

ASSESSMENT OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES FOR HIGH TEMPERATURE STRESS TOLERANCE USING PHYSICO-CHEMICAL ANALYSIS

KHALIL AHMED LAGHARI¹, ABDUL JABBAR PIRZADA², MAHBOOB ALI SIAL¹
MUHAMMAD ATHAR KHAN¹ AND JAMAL UDDIN MANGI²

¹Nuclear Institute of Agriculture (NIA), Tandojam

²Institute of Plant Sciences, University of Sindh, Jamshoro

*Corresponding author: lagharikhalil@hotmail.com

Abstract

High temperature stress would be one of the major threats to wheat productivity due to changing climate scenario. A field study was carried out at Experimental Farm of Nuclear Institute of Agriculture, Tando Jam during 2014-15 by using 20 wheat genotypes (mutants and advance lines) along with four check varieties Sarsabz, TJ-83, TD-1 and Kiran-95. The breeding material was subjected to two different sowing dates viz. sowing date-1 (November 15 as normal sowing) and sowing date-2 (December 25 as late sowing to induce high temperature stress at grain filling). Along with agronomic, breeding data, physiological parameters i.e., SPAD chlorophyll content, proline, osmotic potential and leaf area were assessed in both normal sown and late sown crop to see the affect of high temperature stress at different traits including physico chemical parameters. Results revealed that seven genotypes NIA-8/7, NIA-AMBER, BWS-78, NIA-10/8, NIA-28/4, DH-12/1 and check KIRAN-95 were found tolerant to heat stress and produced higher grain yields (>4000 kg ha⁻¹) under heat stress conditions. While out of seven genotypes, five genotypes DH-12/1, BWS-78, NIA-10/8, NIA-28/4, and Kiran-95 possess one or the other efficient physiological mechanism to cope with heat stress. Whereas NIA-8/7 and NIA-Amber were found having superiority in agro-morphological traits under stress conditions. Most of the physiological traits SPAD chlorophyll and leaf area, proline and osmotic potential had no any significant correlation with grain yield in normal sown crop and highly significant correlation of these traits were calculated under heat stress conditions.

Key words: Wheat, High temperature stress, Physico-chemical approaches.

Introduction

Wheat crop is one of the major staple food crop of Asia and Africa. This crop is highly vulnerable to high temperature stress in both these continents. Wheat in Pakistan is grown over 8.825 million hectares with annual production of 24.946 million tones and average yield 2827 kg ha⁻¹ (Anon., 2019-2020).

High temperature stress is a major wheat yield decreasing factor in central and south east Asia, North Africa, Europe, Australia, and the United States (Semenov & Shewry, 2011; Lott *et al.*, 2011). It is assessed that by 2050, around 60% rise in wheat productivity will be required as to feed increasing population (Rosegrant & Agcaoili, 2010). During the coming decades, future climate changes are predicted to decrease wheat yield in developing countries by 20–30% (Lobell *et al.*, 2008).

Wheat crop will be facing severe terminal heat shocks (Mitra & Bhatia, 2008; Semenov, 2009). Temperature is documented to be one of the major factors controlling and affecting each plant developmental stage. Increase or decrease in temperature will produce huge impact on plant productivity (Hatified & Prueger, 2015). Each plant specie has certain range of minimum, maximum and optimum temperature beyond which plant developmental process is handicapped. Likewise, temperature affects yield and yield contributing traits of wheat, it also affect vegetative and reproductive stages of wheat crop. All enzymatic activities, gene response and expression are also governed by the temperature. Different species and different genotypes within same specie have different mechanisms such as rolling, shedding and thickening of leaves, reduction of leaves size and duration growth are adjusted to diminish affect of heat stress at morphological level (Wahid *et al.*, 2007).

