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*Mohtasham MOHAMMADI and Rahmatollah KARIMIZADEH<sup>1</sup>***SCREENING OF VARIOUS MORPHO-PHYSIOLOGICAL  
CHARACTERS IN RECOMBINATION INBRED LINES GROWN  
UNDER TERMINAL HEAT AND DROUGHT CONDITION****SUMMARY**

Progress in heat and drought stress tolerance has been limited due to lack of suitable selection criteria. To assess the feasibility of utilizing secondary selection criteria to identify high-yielding hexaploid wheat genotypes, 167 recombinant inbred lines with parents derived from the cross of Seri82 and Babax were evaluated during 2009–2010. The experiment was carried out through a alpha lattice design with two replications in a warm and drought area in Gachsaran agriculture research station located in southwest of Iran. Parental genotypes and recombinant inbred lines showed significant variation and differences. Stronger growth vigor, earliness, more grain filling duration, tall plant height, long peduncle, medium spike length, high 1000 kernel weight and kernel per square meter, low canopy temperature and more chlorophyll content are the best features for optimum grain yield performance under heat combined with drought. The correlations of these criteria with yield performance under field condition are remarkable and significant. Heritability of yield components, due to less sensitive to environmental effects was more than grain yield per se and so, breeder emphasis must be put on these components as selection criteria.

**Keywords:** Genetic variation, Dryland, Tolerance, Warm regions

**INTRODUCTION**

Wheat (*Triticum aestivum* L.) is a temperate cereal with an optimum temperature regime of 15–18°C during the grain filling stage (Stone, 2001; Streck, 2005; Lobell et al., 2011) but daily high temperature of 25–30°C or greater is common across many regions where wheat is grown (Mohammadi, 2012).

Wheat breeding worldwide in the last 50 years has had many priorities, of which yield potential gains, maintenance of biotic resistance, and increased abiotic tolerance, especially manipulation of traits for drought and heat, have been given a lot of attention (Rajaram and Braun, 2006).

Under Mediterranean conditions, post-anthesis heat and drought stresses are the major grain yield limiting factors in winter sown wheat genotypes. All vegetative and reproductive stages may be affected by heat and drought stress to some extent (Wahid et al., 2007), however, these two main environmental stress mainly affects assimilates availability, translocation of photosynthates to the

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grain and starch synthesis and deposition in the developing grain during post-anthesis growth stages. The net result is a lower grain yield due to lower grain weight (Gibson and Paulsen, 1999; Rao et al., 2002).

There has not been a parallel phenomenon in relation to combining yield potential and tolerance to drought, heat, and other abiotic environmental stresses. Breeders developing cultivars for abiotic stress environments have mostly ignored yield potential and focused on stress tolerance. However, there is a need for stress tolerant cultivars with high yield potential in years with high rainfall (Rajaram and Braun, 2006).

A number of studies have been carried out at field condition to evaluate the genetic diversity for heat or drought adaptation and establish the traits best associated with yield under heat and drought stress. However, simultaneous evaluation under natural heat and drought stress in field condition on grain yield are scarce.

Indirect selection methodologies that assist in evaluating secondary traits may have acquired increased importance in breeding programs because of a greater understanding of their relative contribution to yield (Richards et al., 2001; Araus et al., 2002; Rebetzke et al., 2002; Reynolds et al., 2005). They are not only practical, but increasingly cost efficient tools that can support breeders in screening, early generation or advanced-line selection (Araus et al., 2001, 2002; Richards et al., 2001; Reynolds et al., 1994, 2005). There are no easy solutions and the occurrence and severity of drought and other environmental stresses is like a lottery –unpredictable - making breeding progress extremely difficult (Richards et al., 2009, Mohammadi and Karimizadeh, 2013). However, there has not been high systematic evaluation of these traits in large populations of sister lines in varying heat and drought environments. It helps the feasibility of utilizing secondary selection criteria to identify tolerant hexaploid wheat genotypes.

This study was performed with the following objectives: (1) to assess several traits related to heat and drought adaptation in the Seri/Babax recombinant inbred line (RIL) population (2) to find more reliable screening traits for drought and heat tolerance.

## MATERIAL AND METHODS

In the current study, 167 RILs plus parents were phenotyped under dryland condition. The population was derived from a reciprocal cross between the related elite lines: semi-dwarf spring wheat variety Seri M82 from the “Veery” cross (Kauz/Buho//Kal/BB) and a fixed line (Babax) derived from the “Babax” cross (Bow/Nac//Vee/3/Bjy/Coc). Seri M82 carries the T1BL.1RS (rye) translocation, and is characterized by moderate tolerance to drought conditions and high yield potential. The Babax parental line has a coefficient of parentage of 0.3316 with Seri M82 (Mathews et al. 2008). It is a sister line of the elite variety Baviacora M92 (recognized for drought tolerance and also has a high yield potential).

