

## PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE OF WHEAT GENOTYPES UNDER TEMPERATURE STRESS

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### Abstract

This paper focuses on the evaluation and performance of newly developed wheat genotypes (DH-7, DH-8, DH-11, DH-12, DH-18, DH-20) along with two local check varieties (Lu-26s and Kiran-95) under three different sowings at various dates viz. November 07 (optimum condition), November 27 (heat stress condition) and December 17 (high heat stress condition) under wire gauze house during 2014-15. All the genotypes showing sensitive, tolerant, medium sensitive, medium tolerant responses to heat stress conditions were selected for various physiological and biochemical plant characters (osmotic pressure, proline, Glycine betain, Sugar, Sodium, Potassium, K/Na ratio, Leaf area, Chlorophyll-a, Chlorophyll-b, total chlorophyll, cell membrane stability and grain yield) with the objective to determine their impact on the grain yield. All the physio-chemical plant characters revealed significant variation among genotypes and temperature conditions in their interactions and means. Heat stress conditions showed significant increase in osmotic pressure, proline, glycine betain, sugar, sodium and cell membrane stability and significant decrease in potassium, K/Na ratio, leaf area, chlorophyll a, chlorophyll b, total chlorophyll and grain yield over optimum condition. Osmotic pressure, proline and glycine betain showed negative and non-significant correlation with the grain yield whereas sodium and cell membrane stability had negative and significant effect on the grain yield. The overall investigations showed that potassium, K/Na ratio, leaf area, chlorophyll-a, chlorophyll-b and total chlorophyll displayed positive and significant correlation with the grain yield. Multiple linear regression analyses of variance revealed that potassium contents in the leaves have highest impact i.e. 25.5 percent in grain yield change followed by proline, sodium and glycine betain contents showing 16.0, 13.7 and 13.2 percent impact on yield fluctuation. It is concluded that four genotypes (DH-7, DH8, DH-11 and DH-18) and one local check Lu-26s were identified to have the potential to perform economically under high heat stress condition.

**Key words:** Physiology, Biochemical, Wheat, Temperature, Chlorophyll.

### Introduction

Wheat is the leading food grain of Pakistan and being the staple diet of the people and occupies a central position in agricultural policies. Wheat contributes 9.1 percent to the value added in agriculture and 1.7 % to GDP of Pakistan. Wheat area cultivated 8734 thousand hectares witnessing a decrease of 2.6 percent compared over last year's area 8972 thousand hectares. Wheat production was estimated at 25.492 million tons during 2017-18, recorded to decline of 4.4 percent over the last year's production of 26.674 million tons (Anon., 2018).

Wheat is grown on larger area than any other crop and its world trade is greater than for all other crops combined. It is easily stored and transported (Slafer and Satorre, 1999). Wheat grain production widely fluctuates as a result of its interaction with weather conditions because grain yield is a complicated parameter quantitatively. Development of productive genotypes is the cause of increase in wheat yield which better adapt various agro-climatic conditions and also resist all types of stresses. Presence of sufficient genetic variability in the breeding materials can facilitate for the selection of grain yield improvement genotypes (Ali *et al.*, 2008).

The effects of risen temperature on development and growth of wheat has been studied by many researchers like Al-Khatib and Paulsen, 1984; Porter and Gawith, 1999; Wheeler *et al.*, 2000 they reported that cause of damage to the membranes responsible for photosynthesis and loss of chlorophyll. Which in turn increase the risk of embryo abortion, reduces the rate of photosynthesis and lower number of grains (Saini *et al.*, 1983)

A high level temperature negatively affects cell metabolism (Levitt, 1980), initiates changes in the design of protein synthesis (Larkindale *et al.*, 2005). The temperatures (supra-optimal) bring under control the cellular proteins synthesis of the normal condition and in the same position induce synthesis and accumulation of several new proteins including heat shock proteins (Feder & Hofmann, 1999; Law & Brandner, 2001).

Leaf chlorophyll maintenance under hot atmosphere condition is repeatedly regarded as an expression of high temperature tolerance and delayed leaf senescence (Khan *et al.*, 2007). The high heat stress at the grains filling point accelerates senescence process method and therefore untimely damage the chlorophyll and down ratio of chlorophyll (a & b) (Al-Khatib & Paulsen, 1984 and Harding *et al.*, 1990).

The objective of the current study was to determine the character of various physio-logical and bio-chemical responses of wheat genotypes grown under high heat stress and normal sowing dates on the grain yield in wire netted-house (cemented tanks in semi-controlled condition).

### Materials and Methods

**Experimental details:** Temperature stress studies in wheat genotypes were conducted under glass house (net house) conditions at Nuclear Institute of Agriculture (NIA), Tandojam, Sindh, Pakistan during

the period of November 2014 to April 2015. The experiment was laid out in RCBD (factorial) with three replications. Eight genotypes of wheat selected from 20 genotypes showing has tolerant (DH-8, DH-11, Lu-26s), medium tolerant (DH-7, DH-18), sensitive (Kiran 95) and medium sensitive (DH-12, DH-20) responses based on various biological plant growth parameters were sown in three dates viz. S1 = Optimum condition (7<sup>th</sup> November 2015), S2 = heat stress (27<sup>th</sup> November 2015; after 20 days gaps from optimum condition) and S3 = high heat stress (17<sup>th</sup> December 2014; after 40 days gaps from optimum condition) The distance was maintained in plant to plant (10 cm) and row to row (20 cm) and the row length was maintained as 1.5-m (Fig. 1; Tables 1 and 2).

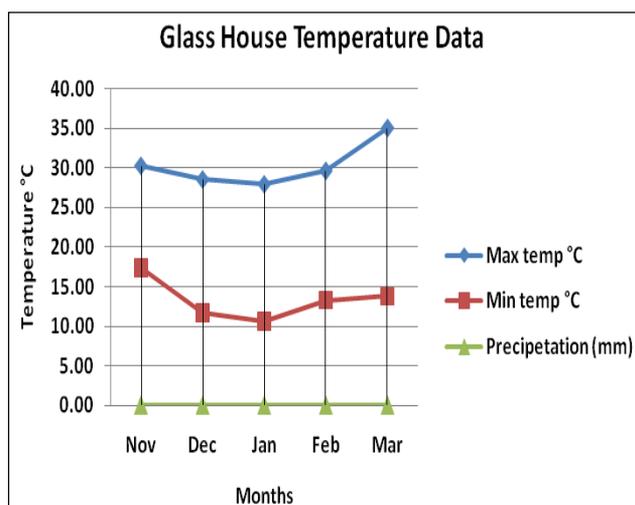


Fig. 1. Temperature data recorded of glass house experiment at NIA, Tandojam wheat season 2015-16.