Reynolds & Trethowan (2007) found strong positive association of number of physiological parameters such as canopy temperature depression (CTD), leaf chlorophyll, grainfilling period, cell membrane stability and osmotic potential with grain yield under high temperature prone environments. Amani *et al.*, (1996) showed CTD to be correlated to yield under hot, dry, irrigated conditions in Mexico. Other researchers have found that there are many plant growth regulator (PGR) which include new chemistry agro chemicals such as glycine betaine, salicylic acid, vitamin E, proline and choline help plant to mitigate temperature, drought and salinity affects (Mohammed & Tarpley, 2009). These scientists are in opinion that physiological traits would help to select heat tolerant genotypes and also will supplement future breeding. Semenov & Halford (2009) highlighted that genotypes with particular agro-morphological ideotypes, special physiology and unique molecular markers should be breed for heat tolerance keeping in view future changing climate scenario. Apart from this, many strategies such as agronomic and other management applications also needed to be considered to reduce high temperature affects (Farooq *et al.*, 2011). Halford (2009) concluded that in order to select better adapted varieties or genotypes in present changing climate scenario, it is crucial that over all crop response to raised temperature and other agro-morphological and physiochemical mechanisms involved in a genotype to cope with stressed condition should be main focus to identify best high temperature tolerant genotypes. Tahir *et al.*, (2009) were of view that presently available high yielding varieties as well as varieties released in past are losing their potential yield performance with changing soil as well climatic factors. Continuous evaluation of new germplasm and selection of most suitable genotypes for wide range of edaphic as well environmental factors is crucial to increase

per hectare yield for targeted environments. Hence, it has been cleared that all possible integrated approaches can be undertaken to identify heat tolerant genotypes to assess mechanisms involved in plant performance under increasing temperature stress. This study is also part of such integrated approaches and to see effect of high temperature stress on physiochemical traits and genotypes mechanisms involved. Correlation studies will predict contribution of all such mechanisms which help plant to reduce heat stress affects and produce higher productivity under temperature stress conditions.

Materials and Methods

A field study was carried at Experimental Farm of Nuclear Institute of Agriculture, Tandojam in 2014-15 to address main issue of high temperature stress tolerance in wheat crop. Twenty wheat genotypes mutants/ advance lines along with four check varieties (Sarsabz, TJ-83, TD-1 and Kiran-95) were exposed to different temperature regimes by sowing on two different dates i.e. November 15 (as normal sowing, without temperature stress at grain filling stage) and December 25 (as late sowing to induce high temperature stress at grain filling stage). Experiment was conducted in Randomized Complete Block Design (RCBD) with three replications. Each experiment was sown with 4 rows of 3 m length and row to row distance of 30 cm.

Metrological data: Metrological data was recorded through out crop season from November to April in 2014-15 wheat cropping season with hygrothermograph, installed in front of wheat field of NIA office.

Minimum, maximum temperature and relative humidity data was recorded by using hygrothermograph. Data was automatically recorded on sheet of hygrothermograph which was changed every week. Mean of minimum and maximum temperature of each month was calculated along with minimum lowest and maximum highest temperatures are shown in Table 1.

Grain yield g plot⁻¹: The observations on yield and yield related data were also recorded. Two cental rows were harvested and threshed with vogel thresher and weighed on electronic balance to record grain yield data (Fig. 1).

The physiochemical data viz SPAD chlorophyll, osmotic potential, proline and leaf area were assessed both in normal and high temperature stressed trials. Physiochemical parameters were measured through following standard methods.

Determination of SPAD Chlorophyll: SPAD Chlorophyll

reading were recorded from 5 flag leaves of each genotype in each replication. Three reading each from top mid and bottom were taken from each leaf and mean SPAD chlorophyll value of each leaf were noted. This chlorophyll were recorded through non destructive process with help of SPAD chlorophyll meter (Minolta SPAD (520) Meter made by Minolta Camera Co., Tokyo, Japan).