The experiment was carried out through a alpha lattice design with two replications in a warm area in Gachsaran Dryland Agriculture Research Station (30°.20' N, 50°.50 E, altitude 710 m), Iran in 2009-10. Soil type was sandy clay loam and PH was about 7.5-8. Each genotype was planted in 6 rows by 7.05 m long, spaced 17.5 cm apart. These germplasms were planted in the beginning of December (optimum planting date).

All physiological and agronomic measurement on the Seri/Babax population included the parents. Early growth vigor (EGV) was recorded in five leaf stage by visual score from 1 (weak) to 5 (very strong). Days to heading (DHE) was determined when 50% of the spikes had half florets with anther extrusion starting from sowing date and days to physiological maturity (DMA) was recorded when 50% of the spikes in a plot showed a total loss of green color. The grain filling period (GFP) was also calculated as the number of days from exhibit heads to physiological maturity. Canopy temperature was measured at the mid-grain-filling stage using a portable infrared thermometer (Model LT-300, Sixth sense). Thermometer held 0.5-1 m from the edge of the plot and approximately 50 cm above the canopy at a 30° angle from the horizon on cloudless days with low wind between 12 and 14 h. Chlorophyll content was measured at anthesis with a self-calibrating chlorophyll meter (Model SPAD 502, Minolta Camera Co., Ramsey, NJ, USA). in five flag leaves per plot. Chlorophyll content measurements per leaf from five randomly selected plants per plot were taken and the average reading recorded. For plant height, five measurements (in cm) per plot were made during late grain filling on representative plants in each of the plot, from the ground to the top of the ear. Kernel per square meter was recorded by Sayre et al., (1997) procedure. Heritability estimates were obtained using variance components.

## RESULTS AND DISCUSSION

Climatic data in Gachsaran field research which is typically Mediterranean with a long term average annual rainfall of 431mm are presented in Table 1. It is showed that current temperature during anthesis and grain filling period (end of March and April) is more than optimum temperature that is coincide with lack of water requirement.

The response of parental lines to heat and drought stress seems to be different as evidenced from their grain yield, agronomic and physiologic performance when comparing. In this research, Babax was less affected by water stress (21%) than Seri. The RILs average yield was 1650kg/ha. Babax had higher yield performance than Seri's. Yield parameters of Seri were more affected by high temperature and drought stress. When comparing parental agronomic performance showed that, Seri's kernel weight is reduced almost 10% more than babax, and also, Seri's KPS is reduced by 9.5 % in contrast to babax's reduction. Such 9.5% difference in kernel set suggests an early reproductive response under drought (Table). It is well established that high temperatures and drought can affect many of the processes involved in photosynthesis (Reynolds et al., 2000).

The link between phenology and TKW suggested that early maturity would favor the post anthesis grain growth periods resulting in increased grain weight and yields under terminal stress

Table 1. Crop growing season metrological details for Gachsaran station (2009-2010).

Period	Temperature (°C)					Mean relative Humidity (%)	Evaporation (mm)	Rainfall (mm)
	Absolute		Mean					
	Min.	Max.	Min.	Max.	Average			
Oct.2009	12.7	37.6	14.8	34.4	24.6	39.6	260.4	0.6
Nov.2009	6.6	30.3	10.9	24.9	17.9	55.7	127.0	121.1
Dec.2009	3.9	21.2	7.0	18.5	12.7	71.4	30.3	141.2
Jan.2010	2.4	24.3	6.1	20.1	13.1	62.9	69.5	36.9
Feb.2010	2.2	25.7	7.1	20.9	14.0	62.4	48.4	34.8
Mar.2010	5.2	33.3	10.1	28.0	19.0	48.7	153.2	7.3
Apr.2010	9.3	35.3	14.3	30.4	22.4	44.2	203.5	25.0

When comparing the parents, Seri reached anthesis 2 days after Babax and it was entered to physiological maturity 1 day after Babax. However, there was significant variation among RILs (Table 2). Phenological variation among RILs showed a range of 100–108 days to anthesis and 126–141 days to maturity. The average anthesis date among the RILs was similar to the later parent Seri. Relationships between anthesis and maturity for RILs clearly was significant ( $r=0.68$ ,  $P>0.01$ ). However, the relationship between days to heading and duration of grain filling remains significant ( $r=0.35$ ,  $P<0.05$ ).

