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Evaluation of Bread Wheat (*Triticum Aestivum* L.) Genotypes for Heat Tolerance at Middle Hawash, Ethiopia

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Abstract:
The objective of the study was to evaluate bread wheat genotypes for their tolerance to heat stress. This research involved 49 wheat genotypes obtained from CIMMYT and Ethiopia's National Wheat Research Programme. The experiments were planted at middle Hawash in a partially balanced simple lattice design in two sowing dates under irrigation condition. The combined ANOVA for heat stress levels revealed highly significant differences (P=0.01) for heat stress levels (HL), genotypes (G) and G x HL interaction for all the traits studied. Most traits showed a reduced performance due to higher heat stress. There were highly significant correlations among all heat tolerance indices except heat tolerance efficiency with mean and geometric mean productivity, and percent yield reduction with mean grain yield. Under higher heat stress, grain yield was negatively correlated with canopy temperature (r=-39), days to flowering (r=-0.49) and days to maturity (r=-0.26). Yield was positively correlated with leaf area (r=0.48) and plant height (r=0.45). Most of the high yielding genotypes were tolerant to heat stress. Some of the low yielding genotypes were also tolerant to heat stress. The genetic variability observed among the genotypes could be exploited for future breeding aimed at the development of heat tolerant wheat varieties.

Keywords: *Genotype, heat stress, physiological traits, yield.*

1. Introduction

Wheat (*Triticum* spp.) is a cereal grain, originally from the Levant region of the Near East and Ethiopian Highlands, but now cultivated worldwide.The optimum temperature for wheat growth is 25^{0}C with minimum and maximum growth temperatures of 3^{0}C to 4^{0}C and 30^{0}C to 32^{0}C, respectively (Briggle, 1980). Ethiopia ranks 22th in production in the world and 3rd both in production and area coverage in Africa (FAO, 2012). Wheat is one of the major cereal crops in the Ethiopian highlands, which range between 6 and 16°N, 35 and 42°E, and from 1500 to 2800 m. But the country production area is limited to the central mid to high land part of the country (Kate & Leigh, 2010). In Arsi, Bale and Shewa areas, in the 1900-2300m a.s.l altitude zone are favourable for the production of early and intermediate maturing varieties of bread wheat. This is estimated to comprise 25 % of the total wheat area, while the remaining 75 % falls in the 2300-2700 m altitude zone (Hailu Unpublished data cited CSA, 1990). In Ethiopia wheat is the fourth important cereal crop in terms of production and area coverage as estimated 1.63m ha and 3.4m tons respectively (CSA, 2013).

Accounting for a fifth of humanity's food,provides 20 percent of world dietary calories, is second only to rice as a source of calories, and it is the first as a source of protein. It is a major diet component because of the wheat plant's agronomic adaptability with the ability to grow from near arctic regions to equator, from sea level to plains of Tibet, approximately 4,000 meter above sea level (http://en.wikipedia.org/wiki). Wheat contains minerals, vitamins and fats and with a small amount of animal or legume protein added is highly nutritious. It contains 70, 22, 12, 12, 2 and 1.8% of carbohydrates, crude fibers, protein, water, fat, and minerals, respectively (Farooq and Tabassum, 2011).

Climate-change-induced temperature increases will likely to reduce wheat production in developing countries by 20-30 per cent (Hans, 2013). The climate change effect rise in temperature that leads to decreased moisture content canshift the importance of wheat to the first rank followed by rice. So heat and drought tolerant wheat variety can minimize the risk of climate change effect (Personal communication). An agro-climatic study on Ethiopia (White *et al*., 2001) concluded that the current wheat area is largely decreased by high temperature and that warming would greatly reduce the area suitable for wheat production. If heat tolerance of currently grown cultivars could be enhanced by 2°C, the wheat area in the periphery of the highlands could be nearly doubled (Reynolds *et al*., 2012).For temperature increases up to 2°C, this trend may be partially offset by CO_2 driven increases in productivity and water use efficiency (Reynolds *et al*., 2012). Therefore, developing heat tolerant wheat varietyis the best option to be done for exploiting the available lowland irrigation potential of Ethiopia and preparing a choice for coming climate change effect.

The difficulty of selecting for improved adaptation to heat stress makes the use of physiological traits attractive to plant breeders (Alderman *et al*., 2013). Multidisciplinary research involving genetic resources enhancement and crop physiology at CIMMYT have led to a physiological trait-based approach to breed for abiotic stress on wheat (MohaMMadi *et al*., 2012). Physiological traits used to examine the response to selection on canopy temperature, leaf chlorophyll content, to evaluate the response of some wheat genotypes facing high temperatures during and after anthesis under field conditions (Mohammad *et al*., 2012b). The physiological breeding approach aims to combine traits associated with all three drivers of yield to result in a cumulative genetic effect on yield (Cossani and Reynolds, 2012). Warmer temperatures are typically associated with a reduction in leaf area index and green area duration. Rapid ground cover (RGC) or early vigour characterizes the capacity of genotypes to develop leaf area or aboveground biomass. While stay green (SG) is recognized as an adaptive plant tolerance (PT) for stress conditions (Vijayalakshmi *et al*., 2010).Complexities in mechanisms of tolerance to high temperatures and individual contribution of several traits so far reported. Further, valuable traits from wild relatives of wheat can be utilized to improve thermal tolerance in wheat by employing appropriate molecular tools (Jagadish, 2012). Genetic variability in wheat species can be used in increasing tolerance to both heat and drought stress through genetic and physiological screening methods (CIMMYT, 2012). Development of new varieties resistant to the prevalent heat stresses is the main national wheat improvement (Nagarajan, 2013). For Africa in general and Ethiopia specifically heat stress tolerant wheat variety development be one of the strategic work to come with ample of food for continental and country's inclined population growth.

1.1. Objective

To evaluate bread wheat genotypes for heat tolerance and yield by morpho-phenological and physiological traits.

2. Materials and Methods

The experiment was carried out at middle Hawash of Afar regional state which found on longitude 40^o 9' East and latitude of 9^{o}16' North with mean maximum and minimum annual temperature of 34^{0}C and 18^{0}C and monthly temperature of 38.06^{0}C and 21.06^{0}C during main season, respectively. The experimental materials were consisting of 49 different bread wheat genotypes and standard checks of the sources of these genotypes are CIMMYT international heat stress nurseries and the National Wheat Research Program.
The experiment was laid out in partially balanced lattice design. The planting dates were first week of June and after 20 days of the first planting date to catch the micro climatic condition. The plot size was 5.4m^2 (1.8mx3m) and which accommodated six rows at 0.3m interval on 0.6m ridge having two rows each. Seeds were sown on rows with manual drilling at a rate of 100 kg ha^{-1} basis. The fertilizer application was at a rate of 50 kg ha^{-1}P_2O_5 (DAP) and 100kg of N_2 (Urea) ha^{-1}. P_2O_5 (DAP) was applied once at sowing time and N_2 (Urea) was applied in split; half at seedling stage, and the remaining 50% at booting growth stages. The irrigation water was applied at every 10 days' interval using furrow method. Lambda cyhalothrin (Karate) 5% E.C was applied at the rate of 300 ml ha^{-1} into the leaf using a knapsack sprayer to control Aphids. All other agronomic activities were applied for each treatment and planting season equally. The net harvestable area was 3.6m^2 (1.2m x 3m) without the two rows from each side left because of border effect.