Determination of Proline ($\mu\text{mole proline g}^{-1}$ fresh weight): Proline content was determined from flag leaves at the time of grain filling in both stressed and non-stressed experiments by the method described by Bates *et al.*, 1973. Flag leaves were chopped and 0.5 g fresh leaf tissue was weighed and then homogenized in 10 ml of 3% sulfo-salicylic acid. The homogenate was taken and filtered through Whatman filter paper. Two ml quantity of filtrate were taken, 2 ml of freshly prepared ninhydrin acid was added into it and the solution was kept at 4°C in a refrigerator and then 2 ml of glacial acetic acid was added in test tubes. The mixture was allowed to react in water bath for one hour at 100°C. The reaction was terminated in an ice bath, 4 ml toluene was added to extract proline through mixing vigorously on stirrer for 1-2 minutes. The chromophore containing proline were taken from the aqueous phase, warmed at room temperature and the absorbance was read at 520 nm using toluene as a blank. The proline concentration was measured from a standard curve and calculated on fresh weight basis using formula:

$$\text{Proline (umole/g .fresh weight)} = (G.R \times 4 \times 10) / S.wt. \times 2 \times 115.5$$

Leaf area (cm²): Leaf area is one of the important parameter which play main role in photosynthetic activity, sun light radiation use efficiency, leaf transpiration and anatomical studies. Three flag leaf from each genotypes as per replication were brought to laboratory. Leaf area of each leaf was measured through automatic leaf Area Meter (LI-COR - LI-3100, USA).

Osmotic potential (-megapascal): Fresh leaf samples (flag leaf) were taken and immersed in a glass tube. A swab of cotton containing chloroform was placed in the test tube. The test tubes were then kept in freezer to kill the leaf tissues. These test tubes were taken out after 24 hours, acclimatized at room temperature and the cell sap was extracted with the help of syringe. This cell sap was taken in a PCR Tube and osmotic potential (OP) was measured by Osmometer (Model-030, Germany).

Data were statistically analyzed for mean square values and interaction of different factors using computer based software Statistics 8.1. Error bar on graph were made using Microsoft Excel.

Table 1. Mean monthly temperature minimum, maximum and minimum lowest and maximum highest temperature during wheat cropping seasons 2014-15.

Year	Month	Mean minimum temperature	Mean maximum temperature	Minimum lowest temperature	Maximum highest temperature
2014-15	November	21.6	32.4	18	37
	December	15.6	27.96	6	34
	January	6	19.26	0	23
	February	8.1	22.2	1	27
	March	20.2	33.6	6	40
	April	25.7	40	19	43

Results and Discussions

Mean monthly temperature during vegetative growth for both normal and late (high temperature stressed conditions) remained favorable for crop growth. Normal sown crop completed its life cycle upto mid-March hence escaped heat shocks while late sown crop faced heat shocks in the month of March and April upto 43°C due to which many physiological and yield and yield related traits were affected (Table 1). Grain yield data showed that under heat stress conditions wheat genotype NIA-8/7, NIA-Amber, BWS-78, NIA-10/8, NIA-28/4, DH-12/1 and check Kiran-95 produced higher mean grain yield than other contesting entries (Fig. 1).

SPAD chlorophyll: SPAD Chlorophyll values varies widely both in normal and late sowing conditions. At non stressed condition chlorophyll content ranged from 36.3 in MSH-5 to 60.3 in MASR-64. Eight genotypes MASR-64, BWS-78, NIA-Amber, NIA-10/8, NIA-28/4, NIA-8/7, BWM-3 and check Kiran-95 had higher SPAD chlorophyll values at normal sowing. It was found that genotypes producing higher grain yield were found to possess higher chlorophyll SPAD values. Genotypes MASR -64, BWS-78, NIA-8/7, NIA-10/8, DH-12/1, NIA-Amber, NIA-28/4 and Kiran-95 had produced more SPAD Chlorophyll values under stress conditions as compare to other genotypes (Fig. 2). Talkuder (2011) in his Ph.D. thesis has also reported chlorophyll losses due to heat stress. Farooq *et al.*, (2011) and Wahid *et al.*, (2007) had reported that photosynthesis is one of the main sensitive process to heat stress. Heat stress reduces green area of leaf causing leaf senescence and yield losses which ultimately affect chlorophyll content measured by SPAD meter (Wang *et al.*, 2011). Physiological data indicated that heat stress significantly affected SPAD chlorophyll content, Osmotic potential, Proline and leaf area. Genotypes DH-12/1, BWS-78 and MASR-64 had shown strong physiological adjustment in chlorophyll content, leaf area proline and osmotic potential traits in response to heat stress. While NIA 10/8 and NIA-28/4 had responded to heat stress by adjusting leaf area and chlorophyll content and Kiran-95 had responded heat stress by adjusting chlorophyll content and osmotic potential.