There was a slight difference in the height of parental lines in response to soil moisture availability. Seri was 1 centimetre taller than Babax and RILs average height was 68.3 cm. However, the range in height under paralleled by a yield increase ( $r=0.46$ ,  $P<0.01$ ) (Table 2). Plant height had a high positive correlation with grain yield showing that high yielding cultivars in the present study were partially tall (Table 2). This is in close agreement with the results of Mohammadi (2012) and Aydin et al., (2010) under drought condition in our study, Peduncle length showed high correlation with grain yield ( $r=0.36$ ,  $P<0.05$ ). The role of peduncle in heat and drought tolerance was already contributed to its role in photosynthesis and stem reserve remobilization (Asseng and van Herwaarden 2003; Pfeiffer et al., 2005; Tahir and Nakata 2005; Villegas et al., 2007).

Table 3 shows the data representing partial and cumulative R<sup>2</sup> as well as the probability for the accepted limiting three variables in grain yield prediction. These variables are kernel number per square meter (0.88%), Thousand kernel weight) (0.102%) and plant height (0.001%). According to the results, 97.5% of the total variation in grain yield could be attributed to these three variables. The

other variables were not included in the analysis, due to their low relative contributions.

Table 2. Correlation of the traits and their mean, minimum and maximum for the parents and the RILs

Character/Genotype	EGV	DHE	PLH	SL	DMA	GFP	TKW	KPS	GYD	CC	CT
Seri 80	3	105	81.0	10.3	133	28	20.2	7665	1548	52.7	24.0
Babax	5	101	80.0	11.7	132	31	22.4	8371	1875	59.2	22.5
Minimum	3.0	100.0	49.0	3.0	126.0	24	17.6	2278	615	45.9	21.3
Average	4.1	103.3	68.3	9.9	131.8	28.5	25.1	6673	1650	54.6	23.4
Maximum	5.0	108.0	93.0	17.3	141.0	38	34.6	16790	3603	63.2	26.2
Correlation with grain yield	0.31**	-.49**	0.46**	0.35**	-.47**	0.46**	0.38**	0.67**	-	0.41**	0.62**
Heritability		0.80	0.59	0.51	0.76	0.74	0.81	0.73	0.43	0.65	0.62

\*, \*\* Significantly different at 5% and 1% level, respectively.

EGV: early growth vigor, DHE: days to heading, PLH: Plant height, SL: spike length, DMA: days to maturity, GFP: grain filling period, TKW: thousand kernel weight, KPS: kernel per square meter, GYD: grain yield, CC: chlorophyll content and canopy temperature.

Table 3. Relative contribution (partial and model  $R^2$ ) in predicting durum wheat grain yield, F-value and probability by the stepwise procedure analysis.

Characters entered into the model	Regression coefficient	Standard error	Partial $R^2$	Model $R^2$	TOL	F	Significant probability
Kernel No. per square meter	0.235	0.006	0.88	0.88	0.147	1.331	0.000
Thousand kernel weight	69.47	3.358	0.102	0.982	0.507	427.9	0.000
Plant height	4.115	1.497	0.001	0.983	0.193	7.555	0.007

Babax had longer grain filling period than Seri. Grain filling duration and yield were correlated significantly ( $r=0.46 < 0.01$ ). The duration of grain growth in the post-anthesis period is reported as the most significant determinant of yield in wheat (Stone 2001; Wardlaw et al., 2002; Mitra and Bhatia 2008). Canopy temperature showed a transgressive segregation with the coolest RILs on average 1.2°C lower than Babax and the warmest RILs 2.2°C higher than Seri. Babax had a cooler canopy than Seri at heading, while the RILs showed a larger range of CT values.

Grain yield was negatively associated with days to heading (DH), days to maturity (DM), and CT at grain-filling, but positively with early growth vigor, plant height and kernel per square meter (Table 2).

By mid-grain filling, Leaf chlorophyll of Seri showed 11 % less than Babax (Table 2). Among the RILs, the chlorophyll ranged from 45.9 to 63.2 %. Canopy temperature consistently showed negative phenotypic correlations with yield (Table 2). Chlorophyll content was moderately correlated with yield ( $r=0.41$ ,  $P<0.05$ ).

Heat and drought stress occurring after anthesis often has detrimental effects on wheat grain yield by hastening maturity, leaf senescence, shortening grain filling period, reducing net assimilates and 1000 kernels weight. Reynolds et al., (2000) and Hays et al., (2007) reported that grain filling period with chlorophyll loss due to accelerated leaf senescence by heat stress. Greenness a genotype can maintain close to maturity. The results presented here indicated that chlorophyll content was correlated with yield under heat combined with drought. This was expected, as decreased rates of senescence is an attribute of stay green phenotypes (Harris et al., 2007). This is in agreement with previous results in sorghum shown by Harris et al. (2007) and Mohammadi et al., (2009). Greenness a genotype can maintain close to maturity. The results presented here indicated that chlorophyll content was correlated with yield under heat combined with drought. This was expected, as decreased rates of senescence is an attribute of stay green phenotypes (Harris et al., 2007). This is in agreement with previous results in sorghum shown by Harris et al. (2007) and Mohammadi et al., (2009).

