samples were dried in an oven at 65-70 °C for 48 h and dry weights were recorded. RWC was calculated as:

$$RWC = \left[\frac{(fresh\ weight - dry\ weight)}{(turgid\ weight - dry\ weight)}\right] \times 100$$

Chlorophyll *a* and *b* were estimated by extracting the leaf material in 80% acetone. Absorbance was recorded at 645 and 665 nm for chlorophyll *a* and *b* respectively, and was calculated according to the procedure of Arnon (1949). Proline was determined in fully expanded leaves according to Pesci and Beffagna (1984). Two plants from each pot were harvested and left for sun drying. After threshing samples, average grain yield per plant was recorded. Two drought tolerance indices were calculated using the following equations (Fernandez, 1992):

$$GMP = \sqrt{YP \times YS}, \quad STI = \frac{(YP \times YS)}{(\overline{YP})^2}$$

**Table8.** Drought tolerance indices and factor analysis scores in $F_2$ generation.

| No | Cultivar/Line | STI | GMP | Factor scores 1 | Factor scores 2 | Factor scores 3 |
|---|---|---|---|---|---|---|
| 1 | Irena/Babax//Pastor | 67.20 | 8.20 | -0.51 | 1.27 | -0.04 |
| 2 | S-78-11 | 56.00 | 7.48 | -1.57 | 1.52 | 0.35 |
| 3 | Tajan | 24.89 | 4.99 | 0.31 | -1.26 | -1.61 |
| 4 | Chamran | 73.30 | 8.56 | -0.98 | 2.30 | -1.16 |
| 5 | Hamoon | 28.00 | 5.29 | 1.03 | 0.06 | -1.30 |
| 6 | Moghan3 | 43.35 | 6.58 | -1.40 | -0.79 | -2.30 |
| 7 | Veery/Nacozari | 77.84 | 8.82 | -1.42 | 0.18 | -0.21 |
| 8 | Hirmand | 30.00 | 5.48 | 0.36 | 0.77 | -1.14 |
| 9 | Irena ×S-78-11 | 105.54 | 10.27 | -0.02 | -1.91 | 0.02 |
| 10 | Irena× Tajan | 79.31 | 8.91 | 1.83 | 1.00 | -0.37 |
| 11 | Irena× Chamran | 134.29 | 11.59 | -0.28 | 0.30 | 1.96 |
| 12 | Irena×Hamoon | 98.64 | 9.93 | -1.21 | 0.93 | -0.84 |
| 13 | Irena×Moghan3 | 102.69 | 10.13 | 1.14 | 1.74 | 0.65 |
| 14 | Irena×Veery | 96.00 | 9.80 | -1.24 | -0.36 | 0.87 |
| 15 | Irena×Hirmand | 77.84 | 8.82 | -0.81 | 1.04 | -0.95 |
| 16 | S-78-11×Tajan | 35.82 | 5.98 | -0.88 | -0.37 | -0.67 |
| 17 | S-78-11×Chamran | 128.04 | 11.32 | -0.92 | -0.48 | 1.78 |
| 18 | S-78-11×Hamoon | 78.03 | 8.83 | -0.38 | 0.81 | 1.45 |
| 19 | S-78-11×Moghan3 | 104.04 | 10.20 | -0.94 | 0.02 | 2.11 |
| 20 | S-78-11×Veery | 79.31 | 8.91 | 1.43 | 0.91 | -0.31 |
| 21 | S-78-11×Hirmand | 81.84 | 9.05 | 1.37 | 0.92 | 0.55 |
| 22 | Tajan×Chamran | 108.29 | 10.41 | -1.87 | -1.43 | 0.35 |
| 23 | Tajan×Hamoon | 87.96 | 9.38 | 0.43 | -1.12 | -0.86 |
| 24 | Tajan×Moghan3 | 88.69 | 9.42 | 1.04 | -1.07 | -0.55 |
| 25 | Tajan×Veery | 80.63 | 8.98 | 0.86 | -0.91 | -0.16 |
| 26 | Tajan×Hirmand | 77.84 | 8.82 | 1.45 | 0.62 | -0.10 |
| 27 | Chamran×Hamoon | 93.31 | 9.66 | 0.29 | -0.85 | 1.13 |
| 28 | Chamran×Moghan3 | 99.71 | 9.99 | 0.64 | 0.18 | 0.64 |
| 29 | Chamran×Veery | 107.53 | 10.37 | 0.53 | -0.87 | 0.25 |
| 30 | Chamran×Hirmand | 36.00 | 6.00 | -0.56 | 0.27 | -0.71 |
| 31 | Hamoon×Moghan3 | 98.64 | 9.93 | 0.00 | -1.06 | 0.25 |
| 32 | Hamoon×Veery | 95.29 | 9.76 | 1.17 | -0.41 | 0.83 |
| 33 | Hamoon×Hirmand | 63.96 | 8.00 | -0.12 | 0.08 | -0.49 |
| 34 | Moghan3×Veery | 94.57 | 9.72 | 0.58 | -1.11 | 0.01 |
| 35 | Moghan3×Hirmand | 101.18 | 10.06 | -0.14 | -0.72 | -0.30 |
| 36 | Veery×Hirmand | 90.64 | 9.52 | 0.82 | -0.21 | 0.86 |

**Table 9**.Correlation among drought tolerance indices and factor scores.

| Variable | STI | GMP | Factor scores 1 | Factor scores 2 | Factor scores 3 |
|---|---|---|---|---|---|
| STI | 1 | | | | |
| GMP | 0.99** | 1 | | | |
| Factor scores 1 | 0.005 | 0.01 | 1 | | |
| Factor scores 2 | -0.17 | -0.14 | 0 | 1 | |
| Factor scores 3 | 0.71** | 0.69** | 0 | 0 | 1 |

** Significant at 1% statistical level

***Statistical analysis***

All data were subjected to analysis of variance. Data obtained from the 28 hybrids of $F_2$ and eight parents were subjected to analysis by Griffing's method II, model 1. Analysis of variance was performed on variables by SAS (1989). The analysis of combining ability was performed using the DIAL98 software (Yukai, 1989).

**Conclusion**

In this study, GCA and SCA were highly significant; the lines Veery/Nacozari, Tajan, Moghan3 and Hirmand had high GCA values for chlorophyll *a* and *b*, and the cultivar Chamran had a high value for RWC. The most significant, and negative, values for stomatal conductance were obtained for Chamran and Veery/Nacozari; therefore, we recommend these lines and cultivars for drought projects. Based on drought stress indices (STI and GMP), the line Irena×Chamran proved to be a tolerant genotype. According to factor analysis and the relationship between factor scores and drought stress indices, RWC may be a good criterion to identify drought tolerant genotypes with higher yield.

**Acknowledgements**

We would like to thank Dr Mihdzar Abdul Kadir for his helpful comments and recommendations on the manuscript. The authors express their gratitude to the Agriculture and Natural Resources Research Center for preparing the grains of wheat genotypes used in the experiments.

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