Proline content ($\mu\text{mole proline g}^{-1}$ fresh weight): Proline content data pointed out that there were non-significant differences among genotypes for proline content when observed in non stressed crop. Proline content under normal sowing ranged from 6.0 in MASR-64 to 19.3 in NIA-10/8. At normal sowing genotype NIA-10/8, NIA-9/5, and NIA-28/4 had shown higher proline values than other contesting entries. Whereas proline content significantly increased many fold under stress a condition that is during late sowing. At stressed conditions proline content ranged from 49.0 in MSH-5 to 350 in MASR-64. Tolerant genotypes accumulated more proline content than susceptible one. Genotype MASR-

64, BWS-78, DH-12/1 and Kiran-95 produced significantly higher proline content under stress conditions (Fig. 3) Tang *et al.*, (2008) concluded that proline is an important indicator for evaluating different species under stress. Elevated levels of proline are associated with genotypes that are better adapted to stress environments (Ahmed & Hasan, 2011). Hassan *et al.*, (2007); Khan *et al.*, (2013) and Khan *et al.*, (2020) have reported that higher quantity of proline accumulation in wheat under high temperature stress conditions.

Osmotic potential (-megapascal): Osmotic potential (-megapascal) data recorded during experimentation is described in figure 4. Data revealed that genotypes had shown different osmotic potential (op) values both under normal as well under stress conditions. Osmotic values at normal sowing ranged from -1.0 in MASR-64 to -1.9 in DH-11/3. At normal sowing MASR-64, BWS-78, DH-12/1 and Kiran-95 had shown desirable values of op. Whereas at late sowing op values ranged from -1.7 in DH-12/1 and MASR-64 to -2.9 in TJ-83. It is hypothesized that those genotypes which could maintain their osmotic potential under high temperature stress (SD-2, late sowing; stressed conditions) produces higher productivity. Higher yielding genotypes DH-12/1 MASR-64, BWS-78 and Kiran-95 had not maintained their op under heat stressed conditions efficiently. They might have some other mechanisms to cope with high temperature stress (Fig. 4). Balla *et al.*, (2014) is of view that physiological and biochemical parameters need to be jointly considered along with grain yield and other yield related parameters in assessing high temperature stress tolerance in wheat, they reported that heat stress reduced chlorophyll content, transpiration and stomatal conductance.

Leaf area (cm^2): Leaf area of wheat genotypes sown in late and normal conditions are given in figure 5. Leaf area differs in different genotypes both in normal and late conditions. At normal sowing leaf area ranged from 23 cm^2 to 45 cm^2 . At normal sowing MASR-3, BWS-78, NIA-10/8, MASR-23, TJ-83, TD-1 and Kiran-95 had produced larger leaf area as compare to other genotypes. However, leaf area significantly reduced due to heat stress in late sown crop. Genotypes DH-12/1, NIA-28/4, NIA-10/8, BWS-78, MASR-64 and MASR-3 produced statistically significantly more leaf area as compare to other genotypes under late sowing condition (Fig. 5). Hatified & Pruger (2015) had reported non significant affect of temperature on leaf area and biomass under stress heat conditions as compared to normal temperature. In other study conducted by Farida & Ashratunnesa (2014) to assess high temperature tolerance under control condition, they had reported that high temperature reduced leaf area, total crop duration and chlorophyll content. Moaed & Deshmukh (2012) had also found that high temperature stress reduces total leaf area, crop growth rate, chlorophyll values, grain yield and yield components. This reduction was more in genotypes which were recommended for irrigated environments than rainfed conditions.