### Studies on physio-biochemical responses of wheat genotypes under various temperature conditions

**Osmotic Potential (-MPa):** 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 left in freezer for over-night 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) (Khan *et al.*, 1992).

**Proline contents ( $\mu\text{mol g}^{-1}$  fresh wt.):** It was estimated in accordance with Bates *et al.*, (1973). Fresh leaf tissue sample (0.5 g) was homogenized in 10 ml of 3 % sulfo-salicylic acid. The homogenate was filtered through Whatman number 2 filter paper. Two ml of filtrate was treated with 2ml acid ninhydrin (1.25 g ninhydrine in 30 ml glacial acetic acid and 20 ml 6Mortho-phosphoric acid, with agitation until dissolved). Kept the solution cool and stored at 4°C. The reagent remains stable for 24 hour) and 2ml of glacial acetic acid in test tubes. The reaction mixture was allowed to react in water bath 1 hour at 100°C. The reaction was terminated in an ice bath. The proline in reaction mixture was extracted in 4 ml toluene through mixing vigorously by passing a continuous stream of air for 1-2 min. The chromophore containing toluene was aspirated 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 determined from a standard curve and calculated on fresh weight basis as follows:

$$\text{mmole proline g}^{-1} \text{ fresh weight} = (\mu\text{gproline ml}^{-1} \times \text{ml of toluene} / 115.5) / (\text{g sample}/5)$$

where 115.5 is the molecular weight ( $\text{g mole}^{-1}$ ) of proline.

**Table 1. Wheat genotypes categorized on the bases of < 50% reduction at high heat stress (wire gauze house studies during 2014-2015).**

Response	Genotypes	PI	PTP	SL	NSS	NGS	GWP	1000-GW	NV
MT	DH-1	+	+	+	-	+	+	+	6
	DH-3	+	+	+	+	+	-	-	5
	DH-4	+	+	+	+	+	+	-	6
	DH-5	+	+	+	+	+	-	+	6
	DH-6	+	+	+	-	+	+	+	6
	DH-7	+	+	+	+	-	+	+	6
T	DH-8	+	+	+	+	+	+	+	7
T	DH-10	+	+	+	+	+	-	-	5
T	DH-11	+	+	+	+	+	+	+	7
MS	DH-12	+	+	+	-	-	+	+	5
	DH-13	+	+	+	+	+	+	-	6
	DH-14	+	+	-	-	+	+	+	5
	DH-15	+	+	+	+	+	+	+	7
	DH-16	+	+	+	+	-	+	+	6
MT	DH-18	+	+	+	+	+	+	-	6
MS	DH-19	+	+	+	+	+	+	+	7
MS	DH-20	+	+	+	+	+	-	-	5
	DH-21	+	+	-	+	-	-	+	4
T	Lu-26s	+	+	+	+	+	+	+	7
S	Kiran-95	+	+	+	+	-	-	-	4

\*PI = Plant height (g), PTP = Productive tillers plant<sup>-1</sup>, SL = Spike length (cm), NSS = No. of spikelets spike<sup>-1</sup>, NGS = No. of grains spike<sup>-1</sup> (g), GWP = Grain weight plant<sup>-1</sup> (g), 1000-GW= 1000 grain w+eight (g), NV = No. of variables  
T = Tolerant, S = Sensitive, MT = Medium tolerant, MS = Medium sensitive (Khan, 2008)

**Table 2. Physico-chemical properties of the soil used for green house experiment.**

S. No.	Characteristics	Determination
<b>Mechanical analysis</b>		
01	Sand %	56.50
	Silt %	28.50
	Clay %	15.00
	Texture	Sandy
02	EC (dS m <sup>-1</sup> )	0.48
03	pH (1:2 soil water extract)	7.40
04	Organic matter (%)	0.80
<b>Soluble cation (meq L<sup>-1</sup>)</b>		
05	Na (meq L <sup>-1</sup> )	2.22
	K (meq L <sup>-1</sup> )	1.08
	Ca (meq L <sup>-1</sup> )	5.80

Temperature data (daily minimum and maximum temperature) were recorded from each treatment (Optimum, heat stress and high heat stress) at the experimental site as under

**Glycine betaine ( $\mu\text{mol g}^{-1}$  dry wt.):** Glycine betaine was estimated by Grieve and Gratan, 1983. Dried ground leaves tissue (0.5g) were extracted by shaking mechanically with the know amount of toluene water for 24 hours at 25°C and filtered by Whatman filter paper number 1. One ml of the extract was mixed with 1 ml of 2.0 N HCl and was mixed thoroughly. After mixing 0.5 ml of the reaction mixture was pipetted in a glass tube and 0.2 ml of potassium tri-iodide solution was added (7.5 g iodine and 10 g potassium iodide dissolved in 100 ml of 1.0 N HCl by continuous shaking for 30 min then filtered and stored at 25°C).

The contents were shaken and cooled in ice bath for 90 min with occasional shaking. Two ml of ice cooled distilled water was mixed and then 20 ml of 1-2 dichloro ethane (cooled at 10°C) was added. Two layers were formed which were mixed by passing a continuous stream of air for 1-2 mint while tubes were still in ice bath (4°C). The upper aqueous layer is discarded and optical density of organic layer was measured at 365 nm. The concentrations of the betaine were calculated against the standard curve. The blank was developed as above except no betaine standard or extract.

**Total soluble-sugars ( $\mu\text{mole g}^{-1}$  fresh wt.):** The fresh leaves total soluble sugars in according to Riaziet *al.*, 1985. One gram chopped leaf samples were shake with 10 ml of 80 % ethanol (v/v) overnight. In 0.1 ml ethanol extract 3 ml freshly prepared anthrone was added, heated at 97°C for 10 minutes, cooled in ice bath and read in spectrophotometer at 625 nm.

**Sodium (Na<sup>+</sup>) and Potassium (K<sup>+</sup>):** It was calculated according to Ansari and Flowers 1986. In route of inorganic salts (Na<sup>+</sup> and K<sup>+</sup>) analyses, under stressed and non-stressed conditions the flag leaf has selected. In hot-air oven (72°C) the leaf samples were dried and the material of plant was reacted with acetic acid (0.2 mM) in water bath for 01 hour pre-heated (95°C), filtered solution and suitable dilution were made. Na<sup>+</sup> and K<sup>+</sup> conc: were determined by flame photometer (Jenway, Model PFP7).

**Leaf area (cm<sup>2</sup>):** The leaf area is one imperative component in many agro-physiological research studies that included growth of plant, interception of light, efficiency of photosynthesis, evapo-transpiration and, fertilizers and irrigation responses. Fresh leaf samples had been taken from fields and then placed in Leaf Area Meter LICOR-3000 for leaf area measurement.