Canopy temperature has strong association with yield performance (Table 2), as previously reported for fixed lines and other families of RILs, including the International Triticeae Mapping Initiative population (Reynolds et al., 2000) Several physiological and agronomic parameters support the overall conclusion that drought adaptation in the Seri/Babax RILs was largely driven by the ability to access water (Table 7), an strategy that seems to be consistent with a suggested concomitant association between a low CT and a decreased residual soil moisture resulting from a high water extraction capacity of the root system (Reynolds et al., 2005). There was a significant association ( $r=-0.53$ ,  $P<0.01$ ) of height with CT measured, indicating that those RILs able to access more water were less susceptible to environmentally-determined effects on the height of genotypes.

The canopy of Babax not only cooler than that of Seri, showing a higher photosynthetic due to chlorophyll content in Babax was also greater. There were significant associations between CT and leaf chlorophyll with grain yield and with each other, suggesting that warmer canopy and chlorophyll loss might have been a symptom of inability to access water. It seems where water is available in deep soil profiles; the ability to extract it under water-stressed conditions has been identified as the main way for drought adaptation as cleared by a strong association of canopy temperature with wheat yield (Olivares-Villegas et al., 2007).

High heritability estimates were obtained for 1000 grain weight (0.81), days to heading (0.80), days to maturity (0.76) and kernel per square meter (0.73). The estimate was relatively high for chlorophyll content (0.65), canopy temperature (0.62) and Plant height (0.59). The least heritability belonged to grain yield (0.43). High heritability estimates in wheat have been reported in most studies for 1000 grain weight (Ehdaie and Waines, 1989; Ali and Shakor, 2012; Koumber and El-Gammaal, 2012), days to heading (Ehdaie and Waines, 1989; Khan and Naqvi, 2011), and kernels per spike (Ehdaie and Waines, 1989; Mohammadi et al., 2011). Days to heading and 1000 grain weight showed high

heritability due to less sensitive to environmental effects. Lower heritability values for grain yield as compared with the estimates for the yield components indicate the important contribution of environmental effects to the phenotypic variance of this trait. Therefore, selection for grain yield per se in the segregating generations would not be fruitful and emphasis must be put on the components.

Although both parents like each other in the performance of some specific traits, such segregation is observed in the Seri/Babax RILs. They presumably do not possess the same allelic or genetic composition controlling such traits (Lynch and Walsh 1998).

### CONCLUSIONS

In the current study, the value of some morpho-physiological traits in heat and drought stressed environments were addressed and showed yield safety can be improved, if future breeding attempts are based on the valuable new knowledge acquired on the processes determining plant development and its responses to stress. The obtained results in this study, support indirect selection of EGV, DHE, CT, CC, PLH, PL, GFP, KPS and TKW for their use in breeding as important predictors of yield performance or as parts of a selection index under heat and drought stress.

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**SKRINING RAZLIČITIH MORFO-FIZIOLOŠKIH KARAKTERISTIKA  
REKOMBINOVANIH INBRED LINIJA UZGAJANIH POD USLOVIMA  
TERMALNE VRUĆINE I SUŠE**

**SAŽETAK**

Napredak u toleranciji na stres od vrućine i suše je ograničen usled nedostatka kriterijuma za odgovarajuću selekciju. Da bi se ocijenila sprovodljivost upotrebe sekundarnog kriterijuma selekcije kako bi se identifikovali visoko-prinosni heksaploidni genotipi pšenice, tokom 2009-2010. godine je izvršena evaluacija 167 rekombinovanih inbred linija čiji su roditelji nastali ukrštanjem Seri82 i Babaxa. Esperiment je sproveden kroz alfa rešetkasti dizajn sa dva ponavljanja u toplom i sušnom području Gašaran poljoprivredne istraživačke stanice koja se nalazi u jugoistočnom Iranu. Roditeljski genotipovi i rekombinovane inbred linije su pokazale značajne varijacije i razlike. Pojačan vigor rasta, ranostasnost, veće trajanje nalivanja zrna, visoke biljke, dugačak korijen iz kojeg raste cvijet, prosječna dužina klasa, velika težina 1000 jezgra i jezgro po kvadratnom metru, niska temperatura nadzemne krošnje i veći sadržaj hlorofila su najbolje osobine za optimalni prinos žita pod toplim uslovima kombinovanim sa sušom. Korelacija ovih kriterijuma sa učinkom prinosa pod terenskim uslovima su izvanredni i značajni. Nasljednost komponenti prinosa, zbog smanjenje osjetljivosti na okolinu su bitni koliko i sam prinos žita, te naglasak uzgajivača treba da bude na ovim komponentama kao na kriterijumima odabira.

**Ključne riječi:** Genetska varijacija, sušno zemljište, tolerancija, topli regioni.