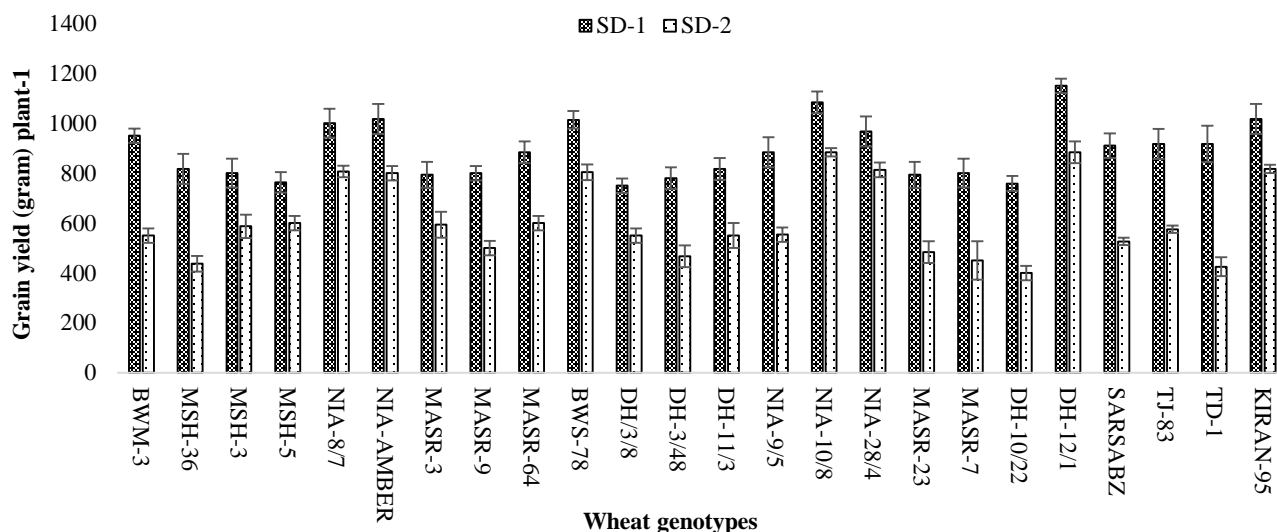


Fig. 1. Grain yield (gram plot⁻¹) of elite wheat genotypes under normal and heat stressed conditions during 2014-15. (SD-1= Sowing date 1; November 15th, SD-2=Sowing date 2; December 25th). Each bar is Mean + S.E (n=3).