2.1. Data Collection

2.1.1. Physiological Traits Data

1. Canopy temperature: Four measurements were taken at ground cover vegetative stage, booting stage, flowering stage and grain filling stage using Fluke-574 Precision Infrared thermometer as per Reynolds et al. (1998) and Olivares-Villegas et al. (2007) and five measurements similar to Zarei et al., (2013). The time of measurement was from 11:00 am to 2:00pm during the peak noon at middle Hawash case with expected high intensity time for temperature similar to Rahmatollah and Mohtasham, (2011).
2. Chlorophyll content: a flag leaf per plant from 15 sample plants per plot was measured using a portable chlorophyll meter SPAD-502 plus (Konica Minolta) at 50% flowering as per Mohammad *et al*., (2012).The averages of the SPAD values from sample plants at flowering were used for analysis in each genotype similar to Moslem *et al*., (2013).
3. Leaf area: Leaf area was measured from ten randomly selected plants in a plot at flowering from the second most top leaf next to flag leaf using leaf area meter (Bio Scientific. Ltd. Area Meter AM 200) and the average of the ten leaves measurements were used for analysis of each genotype in each plot similar to Lack, (2013).
4. Stay green: Rating was done on a 1 to 5 scale based on the proportion of leaf/stem area of normal sized leaves which had prematurely senesced and died (Rosenow, 1993).
5. Lodging percentage: The lodging percentage was estimated visually for each genotype in each plot then average value was calculated and used for comparison of the genotypes interaction.
6. Plant death: The number of dead plants due to heat stress from each genotype.
7. Seed shattering (%): It was calculated value after physiological maturity but before harvest/threshing for the extent/percentage of the seed shattered from the spikelet.

2.1.2. Weather Data

The meteorological data were collected for the experimental site from the early sowing date to the maturity period/harvest time. Temperatures, relative humidity, rain fall, wind speed, sunshine and radiation, evaporation and soil temperature were recorded daily (appendix table.1). These data were summarized and the differences were estimated for the two sowing dates based experiments separately; especially from days to flowering to days to maturity for interpretation of the results. In average the grain filling period for the first sowing date was lower heat stress than the grain filling period of late sowing date. The soil temperature and other meteorological data were also showed similar records that increased the heat stress for the late sowing date.

2.2. Data Analysis

The analysis of variance (ANOVA) was conducted for the data collected using SAS software package version 9.0 (SAS, 2002) and the combined ANOVA of all parameters under observation revealed highly significant difference (table 1). Comparison of means was done based on Duncan multiple range test at 5% of significance (Gomez and Gomez, 1984). Mean values were computed for all traits and these mean values were used in analysis of variance (ANOVA) to test genotypes differences and the significance of genotypes x heat stress interaction effects. Analysis of variance from each planting dates was used to calculate the relative efficiency of partial balanced lattice over randomized complete block design (RCBD)that for most parameters the coefficient of variation and the relative efficiency obtained was better than RCBD. Therefore, data in this study were analyzed using partial balanced lattice as per Gomez and Gomez (1984).

Combined analysis was done after homogeneity test by levene's and barrtlete as per Levene (1960). According to the homogeneity test by levene's test all morphophenological traits except days to flowering and maturity were homogenous. The square root and log transformed value were heterogeneous for days to flowering and days to maturity, so that no need of combined analysis. Simple correlation coefficient of heat tolerance indices/parameters, morpho-physiological traitswere made using SAS soft ware analysis for interpretation of the study.

3. Results and Discussion

3.1. Heat Stress Effects on Physiological Traits

Physiological processes of plants are largely affected by the alteration of surrounded environmental temperature. The ability of plants to cope with extreme temperature is a complex process and is determined by environmental factors and also by the genetic capability of the plant. In general, stability of life processes in most plants is comparatively wide which ranges from several degrees above zero to around 35°C (Zróbek, 2012). The difficulty of selecting for improved adaptation to heat stress makes the use of indirect measures attractive to plant breeders. Physiological traits used to examine the response to selection on canopy temperature, leaf chlorophyll content, and kernel weight and used to evaluate the response of some wheat genotypes facing high temperatures during and after anthesis under field conditions (Mohammad *et al*., 2012a). Results indicated significant difference for all physiological traits and significant phenotypic correlation with grain yield, particularly in more heated environments. Canopy temperature explained 71 and 54.2% of grain yield variation in more and less heated environments, respectively. The lines having tolerance to high temperature during grain filling stage were marked for future breeding program (Mohammad *et al*., 2012b).

In most cases, the thermal times of three phenologil phases, sensitivity to temperature and photoperiod, early vigor, index of storage organs formation, and the potential weight of the storage organs were sufficient to adequately characterize the varieties (Alderman et al.,2013).When LAI 0.75 during the initial stages of development, there is greater control of temperature over the formation of leaf area. One of the simulations the leaf senescence is heat stress and adverse temperatures during the meiosis could also significantly increase sterility (Alderman et al., 2013).

3.2. Analysis of Variance (ANOVA)

The overall combined analysis of the all considered traits under both heat stress levels (HL), Genotypes (G) and HLxG interaction revealed that highly significant difference (table 1). The interaction of the physiological traits mean performance under both swing dates showed significantly different canopy temperature and leaf area reduced by higher heat and similarly for phonological parameters (table 2).