**Chlorophyll contents (Chl-a, Chl-b and Total Chl) (mg g<sup>-1</sup> fresh wt.):** Chlorophyll had been calculated in accordance method of Arnon (1949). Tender leaves had been cut short into 0.5 cm pieces and then, extracted in 80% acetone at -10°C for one night. The extract had been centrifuged at 14000 x g for 5 minutes. The obtained material absorbance had been calculated at 645, 663 nm. Chlorophyll firmness had been determined by the methodology proposed by Sarkar (1993). The contents were calculated as below;

Formulae:

$$\text{Chl - a} = (12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)) \times V / 1000\text{XW}$$

$$\text{Chl - b} = (22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)) \times V / 1000\text{XW}$$

$$\text{Total Chl.} = (20.2 (\text{OD } 645) + 8.02 (\text{OD } 663)) \times V / 1000\text{XW}$$

**Cellmembrane stability:** Cell membrane stability was determined based on electrolyte leakage following the method by Blum & Ebercon (1981). Six leaves of about equal size were immersed in distilled water for 12-h followed by the measurement of electrical conductivity (EC1) of the solution with EC meter. Samples with water were shifted to autoclave for 60 minutes at 50°C and then cooled at room temperature. The conductivity of killed tissues (EC2) was again measured. Cell membrane stability was calculated as the ratio between EC1 and EC2.

### Data analysis

The data were analyzed statistically using Mstat software for analysis of variance. The means were separated by DMR test at  $p= 0.05$ . The physio-biochemical plant characters were correlated with the grain yield. The data were also procedure for multiple linear regression analysis of variance through steps with the objective to find the role of each factor singly and cumulatively in yield fluctuation.

### Result and Discussion

The results (Fig. 2) revealed that various physio-biochemical plant characters i.e. osmotic pressure, proline, glycine betain, sugar, sodium and cell membrane stability were significantly increased under heat stress conditions over optimum condition whereas potassium, K/Na ratio, leaf area, chlorophyll a, chlorophyll b, total chlorophyll and grain yield were decreased under heat stress conditions. The present findings are in conformity with those of Mirza *et al.*, (2012) who also reported that heat stress condition significantly decreased the chlorophyll contents in wheat. The present findings are partially coinciding with those of Almeselmani *et al.*, (2012) who reported that leaf chlorophyll and osmotic pressure were reduced at high temperature whereas in the present findings, osmotic potential was increased on heat stress conditions. The present findings are also in accordance

with those of Evan (2013) who reported that heat stress conditions increased proline contents in wheat crop but are contradicted with those of Ashgan *et al.*, (2014) who found higher amount of proline in heat sensitive genotypes but in the present study heat tolerant genotypes showed higher proline as compared to heat sensitive genotypes. The present findings are however harmonized with those of Ramani *et al.*, (2017) who also found significantly the highest proline and glycine betain contents in heat tolerant genotypes. In the present dissertation, all the biochemical plant characters showed significant difference among genotypes and various temperature conditions based on their interactions. Furthermore, sodium contents ( $r = -0.554^{**}$ ) and cell permeability ( $r = -0.505^{**}$ ) displayed significant and negative correlation with the grains yield. Potassium, K/Na ratio, leaf area, chlorophyll a, chlorophyll b and total chlorophyll had positive and significant correlation with the grain yield showing r-values of 0.743\*\*, 0.625\*\*, 0.757\*\*, 0.632\*\*, 0.485\*\* and 0.518\*\*, respectively. The present findings are in conformity with those of Rahman *et al.*, (1997), Reynolds *et al.*, (1998), Hede *et al.*, (1999) who reported significant and positive correlation between chlorophyll contents and grain yield under heat stress conditions.

In the present study, the results pertaining to grain yield in different genotypes of wheat affected by various temperature stress condition under glass house condition revealed significant variation on the basis of their interactions as well as based on cumulative average in genotypes and temperature conditions (Fig. 3). It is evident from the results that the grain yield was adversely affected in heat stress condition over optimum condition in all the genotypes. On an average effect of temperature, optimum condition, showed maximum grain yield i.e. 30.75 g/15 plant and significantly reduced to 23.58 and 16.63 g/15 plants under heat stress and high heat stress condition, respectively. Furthermore, based on average of genotypes, DH-11 showed maximum grain yield i.e. 33.22 g/15 plants and differed significantly from those of observed in all other genotypes. The minimum grain yield i.e. 15.22 g/15

plants was recorded in DH-20 and also differed significantly from those of observed in all other genotypes. The other genotypes are categorized as intermediate.

The results (Table 3) revealed that potassium contents in leaves showed maximum impact i.e. 25.5 percent in the fluctuation of grain yield. The 100-R<sup>2</sup> value was recorded to be 75.1 and this model (M-6) was found to be fitted the best where proline and osmotic pressure exerted positive and significant effect combined with proline, glycine betain, sugar and sodium contents where the later mentioned biochemical factors showed non-significant effect.

