ASSESSMENT OF GENETIC EFFECTS OF SOME PHYSIOLOGICAL PARAMETERS IN SPRING WHEAT UNDER WATER STRESS

SALMAN SALEEM[1]*, MUHAMMAD KASHIF[2], MUHAMMAD YASIN ASHRAF[4] AND USMAN SALEEM[3]

[1]*Maize, Sorghum, Millet and Fodder Program, National Agriculture Research Center, Islamabad, Pakistan*
[2]*Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan*
[3]*Department of Plant Breeding and Genetics, Agriculture College, University of Sargodha, Sargodha, Pakistan*
[4]*Nuclear Institute for Agriculture & Biology Faisalabad, Pakistan*
**Corresponding author's email: mrsalman146@gmail.com, Call: 00923336500656, Fax: 092519255434*

Abstract

Wheat is one of the most important cereal crops worldwide and drought stress is the major abiotic stress causing decrease in yield. This study was designed to unravel the genetic nature of those physiological parameters which confer drought tolerance in wheat but are mostly ignored by the plant breeders. Contrasting wheat lines for yield under drought were crossed to develop subsequent generation F_1, F_2, BC_1 and BC_2 to study the inheritance patterns. Fixable additive gene action was observed for proline, chlorophyll and total soluble proteins under drought stress which provided the opportunity for the use of these traits in breeding program focused on drought tolerance. In this study, novel work was done to unfold the genetic nature of total soluble sugars and total soluble proteins. Due to epistatic effects complex gene action was observed for grain filling period and grain yield per plant. The information generated from this study could be used as guidelines to chalk out a breeding plan against drought tolerance.

Key worlds: Drought, Epistasis, Gene action, Physiology, Wheat.

Introduction

Wheat is staple food for almost 1/3 of the global population and occupies 1st rank for total area planted under any cereal in the world. However, its per hectare yield and total production is at 3rd rank after maize and rice (FAO, 2014). Concerted efforts of plant breeders on wheat research have brought Pakistan at threshold of self-sufficiency. But expected wheat demand for human consumption by the year 2050 will be 850 million tones as against the 642 million tones today excluding the feed demand for animals across the globe (Sharma *et al*., 2015, Saleem *et al*., 2016b). Worldwide climate changes may affect agriculture production greatly and agitate the food security issues.

To sustain yields there is need to address the factors, limiting potential production or to improve the plant genotypes against the prevalent stresses. Water stress causes severe decrease in grain yield of wheat in semi arid and water scarce regions of the world (Pokharel & Pandey, 2012). Poor plant growth, reduction in yield and decrease in nutrient up take are drought stress features (Hussain *et al*., 2008, Saleem *et al*., 2016a). Germplasm less sensitive to climatic changes, flexible and well adapted to varied environment can help in resolving the issue (Taiz & Zeiger, 2006; Hellin *et al*., 2012). Therefore, physiological and biochemical traits are important for comprehensive understanding of the complex plant response to drought stress and finally their use for varietal development (Rad *et al*., 2013, Saleem *et al*., 2016b). Also these parameters are reasonably reliable selection criteria (Saleem *et al*., 2016b).

The current situation of water availability demand to tailor the genotypes which are physiologically and genetically more stable in stress conditions. This cannot be done without comprehensive understanding of the genetic nature of drought responsive traits. Biometrical quantitative techniques established on generation mean analysis (GMA) are best to investigate phenotypic performance of traits under study (Kearsey & Pooni, 1996, Saleem *et al*., 2016a). The estimation of main genetic effects i.e. additive (d), dominant (h) and epistatic effects like additive × additive (i), additive × dominant (j) and dominant × dominant (l) with generation mean analysis are also very helpful for understanding the genetic makeup of a trait (Saleem *et al*., 2016a,b). The present work will provide basic information about the performance of parents and their crosses which could be used as a guideline in future for parent selection and to lay down a breeding program.