3.2.1. Canopy Temperature

The mean values of canopy temperature were range from 28^{o}c (ETBW5556) to 34.5^{o}c (FARIS-31) with the average of 31.5^{o}c under lower heat stress growing conditions (Table3). There were nine genotypes; ETBW5556 (28^{o}c), ETBW6000 (29.8^{o}c),KAUZ'S'/FLORKWA1//GOUM-RIA-3 (29.9^{o}c), KATILA-3/3/MON'S'/ALD'S'//ALDAN'S'/IAS58 (30.1), GIRWILL-13/2*PASTOR-2 (30.2), KAUZ'S'/FLORKWA-1//GOUMRIA-3 (30.3^{o}c), 5-WON-D 22/JADIDA-2 (30.3^{o}c), LAKTA-1/QAFZAH-21 (30.4^{o}c) and ETBW5957 (30.8^{o}c), with better required canopy temperature reading than local tolerant check Alidoro (31.7^{o}c) and statistically significant different. For the higher heat stress level, the mean values of canopy temperature were range from 28.7^{o}c (OPATA/ RAYON//KAUZ/3/HUD-10) to 32.8^{o}c (GAMDOW-6/ZEMAMRA-8) with the average of 30.7^{o}c. There were fourteen genotypes with better required canopy temperature reading than standard check SHAMISS-3 (30.4^{o}c) but statistically non-significant different (Table3). From the combined analysis of the canopy temperature reading the mean ranges from 29.2^{o}c (ATILLA-7) to 32.4^{o}c (GAMDOW-6/ZEMAMRA-8) with 31^{o}c average. There were ten genotypes showed better tolerance than the susceptible check in their canopy temperature readings of which almost all had better in their yield performance too. Therefore, the CT could one trait used for future wheat breeding under heat stress as it varies for genotypes and correlated well with yield.

These results agreed with the previous study that showed the lower canopy temperature has been used as a selected criterion for tolerance in drought and high temperature stresses (MohaMMadi *et al.*, 2012). Inconsistence this study Reynolds et al., (2007a) reported that under heat stress condition canopy temperature and remobilization of stem carbohydrates suggested potential yield gains of approximately 7 and 9% respectively. Previous study support that in arid, high temperature the heat load received by the leaf under high light conditions can be reduced by increasing leaf reflectance leads to lower leaf temperatures and leaf-air vapor pressure deficits (Ding *et al*., 1983). Similar to present study the result by Rees *et al*., (1993) showed as air-canopy temperature difference correlates better with grain yield measured. There was generally a good relationship between air canopy temperature difference and yield as study showed CT for wheat genotypes selection under heat stress environments negatively and statistically significant correlation to yield at different growth stage similar to (Bilge *et al.,* 2011).

3.2.2. Leaf Area

The mean values of leaf area were range from 29 cm^2 (MOUKA-4/RAYON) to 48.3 cm^2 (ETBW6003) with the average of 38 cm^2 under lower heat stress growing conditions. There were twenty two genotypes with better leaf area than susceptible check UTIQUE 96/FLAG-1 and fourteen genotypes ETBW6003 (48.3 cm^2), GAMDOW-6/ZEMAMRA-8 (48.2 cm^2),KAUZ'S'/FLORKWA1//GOUMRIA-3 (46.9 cm^2), ZAMAMRA-1 (44.2 cm^2), ETBW5898 (44.1 cm^2), HUBARA-8/2*DOVIN-2 (43.9 cm^2), WON-D22/JADIDA-2 (43.9 cm^2), MOONTIJ-3 (43.5cm^2), SHUHA4/FLORWA//HUBARA-3 (42.8 cm^2), ANGI/GIRWILL-5 (42.4 cm^2), ETBW5901 (40.9 cm^2), ETBW5899 (40.2 cm^2), NEJMAH-12 (40 cm^2) and ETBW5954 (39.3 cm^2) were statistically significantly different. The first three genotypes were better leaf area than the best performed standard check for the trait. Even some promising genotypes had less leaf area than the check they were statistically non-significant.

The mean values of leaf area ranged from 15 cm^2 (35) to 30.4 cm^2 (ATILLA-7) with the average of 23 cm^2 under higher heat stress growing condition. There were twenty-two (22) genotypes with better leaf area than local check Alidoro (21.8 cm^2) and standard check SHAMISS-3 (21.5 cm^2) with the three genotypes ETBW5881 (28.9 cm^2), HUBARA-5/ANGI-1 (28.2 cm^2), FARIS-31 (28 cm^2) were statistically significant different. All most all of the out yielded and best performed genotypes were above the checks in their leaf area which implies that this trait is promising selection trait for bread wheat genotypes under heat stress.

The combined analysis of the leaf area revealed that it is important trait for heat stress as much as more than 83% of the out yielded and promising genotypes were better in their leaf area. It was ranged from 26 cm^2 (ETBW6000) to 35.9 cm^2 (ETBW 5898) with the average of 30.3 cm^2. There were ten genotypes had better leaf area than the best performed standard check SHAMISS-3 (32.5 cm^2) and seventeen genotypes were better than the standard check ATILLA-7 (31 cm^2) and eleven genotypes ETBW 5898 (35.9 cm^2), WON-D 22/JADIDA-2 (33.8 cm^2), GAMDOW-6/ZEMAMRA-8 (33.6 cm^2), ETBW6003 (33.5cm^2), HUBARA-8/2*DOVIN-2 (33.5 cm^2), ETBW5881 (33.5 cm^2), ANGI/GIRWILL-5 (33.5 cm^2), FARIS-31(33 cm^2), ATTILA/AWSEQ-4 (32.9 cm^2), ETBW5899 (32.6 cm^2) and KAUZ'S'/FLORKWA-1//GOUMRIA-3 (32.4 cm^2) were performed better than the susceptible check UTIQUE96/FLAG-1 (28.9 cm^2) with statistically significant (P=0.05) (Table 3).

The other findings found similar results like Kumari*et al*., (2007) have pointed out similarly to this study that leaf area under heat stress as a new parameter of the stay green was strongly correlated (r = 0.90) with canopy temperature depression and variations among the genotypes also by (Ataur *et al.,* 2009). Previous study found that marked reduction in growth parameters like plant height, dry matter accumulation and leaf area index by high temperature (Singh, 2011). Higher leaf area helps to cool the under canopy area and conserve moisture by reducing evaporation and evapotranspiration which efficient utilization of resource under heat stress.