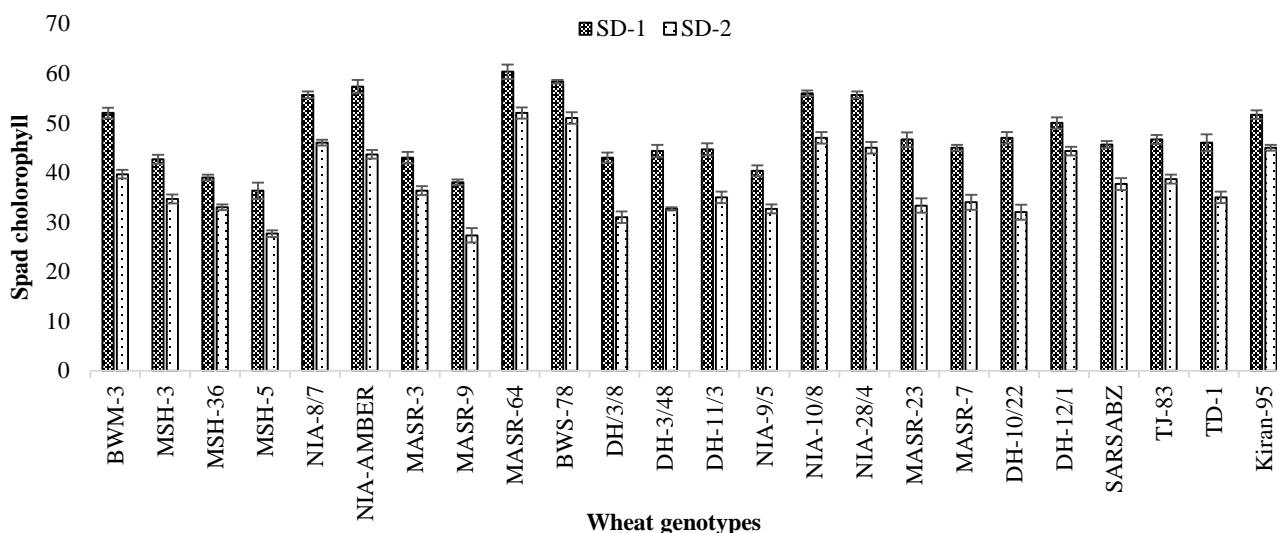


Fig. 2. SPAD chlorophyll content of elite wheat genotypes under normal (SD-1) and heat stressed (SD-2) conditions. SD-1= November 15th; SD-2 December, 25th. Each bar is Mean + S.E (n=3).

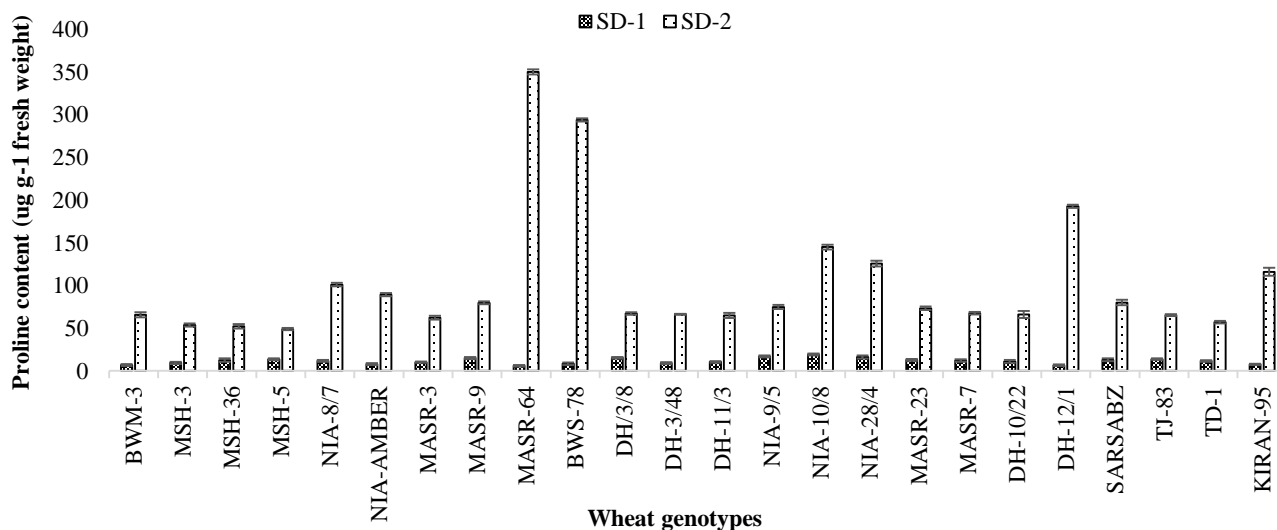


Fig. 3. Proline content (ug g⁻¹ fresh weight) of elite wheat genotypes under normal (SD-1) and heat stressed (SD-2) conditions. SD-1= November 15th; SD=2 December 25th. Each bar is Mean + S.E (n=3).