Materials and Methods

Plant material of this study comprised of two wheat lines divergent for maximum and minimum grain yield under drought conditions identified at Plant Breeding and Genetics Department, University of Agriculture, Faisalabad, Pakistan. Line 9877 (P_1) having maximum grain yield under water stress was crossed with line NR371 (P_2) with minimum grain yield to constitute F_1. The following year P_1, P_2 and F_1 were raised to make BC_1 ($F_1 \times P_1$), BC_2 ($F_1 \times P_2$) and harvested F_2 seed. Next year P_1, P_2, F_1, BC_1 and BC_2 were planted under split plot design having both stress and non-stress treatments in randomized complete block design with three replications. A uniform plant to plant distance of 15 cm and row to row 30 cm was maintained using a dibbler across the three replications and treatments. Non-experimental rows were planted on either side of experimental material. Four irrigations were given to non-stress block at sowing, tillering, booting and grain development stages, while stress block received two irrigations, at sowing and heading stage only. Agronomic practices other than irrigations were kept uniform for both stress and non-stress treatments. The stress plot was strictly under rainfall control shelter to stop any excessive water availability other than the two irrigations. For data recording 30 guarded plants were taken from each of P_1 (9877), P_2 (NR371) and F_1 (First filial generation). From

BC$_1$ (P$_1$ × F$_1$) and BC$_2$ (P$_2$ × F$_1$) 50 plants each, while 250 guarded plants from F$_2$ (second filial generation) were used from each replication for observation of all the characters in this experiment. Proline content, total soluble sugars and chlorophyll were measured in mg/g of fresh weight by the procedures described by Bates *et al.* (1973), Dubois (1951) and Arnon (1949), respectively. Total soluble proteins were also determined in mg/g of fresh weight by the method given by Lowry *et al.* (1951) by using BSA as standard (Fresh). Grain filling period was estimated by taking the difference of days taken to maturity and days taken to heading in all the generations and treatments. Analysis of variance was carried out using the method of Steel *et al.* (1997). A computer program provided by Dr. H.S. Pooni, School of Biological Sciences, University of Birmingham UK. was used for generation mean analysis (GMA) by the method of Mather & Jinks (1982). The narrow sense heritability (h$^2_{ns}$) was estimated by the method illustrated by (Mather & Jinks, 1982 and Saleem *et al.*, 2016a,b).

Results and Discussion

The pooled analysis of variance for all the traits regarding parents (P$_1$ and P$_2$) and generations (F$_1$, F$_2$, BC$_1$ and BC$_2$) was significant under both the treatments indicating the existence of broad genetic variability in studied genotypes (Table 1). This genetic variability indeed is the evidence of the wide range of quantitative trait loci present controlling the traits in parent lines and generations (Saleem *et al.*, 2016b). The presence of contrasting behavior and genetic variability were also observed by Sayar *et al.* (2008), Farshadfar *et al.* (2013), Sehar *et al.* (2015) and Saleem *et al.* (2016a,b) in characters studied here.

The drought stress significantly affected physiological and biochemical traits of the material studied (Figs. 1-4) which influenced the gene expression, it showed that gene expression depended on environmental conditions and physiological response of the plants. Similar genotypic responses are also reported indicating that stress induced physiological and biochemical alterations and affect the gene expression (Saleem *et al.*, 2016a). Proline content increased under drought stress in all wheat material under study and varied from 1.5 to 4.29 mg g^{-1} fresh weight in P$_1$ and BC$_2$.While under normal irrigation it ranged from 0.71 to 1.41 mg g^{-1} fresh weight in P$_1$ and F$_1$. Increase in proline content was minimum in P$_1$ showing lack of genetic potential ability to accumulate enough proline for osmotic adjustment to drought stress. However, a drastic increase in