3.2.3. Chlorophyll Content

The chlorophyll content SPAD value of genotypes under lower heat level evaluation were range from 37.7 (ETBW6000) to 52.3 (ZAMAMRA-1) with the average 45.2. There were five genotypes performed than the best performed standard check ATILLA-7 (47.9) and seven genotypes had more SPAD value than the next standard check SHAMISS-3 (47.4). With comparison to local check Alidoro (45.3) there were fifteen genotypes had more chlorophyll SPAD value and three of them were ZAMAMRA-1 (52.3), ETBW 5898 (49.7) and ETBW5535 (49.4) statistical significant different. There were twenty-three genotypes had higher chlorophyll content reading than the susceptible check UTIQUE 96/FLAG-1 (44.9) from which four in addition to the three above genotypes MOUKA-4/RAYON (48.6) were statistically significant different. For the higher heat stress level, the chlorophyll content SPAD value ranged from 43.1 (PASTOR-2/HUBARA-5) to 56.8 (ACHTAR*3//KANZ/KS85-8-4/3/KATILA17/4/MON'S'/ALD'S'//ALDAN'S'/IAS58) with the average value of 47.5. The higher performed genotypes to the standard check AILLA-7 (50.5) were four and local check Alidoro (49.1) were nine and most of them are statistically significant (P=0.05). There were fifteen genotypes that better performed than the standard check SHAMISS-3 (48.3) and the susceptible check UTIQUE 96/FLAG-1 (47.9). From the combined analysis of chlorophyll SPAD value ranged from 41.1 (ETBW6000) to 52.6 (ACHTAR*3//KANZ/KS85-8-4/3/KA TILA17/4/MON'S'/ALD'S'//ALDAN'S'/IAS58) with the average of 47.4. There were four genotypes with higher chlorophyll content than standard checkATILLA-7 (49.4) and three genotypes in addition superior to the standard check SHAMISS-3 (47.9) and other three genotypes with higher value than Alidoro (47.2). There were three genotypes ACHTAR*3//KANZ/KS85-84/3/ KATILA17/4/MON'S'/ALD'S'//ALDAN'S'/IAS58(52.6), ETBW 5898 (51.1), ETBW5535 (50.1) statistically significant difference (P=0.05) from the mentioned genotypes. About 75% of the promising genotypes were superior for the trait which showed that the trait is important selection parameter for heat stress tolerance bread wheat genotypes (Table 3).

The correlation of chlorophyll content to yield was positive under higher heat stress growing condition. Inconsistency of the study (Mohammad *et al*., 2012c) found that under favourable soil-water conditions and high temperature, less canopy temperature and more leaf chlorophyll content strength photosynthesis activity resulted in increasing of kernel weight. The study result of the flag leaf Chlorophyll Content, TKW showed statistically significant difference among genotypes response to heat stress with its correlation revealed as positively to yield which found in the study significant difference for genotypes under evaluation. In line with current study previous result showed that chlorophyll content, plant development and grain yield, contribute significantly to our understanding of the mechanisms determining potential productivity in superior germplasm under hot conditions (Delgado *et al*., 1994). The other findings were similar to the study by (Mirza et al, 2012) as showed that flag leaf chlorophyll content, grain filling rate and thousand grain weights were associated with stress tolerance special attention on these traits and focus on them in breeding programs (Zarei et al., 2013). Therefore, application in early generation selection, canopy temperature and chlorophyll content can be powerful tools for selecting advanced lines in the breeding program for performance in heat stressed target environments in agreement with the study result implication by (Delgado *et al*., 1994).

3.2.4. Stay Green

The stay green scale used for evaluation of genotypes under evaluation and percent of senescence ranged from 25 for promising genotypes (KAUZ'S'/FLORKWA-1//GOUMRIA-3, KATILA-3/3/MON'S'/ALD'S'//ALDAN'S'/IAS58, and ETBW5556) to 79 % (lowest yielded genotypes) with the 75% of out yielded and promising genotypes were also promising for their stay green trait performance (Table 3). The result had implication that even stay green was important trait in contributing heat tolerance it may not necessarily important for yield potential increment. The effect of stay green with others trait overall effect was more promising. In consistency of the result found different studies by Aziz et al., 2009;Mian et al., 2007 reported the importance of stay green trait for heat stress tolerance event it was not directly related with yield. The pervious study pointed out a positive non-significant correlation (r = 0.332) between grain yield and stay green duration and it was highly negative correlated with Canopy temperature (r = - 0.523, P =0.01) that showed bread wheat genotypes with cooler leaf present high grain yield and have longer stay green duration (Bilge *et al*., 2011). Jagadish, (2012) proved that stay green features contribute to grain yield of thermo tolerant genotypes, however, other factors such as longer crop duration and poor translocation of stem carbohydrates may disqualify stay green for high temperature environments. That is the stay green trait is conditional for its importance to genotypes tolerance to heat stress as it was observed as beneficial for early maturing genotypes.

3.2.5. Lodging

For lodging percent of genotypes under lower heat stress the value ranged from 0 to 25%, but 0 to 15% under higher heat stress and there were promising genotypes resistant to lodging and also out yielded. The result showed the most promising genotypes in their yield showed better and best performed genotypes in stay green were also promising for their resistance to lodging. For combined analysis the lodging percentage ranged from 0 to 18% and general performance of genotype with yield potential and stay green trait is pronounced too. This implies how strong the stay green and lodging resistance correlated for their combined contribution towards yield potential.Lodging was showed genotypic difference which implies that it needs attention so that it can affect yield in addition to heat stress. The supportive studies were discussed as the main causes of yield variation that can be reduced by breeding were identified as lodging and heat and most often yield difference among genotypes is correlated with lodging resistance and early maturing (Saulescu *et al*., 1998). As breeding objectives for a selection group of CIMMYT mega-environments two important traits are lodging tolerance and resistance to grain shattering that save the yield loss (CIMMYT, Unpublished data).

Traits	HL	G(48)	G x HL (48)	Pooled Error (85)	Mean	CV	R2	
CT	35.74**	1.89**	1.90**	0.13	31.1	1.14	0.96	
LA	10290.65**	16.68**	54.43**	4.33	30.31	6.87	0.97	
CLC	257.60**	14.30**	5.28**	2.46	46.36	3.38	0.87	
DMA	1395.56**	34.07**	13.86**	2.96	81.87	2.10	0.94	
SPF	108.76**	19.39**	15.72**	3.43	89.31	2.07	0.88	
YLD	2760820**	410776**	291597**	80449.7	1562	18.16	0.86	
TKW	204.08**	15.71**	13.77**	0.80	26.09	3.42	0.96	

*Table 1: Mean square of traits from combined ANOVA for two sowing dates based heat stress of forty-nine bread wheat genotypes at middle Hawash in 2013. Numbers in parenthesis represent degrees of freedom, ns, *, **, = Non Significant, significant at P = 0.05 and highly significant at P = 0.01 respectively, HL= Heat levels, G= Genotypes, CT=Canopy temperature (Infra red thermometer reading value), LA= Leaf area (cm^2), CLC=Chlorophyll content (SPAD Value), DAM = Days to maturity, SPF=Spike fertility (%), GY= Grain yield (kg/ha), TKW = Thousand kernel weight (g), CV= Coefficient of variation (%)*

Sowing Date	CT	LA	CLC	SG	LOD%	DAF	DMA	GFP	PLH
SD1	31.528^{A}	37.56^{A}	45.21^{B}	53	10	51.62^{B}	84.54^{A}	32.89^{A}	56.60^{B}
SD2	30.674^{B}	23.064^{B}	47.52^{A}	49	5	52.25^{A}	79.24^{B}	26.94^{B}	57.99^{A}
Mean	31.1	30.29	46.36	51	7.5	51.93	81.87	30.18	57.30
CR % (0.05)	0.10	0.59	0.45			0.45	0.49	0.53	0.61
EMS	0.13	4.33	2.46			2.55	2.96	3.52	4.61

Table 2: Grand means comparisons of phenological and physiological traits of BW under heat stress of two sowing dates
First sowing date relatively with lower heat stress (SD1), second sowing date with higher heat stress (SD2) post anthesis, Canopy temperature (CT), leaf area at booting (LA), flag leaf chlorophyll content at flowering (SPADf), stay-green rating (SG), lodging percentage rating (LOD), days to flowering (DAF), days to maturity (DM), grain filling period (GFP), plant height (PH), plant death (PD) and seed shattering (SSH).