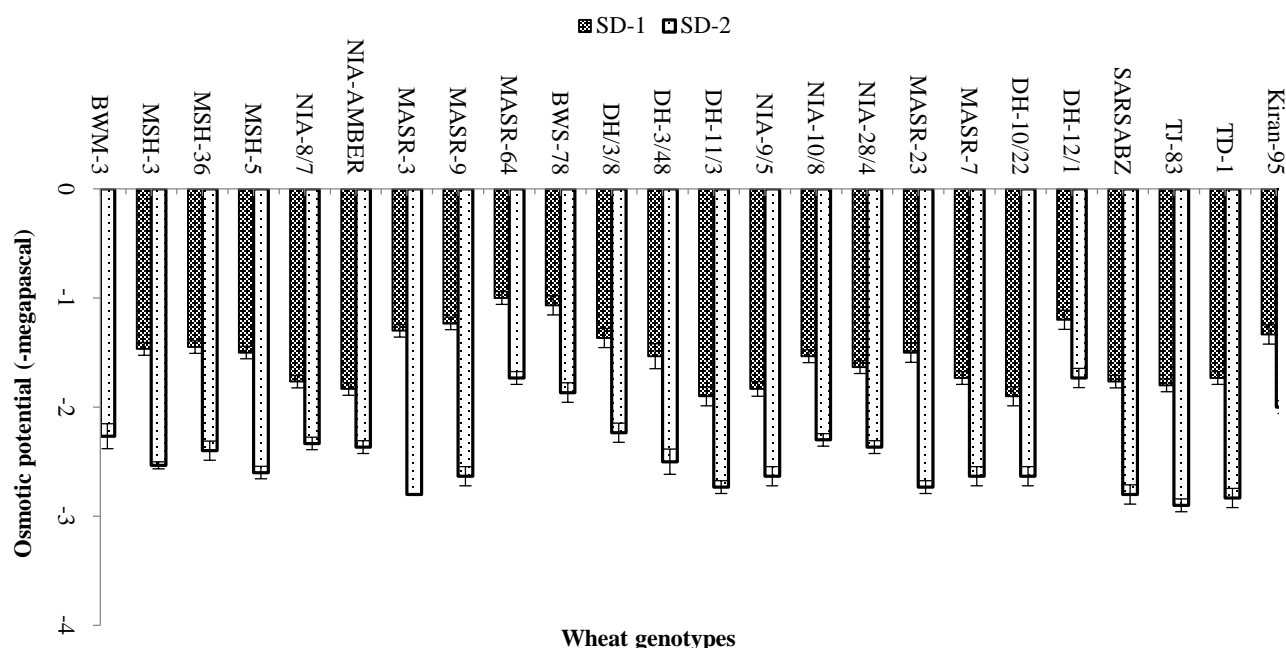


Fig. 4. Osmotic potential (-megapascal) of elite wheat genotypes under normal (SD-1) and heat stressed (SD-2) conditions. SD-1= November 15th; SD=2 December 25th. Each bar is Mean + S.E (n=3).