proline content was observed in rest of the material studied. Similar to proline content, P$_1$ also produced minimum total soluble proteins and soluble sugars under both the moisture regimes, which confirmed the above findings regarding the sensitive response of P$_1$ to drought stress and lack of genetic ability to adapt to the stress environment. Contrary to P$_1$, P$_2$ produced maximum soluble proteins and soluble sugars under both treatments and the generations which showed the genetic ability of P$_2$ to adopt the environmental stress. Above findings clearly indicated the genetic superiority of P$_2$ to P$_1$ for drought tolerance. Total chlorophyll decreased in all studied material under stress conditions but the extent of reduction was different. Maximum decrease of 41% was recorded for P$_1$ which indicated the reduction in its ability to synthesize chlorophyll under stressed conditions and degradation of chlorophyll was high which might be due to the activities of chlorophylase enzyme under stress environment (Taiz & Zeiger, 2006, Saleem *et al.*, 2016a). While in P$_2$ decrease was only 15% under drought which proved that it had genetic potential to tolerate drought stress as a result of which accumulation of osmolytes such as proline and sugars appeared, consequently plants adjusted them osmotically. All the hybrid generations remained in between the parental range but better than P$_1$. Chlorophyll reduction may be due to reduction in EMS as chlorophyll is membrane bounded organelle and literature proves (Tas & Tas, 2007; Ghobadi *et al.*, 2011; Arjenaki *et al.*, 2012,Koscielniak *et al.*, 2014, Saleem *et al.*, 2016) that drought stress affects the membrane stability resulting in decrease in chlorophyll contents. As a result the above mentioned physiological and biological disorders occur, which affect growth and grain filling period under drought stress in P$_1$ followed by BC$_1$ and F$_2$ (Fig. 5). The reduction in grain yield in present study may be due to above indicated physiological disorders. So, P$_1$ has obviously minimum grain yield per plant. The findings of present study confirmed drought sensitive nature responsible for the yield loss of 80% in P$_1$ while it was up to 40% in P$_2$ due to the efficient and stable physiological processes (Fig. 6). However, hybrid generations showed their yield in between P$_1$ and P$_2$, which was an evidence that plants maintaining physiological and biochemical processes as per environmental requirement were producing economical crop yield. The crossed generations produced from these lines have also performed better under stress. Hence, the breeding material which is physiologically active under drought conditions should be used in breeding programs to develop drought tolerant high yielding crop varieties.

Table 1. Estimation of mean square values of yield and physiological parameters studied under water stress and non-stress conditions in wheat.

| Traits	Replications (r)	Treatment (t)	Error (r × t)	Generations (g)	Interaction (t × g)	Error (r × t × g)
Proline content	0.021	0.571**	0.003	0.031**	0.09**	0.006
Total soluble protein	0.004	2.01**	0.006	0.004**	0.12**	0.003
Total soluble sugars	0.003	0.781**	0.0002	0.091**	0.011**	0.002
Total chlorophyll	0.006	3.909**	0.008	0.036**	0.16**	0.006
Grain filling period	0.306	101.936**	0.23	6.07**	3.21**	0.36
Grain wt. plant^{-1}	0.02	1039.27**	0.03	2.31**	0.34*	0.08
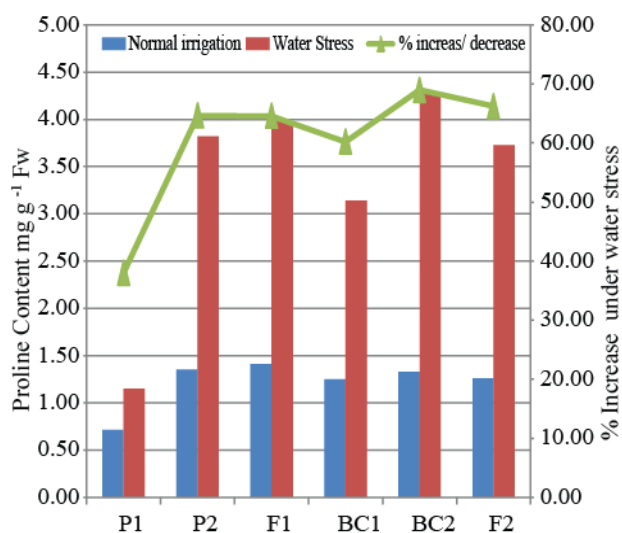
Fig. 1. Comparison of parents and generations under normal and water stress conditions for Proline Content.