Trt	CT		LA		CLC		SG		LOD%	
	HT	LT	HT	LT	HT	LT	LT	HT	LT	HT
1	29.6^{L-P}	28.8^{U}	30.4^{A}	30.2^{R-U}	50.5^{B-E}	47.9^{B-F}	31	38	5	0
2	30.5^{E-L}	30.2^{RTS}	22.6^{C-O}	30.4^{STU}	49.5^{B-G}	45.8^{D-K}	31	31	5	0
3	29.4^{M-P}	30.1^{ST}	26.4^{A-I}	29.6^{TU}	49.0^{B-H}	47.6^{B-G}	25	25	0	0
4	30.9^{B-K}	30.8^{QRS}	25.4^{A-J}	35.4^{J-O}	47.2^{C-K}	46.3^{C-J}	56	62	20	0
5	31.1^{B-I}	30.3^{ST}	23.6^{B-O}	43.9^{B}	47.7^{C-J}	44.5^{G-L}	70	38	15	0
6	30.1^{H-O}	31.4^{J-Q}	27.3^{A-G}	33.1^{N-R}	46.5^{E-K}	44.1^{I-L}	70	31	10	0
7	30.4^{F-M}	31.0^{O-S}	22.8^{C-O}	39.3^{E-H}	46.3^{E-K}	44.4^{G-L}	62	25	15	0
8	32.8^{A}	32.0^{D-K}	19.1^{L-R}	48.2^{A}	44.0^{JK}	44.5^{F-L}	70	70	10	0
9	29.1^{OP}	32.4^{D-G}	21.8^{F-P}	33.7^{M-R}	46.9^{D-K}	43.3^{JKL}	62	38	15	0
10	32.0^{AB}	32.7^{CDE}	22.8^{C-O}	35.1^{J-P}	46.8^{D-K}	44.3^{G-L}	62	62	15	5
11	30.0^{I-O}	31.9^{F-N}	27.3^{A-G}	38.5^{E-I}	46.2^{F-K}	44.8^{E-L}	70	62	20	10
12	30.6^{D-L}	30.4^{R-S}	22.6^{C-O}	35.1^{J-P}	46.8^{D-K}	45.4^{D-K}	70	70	20	15
13	30.8^{C-K}	31.3^{K-Q}	16.7^{PQR}	37.1^{H-K}	43.1^{K}	41.7^{L}	25	38	0	5
14	31.9^{ABC}	29.8^{T}	19.4^{K-R}	32.7^{O-S}	44.0^{JK}	37.7^{M}	62	70	15	15
15	31.7^{BCD}	32.4^{D-I}	19.6^{J-R}	43.5^{BC}	48.4^{C-I}	44.8^{E-L}	70	79	15	10
16	31.5^{B-F}	31.8^{F-P}	24.1^{B-M}	37.6^{F-J}	48.8^{B-H}	46.8^{B-I}	38	62	10	5
17	31.1^{B-J}	29.9^{T}	19.8^{J-R}	36.5^{I-M}	44.0^{JK}	43.3^{JKL}	62	70	15	10
18	30.3^{G-N}	31.8^{F-P}	18.8^{L-R}	48.3^{A}	48.7^{B-H}	44.2^{I-L}	79	62	20	5
19	30.4^{F-N}	32.0^{D-L}	20.7^{I-Q}	44.3^{B}	48.3^{C-I}	47.4^{B-H}	70	38	15	5
20	30.4^{F-N}	31.0^{N-S}	25.1^{A-K}	37.2^{HIJ}	46.6^{D-K}	46.1^{D-K}	38	31	5	0
21	30.6^{D-L}	28.0^{V}	20.7^{I-Q}	36.2^{I-M}	46.5^{E-K}	43.9^{I-L}	31	25	5	0
22	30.0^{I-O}	31.6^{I-Q}	21.1^{H-Q}	42.8^{CB}	46.2^{F-K}	45.2^{E-K}	70	31	10	0
23	30.0^{I-O}	32.2^{D-J}	23.6^{B-O}	36.2^{I-M}	47.8^{C-J}	44.5^{G-L}	79	70	25	5
24	31.1^{B-J}	31.5^{I-Q}	21.5^{G-P}	40.0^{D-G}	47.0^{C-K}	45.0^{E-L}	70	62	20	5
25	31.4^{B-G}	32.0^{D-L}	27.3^{A-E}	44.1^{B}	52.6^{B}	49.7^{AB}	62	38	20	5
26	31.4^{B-G}	33.4^{B}	23.1^{B-O}	43.9^{B}	44.2^{IJK}	44.4^{G-L}	38	38	5	5
27	30.1^{H-O}	30.3^{R-S}	17.8^{O-R}	46.9^{A}	46.3^{E-K}	41.8^{L}	38	62	10	0
28	31.5^{B-F}	31.2^{L-Q}	28.9^{ABC}	38.6^{E-I}	46.8^{D-K}	44.9^{E-L}	38	38	10	10
29	31.1^{B-J}	31.1^{M-R}	15.3^{QR}	38.6^{E-I}	43.9^{JK}	43.7^{I-L}	31	31	5	0
30	30.8^{C-K}	31.1^{M-R}	20.2^{J-R}	37.0^{H-L}	48.8^{B-H}	42.8^{KL}	38	62	10	5
31	31.4^{M-P}	31.9^{F-P}	18.5^{M-R}	34.4^{K-Q}	46.9^{D-K}	46.9^{B-I}	25	38	5	5
32	29.8^{K-O}	31.7^{G-Q}	27.1^{A-G}	32.5^{P-S}	50.8^{BCD}	49.4^{ABC}	31	31	0	5
33	30.9^{B-K}	31.9^{E-M}	18.2^{N-R}	44.2^{B}	46.4^{E-K}	52.3^{A}	70	62	15	10
34	28.7^{P}	30.9^{P-S}	27.7^{A-F}	31.8^{Q-T}	49.4^{B-H}	43.1^{JKL}	62	62	10	0
35	31.2^{B-G}	32.3^{D-J}	15.0^{R}	40.9^{CDE}	44.3^{IJK}	45.0^{E-L}	70	38	15	0
36	29.2^{OP}	31.5^{J-Q}	26.9^{A-F}	34.2^{L-Q}	47.2^{C-K}	43.0^{JKL}	25	25	5	0
37	31.3^{B-G}	32.8^{BCD}	25.5^{A-J}	36.3^{I-M}	49.4^{B-H}	44.9^{E-L}	38	38	10	5
38	30.8^{C-K}	31.7^{F-O}	21.9^{E-P}	35.0^{J-P}	49.1^{B-H}	45.3^{D-K}	38	62	15	0
39	31.9^{ABC}	32.1^{D-J}	23.9^{B-N}	32.1^{Q-T}	50.0^{B-F}	45.4^{D-K}	38	62	10	0
40	30.8^{C-K}	32.4^{D-H}	28.2^{A-D}	31.0^{R-U}	47.0^{C-K}	44.9^{E-L}	38	38	5	0
41	29.9^{K-O}	31.9^{F-N}	25.8^{A-H}	35.7^{J-N}	45.2^{H-K}	44.5^{F-L}	79	70	15	5
42	31.6^{B-E}	31.4^{K-Q}	22.5^{D-P}	37.6^{G-J}	46.0^{F-K}	46.1^{D-K}	70	38	10	0
43	29.8^{K-O}	32.0^{D-L}	24.5^{B-L}	42.4^{BCD}	49.6^{B-G}	47.9^{B-E}	62	62	10	5
44	30.8^{C-K}	32.4^{DEF}	19.9^{J-R}	39.2^{E-I}	45.7^{G-K}	43.9^{I-L}	62	62	5	0
45	31.3^{B-G}	31.6^{H-Q}	26.8^{A-H}	29.0^{U}	51.2^{BC}	48.6^{BCD}	38	62	10	0
46	31.2^{B-H}	33.2^{BC}	25.1^{A-K}	40.2^{DEF}	48.4^{C-I}	44.9^{E-L}	38	62	10	0
47	30.6^{D-L}	32.1^{D-K}	25.0^{A-K}	36.5^{H-M}	56.8^{A}	48.0^{B-E}	70	62	10	5
48	29.9^{K-O}	34.5^{A}	28.0^{AB}	37.3^{G-J}	47.1^{C-K}	46.1^{D-K}	62	38	10	5
49	29.3^{NOP}	32.2^{D-I}	21.4^{G-P}	36.3^{I-M}	47.9^{C-J}	44.9^{E-L}	62	31	5	0
Mean	30.7	31.5	23.0	38	47.5	45.2	53	49	10	5
CR (%)	1.152	0.84	5.925	2.898	4.228	3.38				
EMS	0.217	0.116	5.7396	1.3728	2.9234	1.86				
CV (%)	1.52	1.08	10.40	3.12	3.60	3.02				