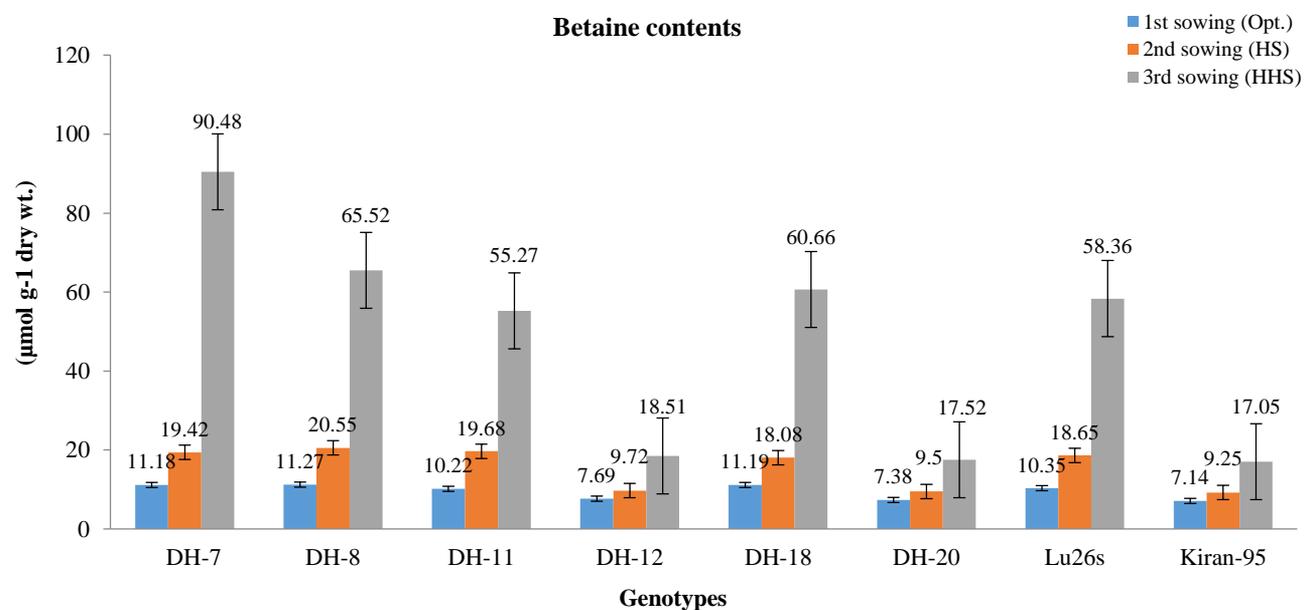
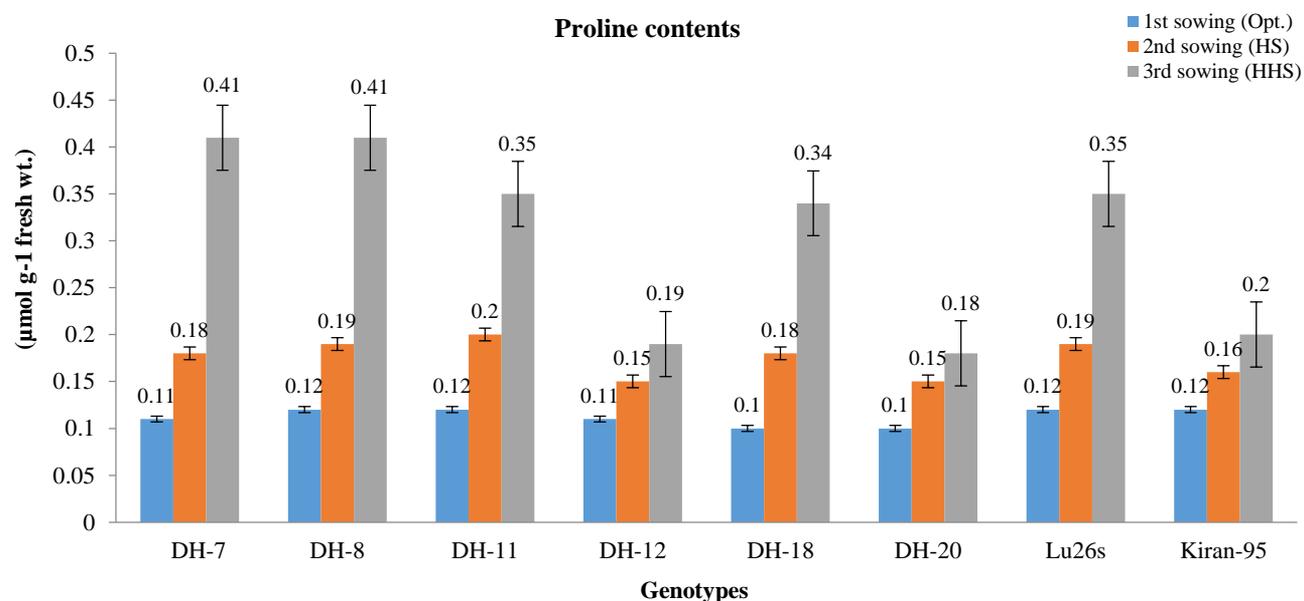
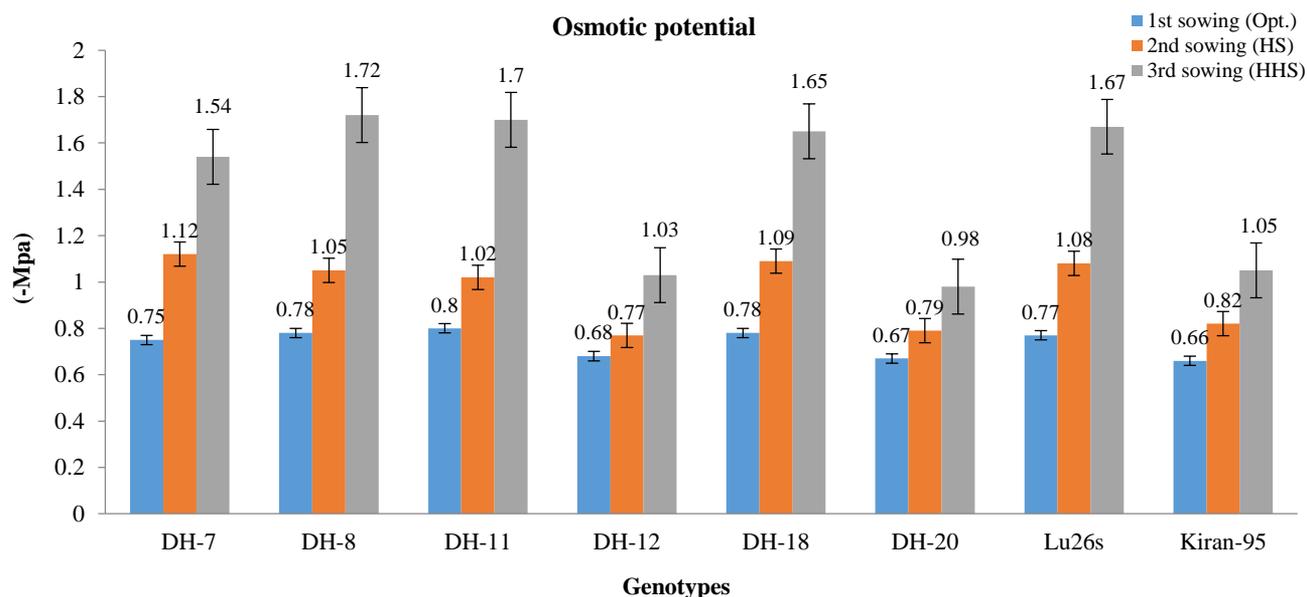
The proline content showed next important factor having 16.00 percent impact in grain yield fluctuation followed by sodium contents exhibiting 13.7 percent impact in grain yield fluctuation in the leaves of various selected genotypes of wheat. Cell membrane stability, leaf area and K/Na ratio were not important factors having negligible impact i.e. 0.00 to 0.3 percent in fluctuation of grain yield. Furthermore all the factors when computed together showed 91.3 percent impact in grain yield fluctuation. The model (M-12) was also found to be fitted the best. In this model total chlorophyll, chlorophyll-b, chlorophyll-a, potassium and proline contents were important.