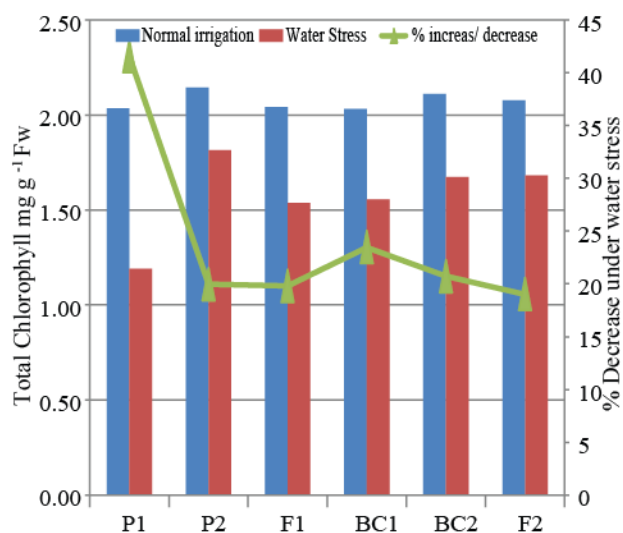Fig. 4. Comparison of parents and generations under normal and water stress conditions for total chlorophy.
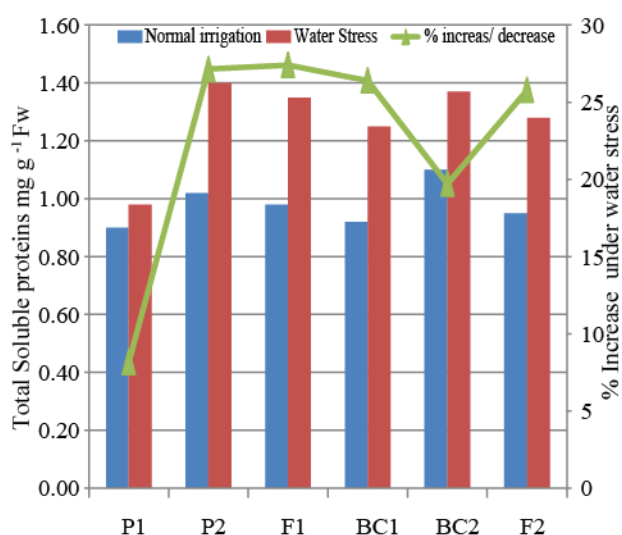

Fig. 2. Comparison of parents and generations under normal and water stress conditions for total soluble proteins.
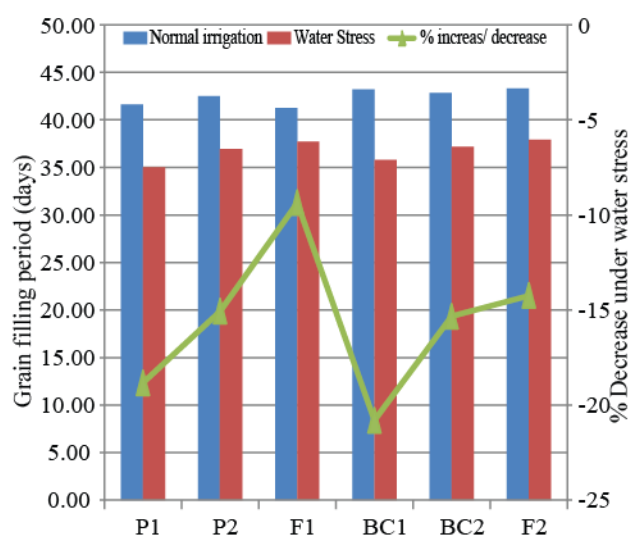