Table 3: Mean comparison of physiological traits for forty nine bread wheat genotypes tested for heat stress under two sowing dates at middle Hawash in 2013.

**lower heat stress (LT), Higher heat stress (HT), Canopy temperature (CT), leaf area at booting (LA), flag leaf chlorophyll content at flowering (SPADf), stay-green rating (SG) (in % leaf/stem senescence), and lodging in percentage (LOD).*

3.3. Correlations among Traits

Under the lower heat stress chlorophyll content negative correlate and highly significant (P=0.01) with days to maturity (r=-0.51. This implies the chlorophyll content trait used directly with yield but inversely with phonological traits under heat stress for wheat under study. Days to flowering and days to maturity correlate positive highly significant (P=0.01) with plant height(r=-0.61), (Table 4).

Under the higher heat stress growing micro climate condition canopy temperature correlate negative and statistically highly significant (P=0.01) with leaf area (r=-0.33), plant height (r=-0.43). Days to flowering correlate negative and statistically highly significant (P=0.01) with GFP (r=-0.62). There are some traits having negative correlation to one another but both correlates to yield positively as revealed by the study result. For overall heat stress interaction, the correlation analysis revealed that canopy temperature correlates negative and statistically highly significant (P=0.01) with plant height (r=-0.46), but positive statistically highly significant (P=0.01) with leaf area (r=0.32). Leaf area correlate positive statistically highly significant (P=0.01) with days to maturity (r=0.45), grain filling period (r=0.49). Days to flowering and maturity correlate negatively even statistically non-significant to yield which implied that the early genotypes were slightly lower in yield performance. The above traits higher value under heat stress contributes to yield potential and direct selection of the trait is important (Table 5).

All of the traits under study were correlated with each other even the magnitude and significance varied as the study of Mohammadi *et al*., (2004). Most of the traits and canopy temperature were correlated negatively which implies the reality of using lower canopy temperature of genotypes required for heat tolerant wheat genotypes selection and some of the traits with days to flowering and maturity also showed a negative correlation which may efficient utilization of resources process of escape mechanism from heat stress (Table 4and 5). Most morph-phonological traits less affected by heat stress; while,leaf area, highly reduced and correlated with reduced yield directly. Similar to the study the positive correlation effect of chlorophyll continent to yield and heat tolerance was found under heat stress conditions, (Lack, 2013). The physiological trait chlorophyll content has positive correlation with yield (Delgado *et al.,* 1994). The study also revealed the genetic variability of genotypes against the interaction of the heat stress with a statistically highly significant different (P=0.01) for most of the traits. Higher correlation coefficient under heat stress conditions showed that CT influences grain yield more strongly in heat stress having strong negative correlation with yield and kernel weight, highly but weak significant negative correlation to grain filling period and kernel per spike. (Mohammadi and Karimizadeh, 2012). In agreement with the study similar correlation coefficient among traits and the grain yield positively significant correlation with biomass and harvest index under heat stress was found previously (Riaz *et al*., 2010).

	CT	LA	CLC	DAF	DMA	PHT	YLD
LA	0.23*						
CLC	0.16ns	0.034ns					
DAF	-0.27**	0.095ns	-0.316**				
DMA	-0.25**	-0.032ns	-0.514**	0.58671**			
PHT	-0.47**	-0.172ns	-0.175ns	0.395**	0.412**		
YLD	-0.16ns	-0.285**	0.121ns	-0.268**	-0.101ns	0.316**	
GFP	0.078ns	-0.144ns	-0.131ns	-0.607**	0.287**	-0.063ns	0.2181*

Table 4: Estimates of correlation coefficient among different traits of forty-nine bread wheat genotypes evaluated under first sowing date lower heat stress at middle Hawash 2013.