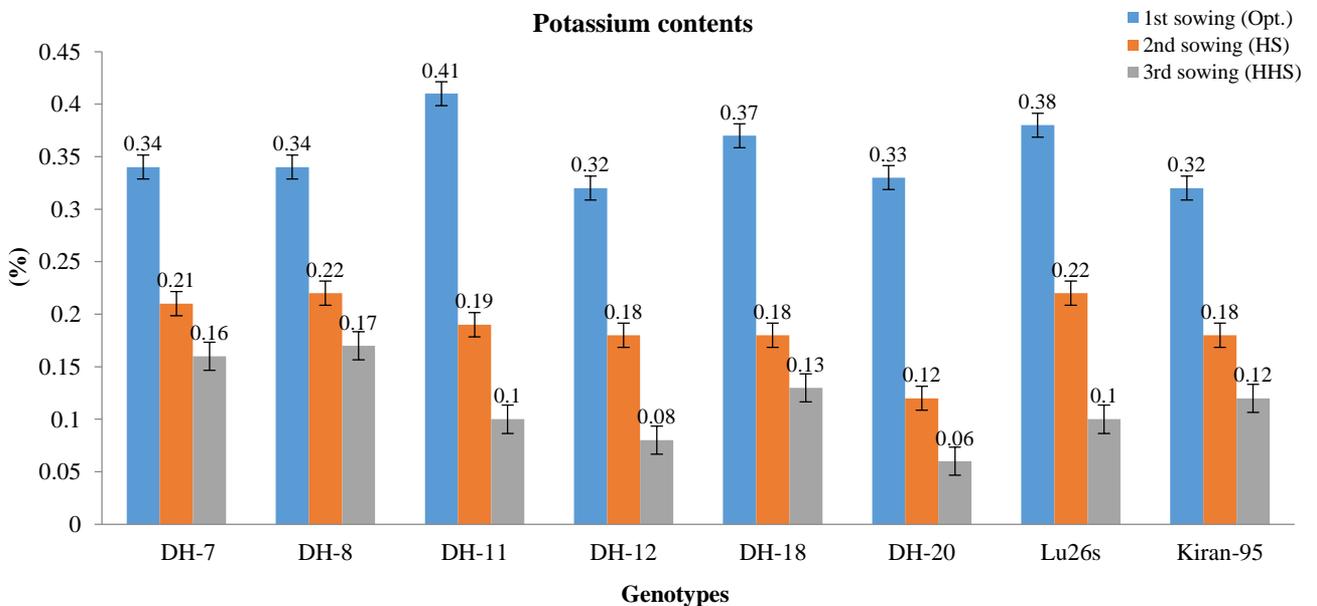
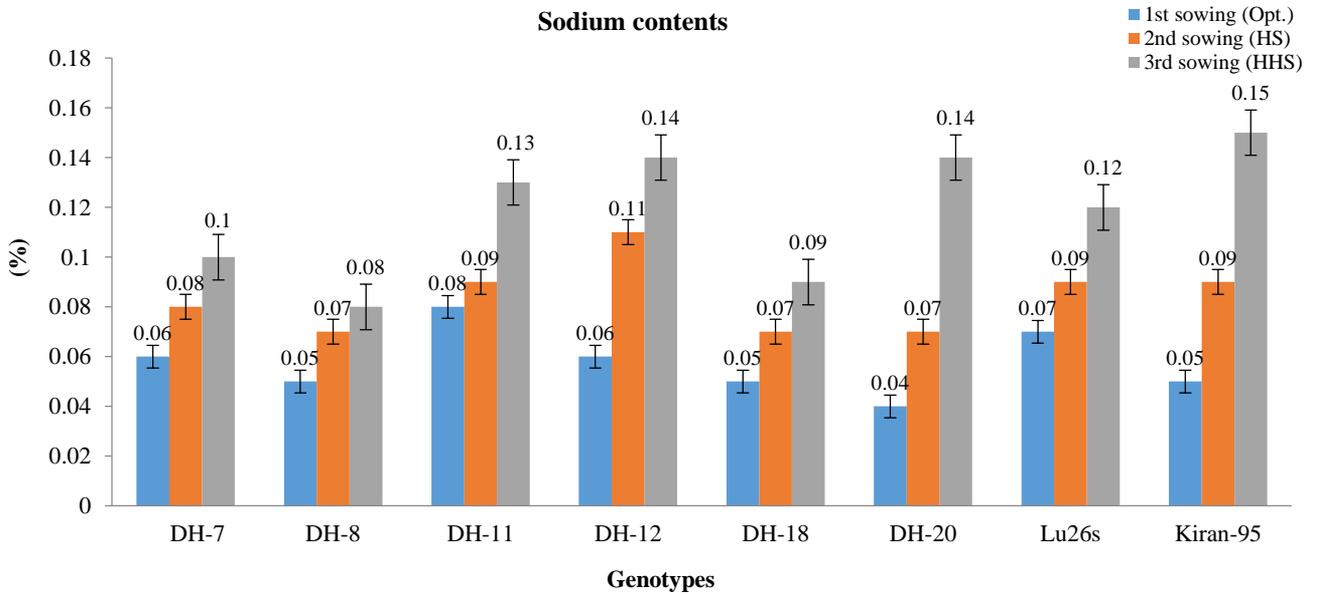
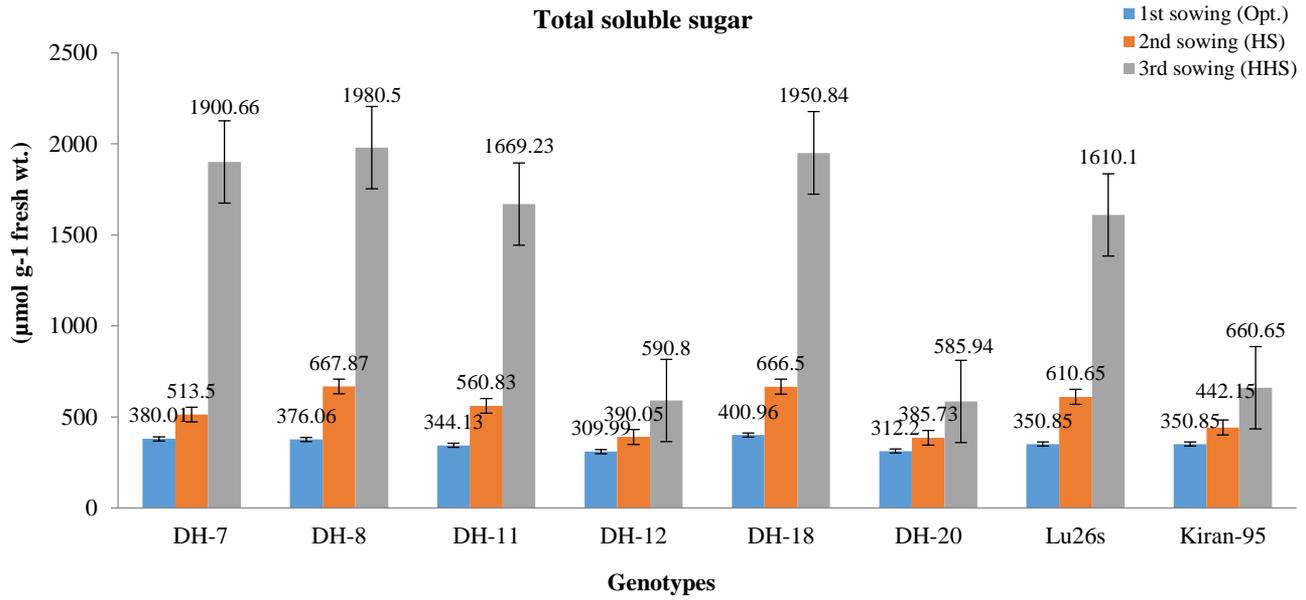
From these results it was evident that potassium contents showed maximum impact i.e., 25.5 percent in the fluctuation of grain yield followed by proline (16.0%), sodium (13.7%), glycine betain (13.2%), and total chlorophyll (9.2%). The others biochemical factors exerted negligible impact (0.00-5.8%) on the grain yield. The present findings are partially in line but cannot be compared with those of Blum (1988), Al-Khatib & Paulsen (1984 & 1999), Harding *et al.*, (1990), Wardlaw *et al.*, (1980), Blum (1986), Roy *et al.*, (2013), Khan *et al.*, (2015), Ibrahim *et al.*, (2017), Ramani *et al.*, (2017), Saleem *et al.*, (2017) and Amarshettiwar & Berad (2018) because they conducted experiments under different materials and methods as well as different ecological conditions.