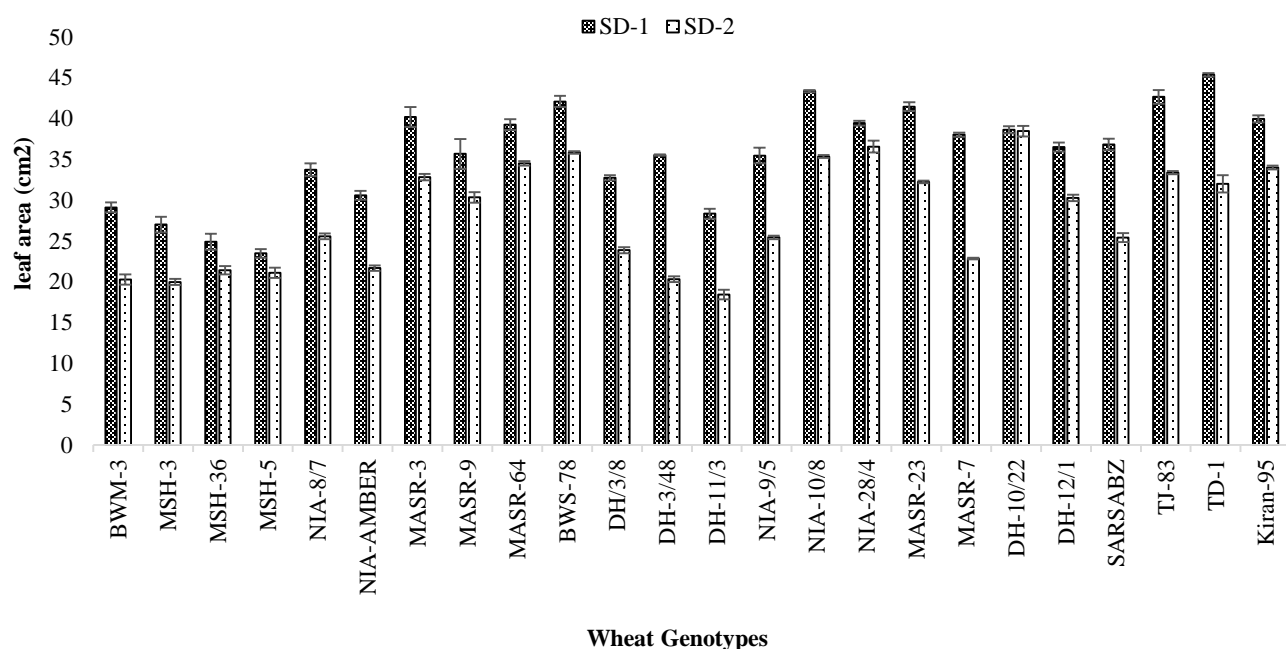


Fig. 5. Leaf area (cm²) of elite wheat genotypes under normal (SD-1) and heat stressed (SD-2) conditions. SD-1= November 15th; SD=2 December 25th. Each bar is Mean + S.E (n=3).

Correlation studies of various physiological parameters:

Correlation studies of various physico-chemical parameters with grain yield under normal sowing time are described in table 2. Most of the physiological traits spad chlorophyll and leaf area had non-significant interaction with grain yield under normal sowing conditions. Hence it depicts the little role of these parameters for selection of genotypes under non stressed conditions (Table 2).

Table 2 describes the correlation of various physiological traits under heat stressed conditions. Physiological traits were highly significantly correlated with grain yield under stress conditions.

Grain yield was highly significantly correlated with SPAD Chlorophyll (0.745**), osmotic potential (0.606**) and proline (0.706**) (Table 3). SPAD chlorophyll had positive correlation with proline, op and leaf area (0.782**, 0.668** and 0.431* respectively). Proline had significant positive correlation with op (0.783**) and leaf area (0.477**) whereas op has positive correlation with leaf area (0.477**). Reynolds *et al.*, (1994) described a significant positive correlation of physiological parameters under heat stress environment.

Table 2. Correlation of various physiological characters with grain yield under normal non stressed conditions (SD1= 15th November sowing).

Traits	Grain yield	Spad chlorophyll	Proline	Osmotic potential – megapascal	Leaf area
Grain yield	---	0.734**	-.176	0.121	0.321
Spad chlorophyll	0.734**	----	-.0.303	0.176	0.445*
Proline	-.0.176	-.0.303	---	-0.285	0.125
Osmotic potential	0.121	0.176	-.0.285	----	0.176
Leaf area	0.321	0.445*	0.125	0.176	----

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 3. Correlation of various physiological characters with grain yield under heat stressed conditions (SD-2= 25th December sowing).

Traits	Grain yield	Spad chlorophyll	Proline	Osmotic potential- megapascal	Leaf area
Grain yield	----	0.745**	0.484*	0.606**	0.288
Spad chlorophyll	0.745**	----	0.782**	0.668**	0.431*
Proline	0.484*	0.782**	----	0.783**	0.477*
Osmotic potential	0.606**	0.668**	0.783**	---	0.198
Leaf area	0.288	0.431*	0.477*	0.198	---

**Correlation is significant at the 0.01 level (2-tailed)

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