**, **, = significant at P = 0.05 and significant at P = 0.01respectively. CT= Canopy temperature, LA = Leaf area (cm2), CLC= SPAD value, DAF= Days to flowering, DAM = Days to maturity, GFP = Grain filling period, PH = Plant height (cm), YLD= Grain yield (kg/ha).*

	CT	LA	CLC	DAF	DMA	PHT	YLD
LA	-0.33**						
CLC	-0.10ns	0.38**					
DAF	-0.03ns	-0.15ns	0.09ns				
DMA	-0.09ns	-0.11ns	0.19ns	0.59**			
PHT	-0.43**	0.34**	0.26**	-0.10ns	-0.16ns		
YLD	-0.39**	0.48**	0.16ns	-0.49**	-0.26**	0.45**	
GFP	-0.05ns	0.07ns	0.08ns	-0.62**	0.27**	-0.04ns	0.33**

Table 5: Estimates of correlation coefficient among different traits of forty-nine bread wheat genotypes evaluated under second sowing date higher heat stress at middle Hawash 2013.

**, **, = significant at P = 0.05 and significant at P = 0.01 respectively. CT= Canopy temperature, LA = Leaf area (cm2), CLC= SPAD value, DAF= Days to flowering, DAM = Days to maturity, GFP = Grain filling period, PH = Plant height (cm), YLD= Grain yield (kg/ha).*

	CT	LA	CLC	DAF	DMA	PHT	YLD
LA	0.32**						
CLC	-0.15*	-0.24**					
DAF	-0.17*	-0.06ns	-0.09				
DMA	0.08ns	0.45**	-0.36**	0.44**			
PHT	-0.46**	-0.04ns	0.11ns	0.15*	0.04ns		
YLD	-0.15*	0.24**	0.03ns	-0.37**	-0.01ns	0.35**	
GFP	0.23**	0.49**	-0.27**	-0.53**	0.53**	-0.10ns	0.35**

Table 6: Estimates of correlation coefficient among different traits of forty-nine bread wheat genotypes tested under different heat stresses combined analysis at middle Hawash 2013.

**, **, = significant at P = 0.05 and significant at P = 0.01 respectively. CT= Canopy temperature, LA = Leaf area (cm2), CLC= SPAD value, DAF= Days to flowering, DAM = Days to maturity, GFP = Grain filling period, PH = Plant height (cm), YLD= Grain yield (kg/ha).*

4. Summary and Conclusion

The current climate change and global warming effect heat stress on wheat production in tropical developing countries is the challenging problem for sustainable wheat production. Bread wheat is above all the staple food crop worldwide and most climate change affects its productivity. The principle of breeding against the facing problem is well understood but the status of breeding for abiotic stress; especially heat stress in developing countries like Ethiopia has not received much attention. In general, abiotic stress specifically heat, stress tolerance wheat genotypes evaluation and tolerant variety development and further utilization for breeding purpose is the important breeding strategy currently for eastern and southern Africa.The objective of the study was to evaluate bread wheat genotypes for heat tolerance and yield performance. The combined ANOVA for heat stress levels at post anthesis revealed that highly significant difference (P=0.01) by heat stress Levels (HL) for all physiological traits 49 entries evaluated. The result revealed that the genotypes variability in tolerance to heat and importance of different traits for selection criteria under heat stress. The present study showed the promising importance of these techniques for selecting tolerant genotypes which can reduce the gap of food security problem in a country. The physiological traits used canopy temperature is negatively but flag leaf chlorophyll content and leaf area were positively correlated to yield which implies how much it is required and important as evaluation criteria for bread wheat genotypes under heat stress. All traits were correlated and their importance to the study were estimated. There were some genotypes found both tolerant to heat stress and out yielding than most other genotypes from the result of study. Working with abiotic stress in Ethiopian and other developing countries is unquestionable and be considered as an important breeding strategic activity in future to address climate change effect. The direct and indirect effect difference of the different traits to yield under heat stress may need further study and the percent estimate of each and every trait impact to heat tolerance, high yield and quality grain also that require due attention for the coming climate change effect. Physiological traits study facility accessibility especially for developing countries for the success of strategic breeding programme and international researchers, officials, stock holder’s interaction and integrity is important issue for future.

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Appendix

SD	Daily	R.F (mm)	Temperature °C			Daily To.°C	R.H (%)	D.pt. °C	Evap. (mm)	SunS. hrs/day	WS	Soil temp. at	
			Min	Max.	Mean						2m	5cm	20cm
1	Mean	4.10	24	33.85	27.91	27.69	56.49	19.45	3.52	6.24	1.51	33.35	29.18
	Max	63.0	24	37.50	30.75	30.70	76.80	24.14	14.44	10.70	3.37	39.20	30.76
	Min	0.0	24	27.30	18.10	22.50	0.00	7.90	-44	0.00	0.00	26.16	26.96
2	Mean	2.02	24	34.27	28.31	28.39	32.72	19.50	2.91	7.49	1.65	34.67	29.81
	Max	21.4	24	37.50	30.75	30.70	76.80	24.14	9.70	10.70	2.87	41.36	31.40
	Min	0.0	24	30.50	26.00	25.20	0.00	15.90	-46	0.00	0.00	28.80	28.40
1-2	Mean*	2.08	0.0	-0.42	-0.39	-0.70	23.77	-0.06	0.60	-1.24	-0.1	-1.31	-0.63
	Max	41.6	0.0	0.00	0.00	0.00	0.00	0.00	4.74	0.00	0.49	-2.16	-0.64
	Min**	0.0	0.0	-3.20	-7.90	-2.70	0.00	-8.00	1.90	0.00	0.00	-2.64	-1.44
*&** (1-2)/2		1.0	0.0	-1.8	-4.1	-1.7	11.9	-4.0	1.3	-0.6	-0.1	-2.0	-1.0
Aver. (1-2)		14.6	0.0	-1.21	-2.76	-1.13	7.92	-2.69	2.41	-0.41	0.12	-2.04	-0.90

Table 1: Maximum, mean and minimum daily meteorological data record difference between the first and late sowing date during grain filling period at WARC, 2013
Source: Werer Agricultural Research Center (WARC) (Unpublished data)