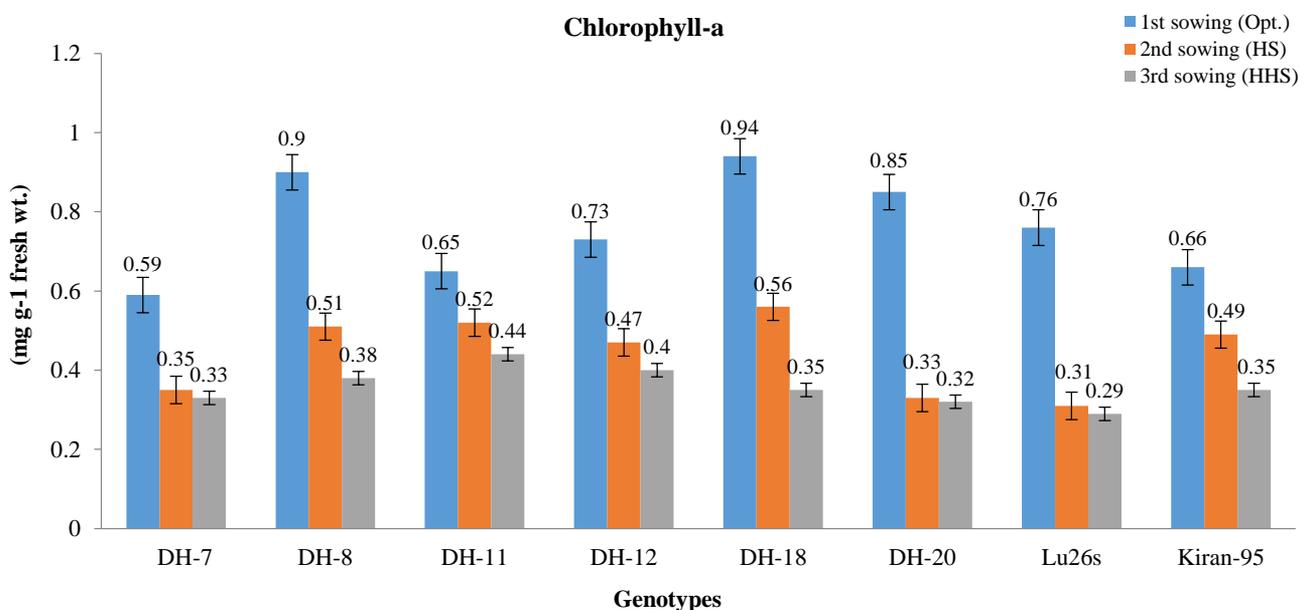
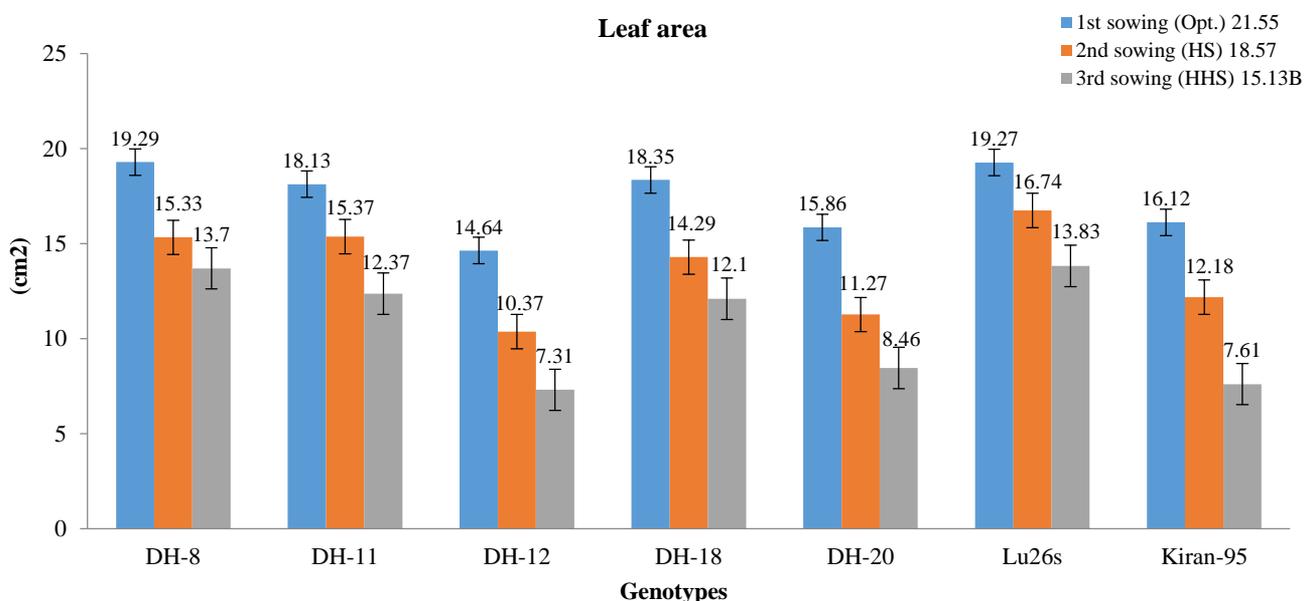
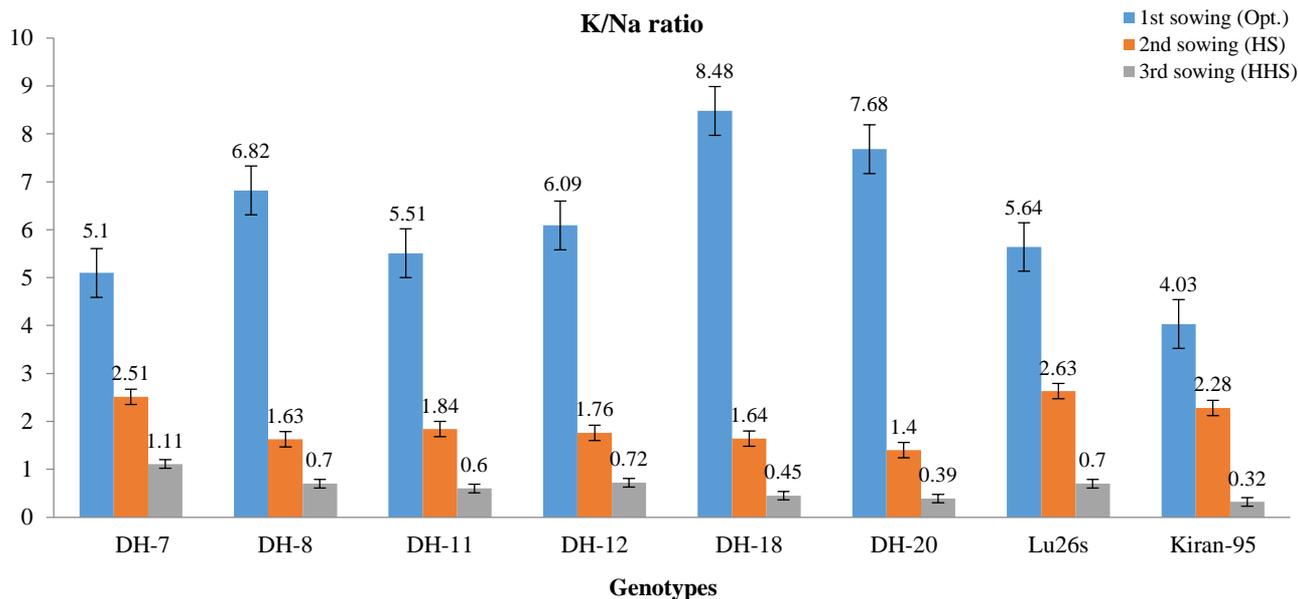
**Table 3. Multiple linear regression models along with coefficient of determination showing the impact of various biochemical plant characters (independent factors) on yield (dependent factor) in wheat genotypes under glass house experiment.**