Fig. 5. Comparison of parents and generations under normal and water stress conditions for Grain filling period.
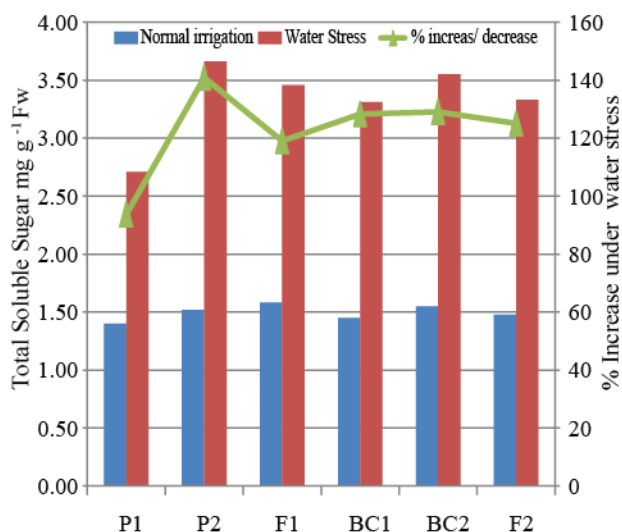

Fig. 3. Comparison of parents and generations under normal and water stress conditions for Total Soluble Sugar.
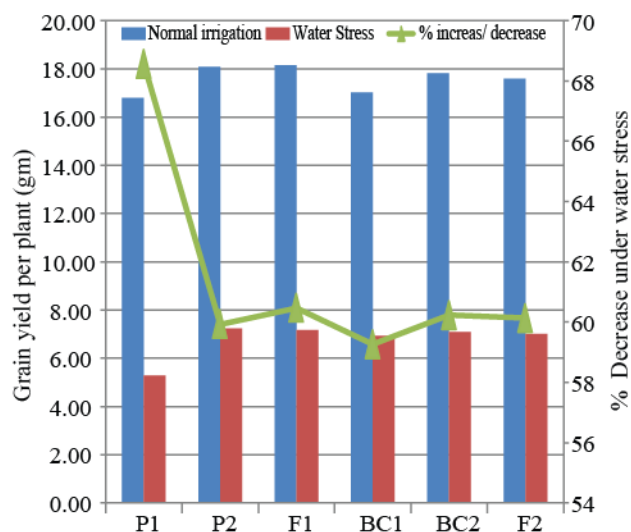

Fig. 6. Comparison of parents and generations under normal and water stress conditions for Grain yield per plant.

Under normal irrigation regime

Plant traits	m ± S.E	[d] ± S.E	[h] ± S.E	[i] ± S.E	[j] ± S.E	[l] ± S.E	χ^2 (df)	h^2ns
Proline content	1.29±0.13	0.73±0.06			0.29±0.04		2.11(3)	0.55
Total soluble protein	0.91±0.05	0.52±0.14	0.24±0.06				1.47(3)	0.61
Total soluble sugars	1.41±0.34	0.64±0.21	0.37±0.11		0.25±0.13		1.21(2)	0.54
Total chlorophyll	2.09±0.67	1.11±0.001	0.26±0.02				1.99(3)	0.48
Grain filling period	37.94±0.16	0.97±0.18		-2.84±0.25	-4.82±1.09		1.34 (2)	0.36
Grain wt. plant-1	15.98±0.45	3.07±0.13	5.81±0.62	5.17±0.48	-3.33±0.85		0.321(1)	0.58

Under water stress regime

Proline content	3.48±0.97	1.12±0.24		0.94±0.03			1.37(3)	0.74
Total soluble protein	1.35±0.36	0.46±0.09		0.31±0.03			2.75(3)	0.69
Total soluble sugars	3.12±0.52	1.22±0.39			0.61±0.11		3.22(3)	0.63
Total chlorophyll	1.58±0.41	1.15±0.01		0.45±0.01			2.44(3)	0.68
Grain filling period	42.22±0.17	1.10±0.16		-1.70±0.24			1.06 (3)	0.61
Grain wt. plant^{-1}	7.12±0.055	0.99±0.061		-0.84±0.08	-2.25±0.40		3.07 (2)	0.62

Where: m = Mean, [d] = Additive, [h] = Dominance, [i] = Additive × additive, [j] = Additive × dominance and [l] = Dominance × dominance effects. χ^2 = Chi square and (df) = Degree of freedom, h^2ns = Heritability in narrow sense.