Year recorded	Total R.F(mm)	Max Temp(°c)	Min Temp(°c)	Temp (°c)	Aver R.H(%)	Evaporation (mm)	Sunshine (hrs/day)	Soil temp at	
								5cm	20cm
2000	564.9	34.4	18.5	28.1	48.8	2528.4	8.8	34.6	29.2
2001	601.5	34.6	18.9	28.3	50.2	2558.1	8.7	35.6	29.6
2002	360	35.5	20.2	29.2	46.1	3111.2	8.7	36.5	30.5
2003	543.3	35.3	19.6	28.7	50.9	3026.5	8.6	34.2	29.6
2004	238.8	34.5	19.5	28.4	50.8	2882.7	8.7	32.3	28.4
2005	791.1	34.4	19.3	28.3	52.8	2806.2	8.7	32.0	28.5
2006	570.1	34.4	19.9	28.5	53.5	2681.3	8.6	33.0	29.2
2007	641.6	34.5	19.0	27.9	52.3	2801.9	8.5	34.2	29.5
2008	488.5	34.3	18.5	28.1	50.0	2888.2	8.9	35.3	28.8
2009	437.7	35.1	20.0	28.9	48.4	3161.2	8.7	35.9	29.8
2010	717.2	34.4	20.1	28.1	55.3	2669.0	8.1	32.5	29.4
2011	531.4	35.0	21.4	28.1	49.3	2745.1	8.4	31.2	29.2
2012	508.2	35.4	20.1	28.4	48.7	2774.3	8.6	32.4	29.2
Mean	538.0	34.8	19.6	28.4	50.5	2818.0	8.6	33.8	29.3

Table 2: Thirteen year's meteorological data record of study site at middle Hawash.
Source: Werer Agricultural Research Center (WARC) (Unpublished data)

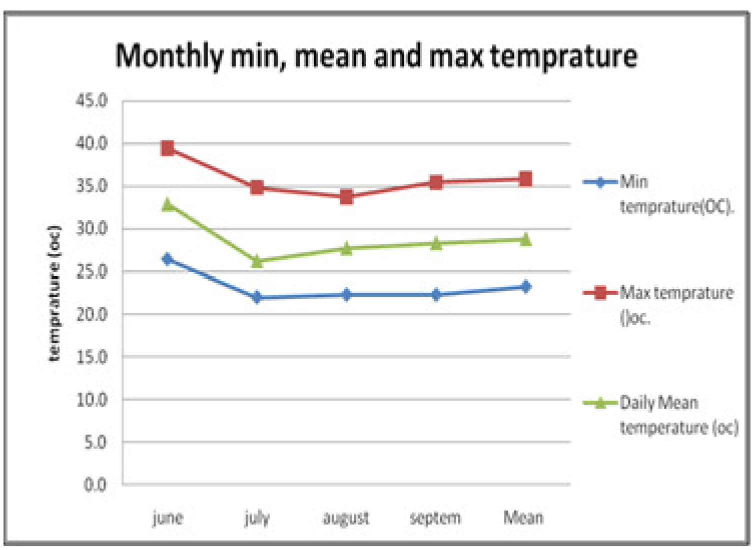

Figure 1: Mean monthly maximum and minimum temperature of study site at middle Hawash during the experimental period 2013.
Source: WARC (Unpublished data)

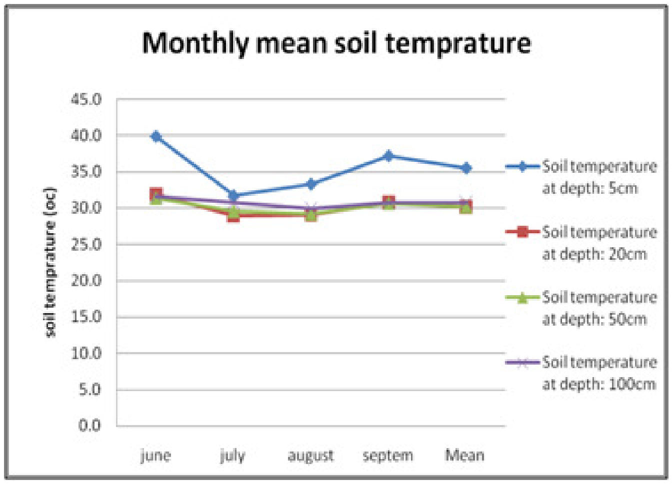

Figure 2: Soil temperature during cropping time for forty nine bread wheat genotypes grown under heat stress growing condition at middle Hawash 2013
Source: WARC (Unpublished data

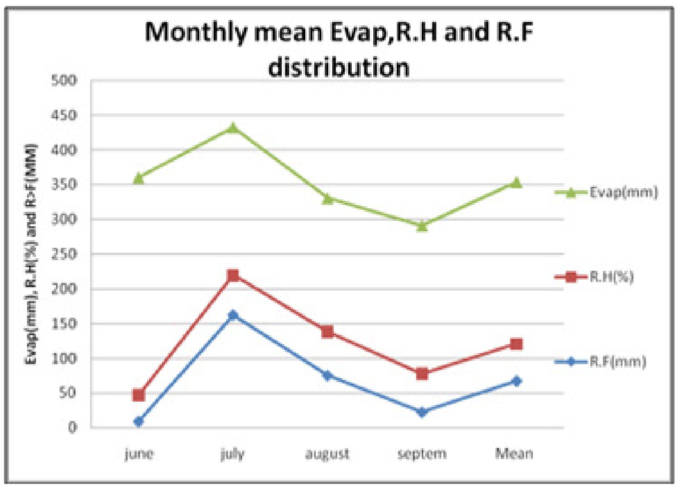

Figure 3: Mean monthly RF, RH and evaporation during cropping time for forty nine bread wheat genotypes grown under heat stress at middle Hawash 2013.
Source: WARC (Unpublished data)

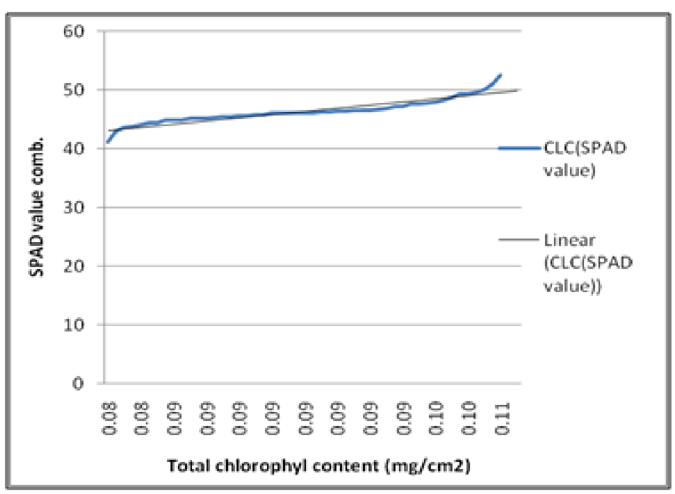

Figure 4: The relationship of the flag leaf chlorophyll (SPAD value) and total chlorophyll per unit area (mg/cm^2) at 50% flowering stage comb.