Mod #	Regression equations	100R <sup>2</sup>	Impact	F-value
M1	$Y = 30.013 - 6.114x_1$	5.8	5.8	1.36
M2	$Y = 18.250 + 32.490x_1 - 144.06x_2^*$	21.8	16.00	2.93
M3	$*Y = 39.073 + 12.394x_1 - 244.40x_2^{**} + 0.860x_3^*$	35.0	13.2	3.60
M4	$Y = 41.534 + 8.110x_1 - 226.97x_2^{**} + 1.263x_3 - 0.0142x_4$	35.9	0.9	2.66
M5	$*Y = 33.915 + 27.928x_1 - 164.61x_2 + 0.706x_3 - 0.0143x_4 - 143.02x_5^*$	49.6	13.7	3.54
M6	$**Y = -28.597 + 52.223x_1^{**} - 48.99x_2 - 0.561x_3 + 0.0018x_4 - 8.755x_5 + 91.504x_6^{**}$	75.1	25.5	8.56
M7	$**Y = -24.844 + 50.108x_1^{**} - 63.919x_2 - 0.435x_3 + 0.00057x_4 - 13.536x_5^{**} + 97.797x_6 - 0.5306x_7$	75.4	0.3	6.99
M8	$**Y = -27.888 + 47.566x_1^* - 66.773x_2 - 0.583x_3 + 0.0068x_4 + 5.938x_5 + 85.242x_6^* - 0.414x_7 + 0.389x_8$	75.7	0.3	5.84
M9	$**Y = -36.325 + 41.264x_1^* - 61.298x_2 - 0.4169x_3 - 0.003x_4 + 27.007x_5 + 70.510x_6 - 2.113x_7 + 0.828x_8 + 24.904x_9$	79.5	3.8	6.02
M10	$**Y = -26.394 + 25.231x_1 - 10.136x_2 - 0.112x_3 + 0.0054x_4 + 63.947x_5 + 72.192x_6 - 3.191x_7 + 1.386x_8 + 36.189x_9^* - 12.474x_{10}$	82.1	2.6	5.98
M11	$**Y = -11.448 + 20.623x_1 - 167.30x_2^{**} - 0.0152x_3 + 0.016x_4 + 82.609x_5 + 85.116x_6^{**} - 1.664x_7 + 0.9703x_8 + 57.023x_9^{**} + 29.003x_{10}^* - 42.049x_{11}^{**}$	91.3	9.2	14.40
M12	$**Y = -9.148 + 24.224x_1 - 163.18x_2^* - 0.075x_3 + 0.0166x_4 + 80.336x_5 + 81.677x_6^{**} - 1.811x_7 + 1.027x_8 + 57.418x_9^{**} + 28.488x_{10}^* - 42.241x_{11}^{**} - 0.0706x_{12}$	91.3	0.0	9.66

where:  $x_1$  = Osmotic pressure;  $x_2$  = Proline;  $R^2$  = Coefficient of determination;  $x_3$  = Glycine betain;  $x_4$  = Sugar;  $x_5$  = Sodium ;  $x_6$  = Potassium;  $x_7$  = K/Na ratio;  $x_8$  = Leaf area;  $x_9$  = Chlorophyll a;  $x_{10}$  = Chlorophyll b;  $x_{11}$  = Total Chlorophyll;  $x_{12}$  = Cell stability; \* = Significant at  $p \leq 0.05$ ; \*\* = Significant at  $p \leq 0.01$