Gene action studies: Additive as well as non-additive components of inheritance were responsible for the expression of traits in generations studied. Epistatic effects of one or the other type were involved in almost all the traits except Dominant × Dominant [l] effect which was not found in any of the traits under study. Three parameter model was found best fit to explain genetic variability for Proline content under water stress and non-stress treatments. Under non-stress treatment additive [d] and additive × dominant [i] components of inheritance controlled the trait expression. The presence of considerable additive × dominant epistasis indicated that it would be difficult to fix the trait under non-stress regime. Whereas, in stress regime additive [d] and its complementary epistasis, additive × additive [i] provided the opportunity for early generation selection to fix the trait (Table 2). In a similar study additive nature was reported by Maleki *et al.* (2010) while, Rad *et al.* (2013) observed non-additive nature of the trait. Three parameter models [mdi] appeared best fit to explain genetic variability of total soluble proteins and leaf chlorophyll under water stress regime. The presence of fixable additive [d] main effect and its complimentary additive × additive [i] epistasis were responsibly high to control the inheritance of these traits. The success in selection increased due to the supremacy of additive genetic control. Farshadfar & Amiri (2015) in their study had similar results for chlorophyll content. While non-additive genetic control was reported by Rad *et al.* (2012) where differences could be due to the difference in genetic material or environment. The total soluble sugars were governed by four parameter model [mdhj] under normal irrigation and three parameter model [mdj] under water stress. In both the cases high magnitude of additive × dominant component made it difficult to fix the trait expression. It suggests for improving the trait before using in a breeding program or delay the selection procedure to later generations. Literature regarding the

gene action of total soluble proteins and soluble sugars was missing or silent. Grain filling period also under the influence of epistatic effects. The chances of segregation are more and important epistasis are of duplicate nature therefore, selection may not be useful. It would be better to improve the trait using some other suitable technique. Zara-Kohan & Heidri (2012) found non-additive gene action controlling the trait and recommended delayed selection. Similarly, Nazeer *et al.* (2004) found the trait under the influence of partial dominance. The grain yield per plant also exhibited a complex gene action with five parameter model under non-stress [mdhij] and four parameter model under stress regime [mdij] to best fit for explaining the nature of inheritance for grain yield per plant. The involvement of epistasis at high magnitude makes it difficult to manipulate the trait. Strong epistatic effects were also noted by Novoselovic *et al.* (2004), Munir *et al.* (2007) Saleem *et al.* (2016a,b). Grain yield per plant is considered to be a complex trait governed by many genes and their interaction, therefore the selection of component traits may be a better way to improve yield under given circumstances (Majid *et al.*, 2007, and Saleem *et al.*, 2016a,b). The above discussion suggests to focus on physiological parameters for the improvement of grain yield through these parameters along the other yield component traits.

Conclusion

In majority of the studied traits, epistatic effects were involved but the presence of additive gene action and its complementary epistasis in proline content, total soluble proteins and total chlorophyll provide an opportunity to use these traits for breeding drought tolerant genotypes. Presently these traits have not been extensively used by plant breeders for developing high yielding and drought tolerant varieties, whereas the findings of present study suggest that these traits confer tolerance against drought

stress. So, for efficient breeding utilization of these physiological traits/markers like proline, total soluble proteins and total chlorophyll are necessary to improve tolerance potential and crop yield for drought prone areas.

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