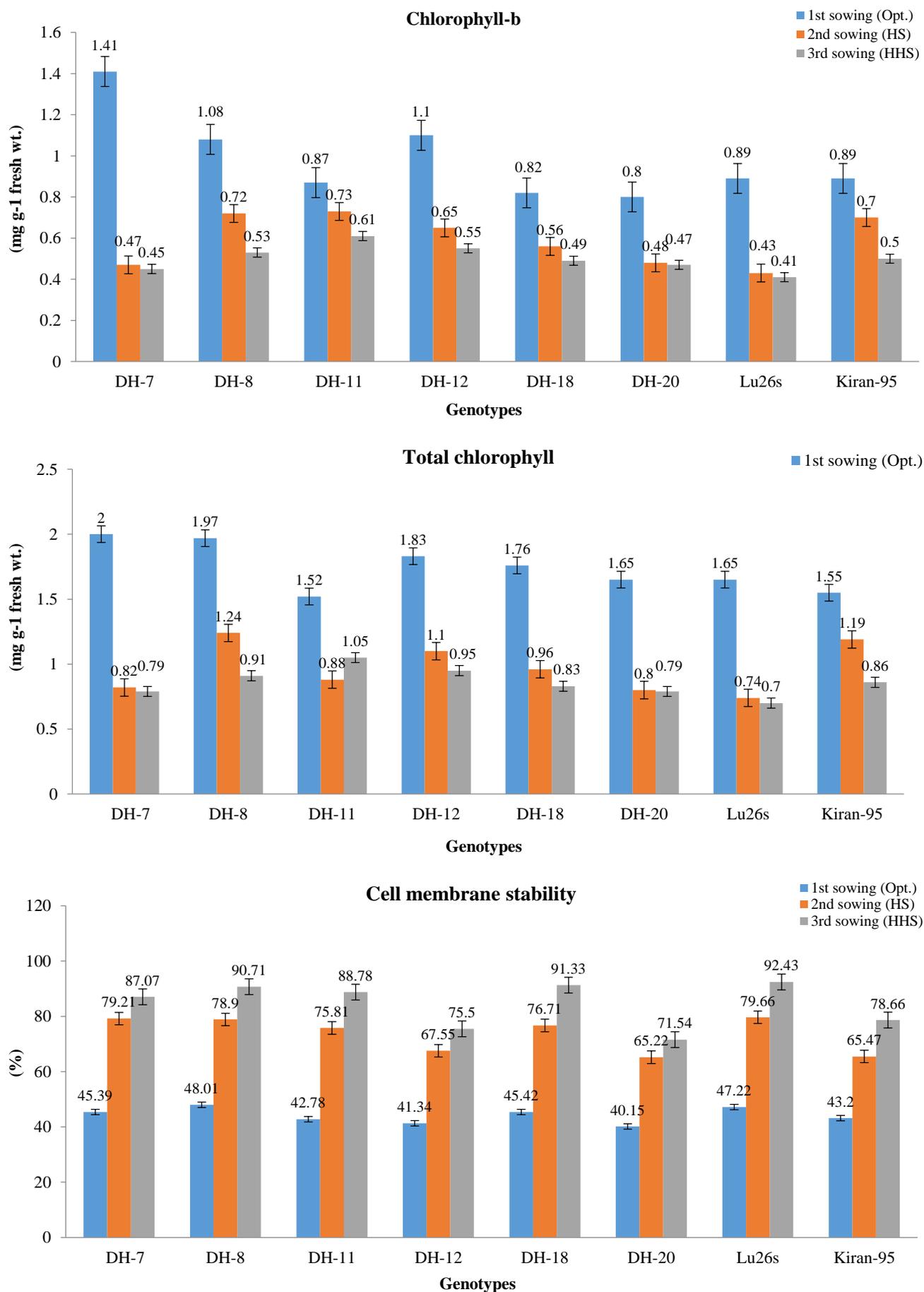


Fig. 2. Physico-chemical plant factors in various selected genotypes of wheat.

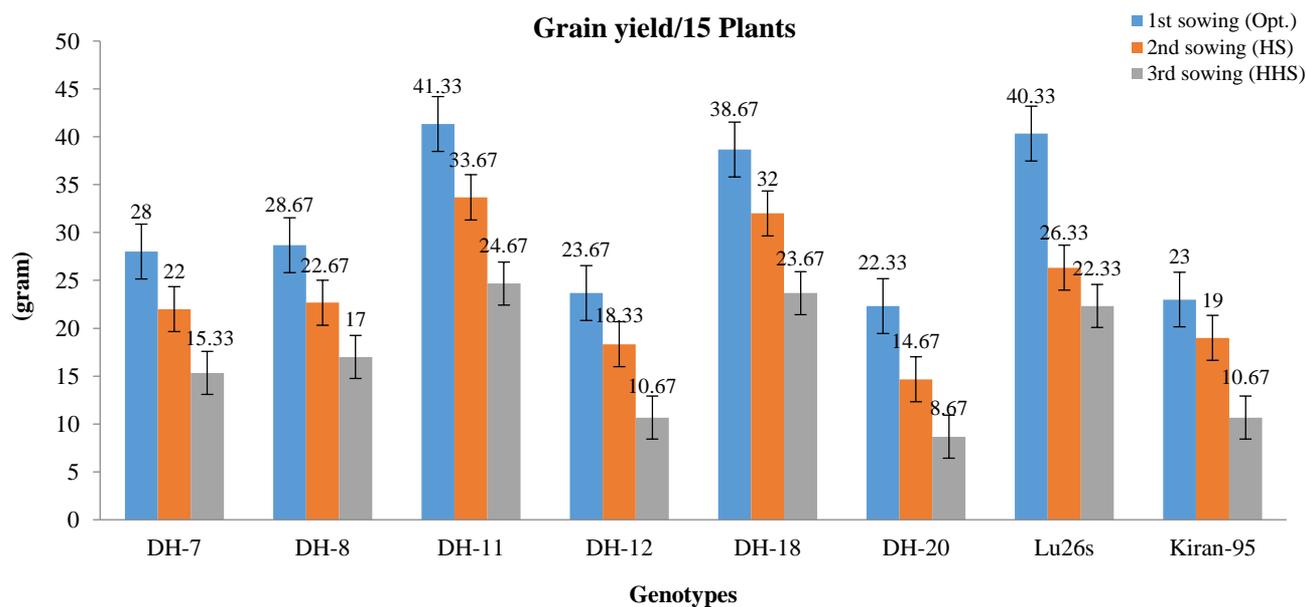


Fig. 3. Effect of different temperatures on grains yield/15 plants (g).

## Conclusions

Osmotic pressure, proline, glycine betain, sugar, sodium and cell membrane stability were significantly increased while potassium, K/Na ratio, leaf area, chlorophyll a, chlorophyll b, total chlorophyll and grain yield were significantly decreased in heat stress conditions.

Sodium and cell membrane stability had negative and significant effect with the grain yield while potassium, K/Na ratio, leaf area, chlorophyll a, chlorophyll b and total chlorophyll displayed positive correlation.

Potassium contents in the leaves had maximum impact i.e. 25.5 percent in grain yield fluctuation followed by proline, sodium and betain showing 16.0, 13.7 and 13.2 percent.

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