

Effect of Heat Stress on Inter-relationship of Physiological and Biochemical Traits with Grain Yield in Wheat (*Triticum aestivum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author AK conducted the experiments and related field and laboratory work. Author RDR performed the planning of the experiment and wrote the manuscript. Author CR did the experimental planning and data analysis. Author AKP guided the laboratory analysis. Author SK checked the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Heat stress, particularly the stress appears at the time of flowering to grain filling stages causing severe yield loss in wheat. Heat tolerance is complex phenomena that include adjustment in morphological, physiological and biochemical traits of the crop. Present investigation was carried out to understand the effect of terminal heat stress on different traits of wheat. The experiment was conducted in three dates of sowing as timely sown, late sown and very late sown to expose the crop to heat stress at later stages of the crop growth. Significant genetic variations for all the traits evaluated under three conditions indicated the presence of variability for the traits. Trait association analysis revealed that flag leaf chlorophyll content and MSI at seedling stage; MDA at reproductive stage had direct relationship with grain yield. While under very late sown condition MDA and RWC

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at seedling stages were found to be highly correlated with grain yield. It indicates that MDA, RWC at seedling stage and days to booting, days to milking plays important role in very late sown condition that can be used as selection criteria in breeding programme.

Keywords: Chlorophyll; heat stress; relative water content and wheat.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most cultivated food crop worldwide due to its wider adaptability to different agro-climatic conditions. Wheat production is vulnerable to climatic changes particularly under temperature and drought stress. High temperature either at early or late stages is a major yield production constrains. Globally, about 7.0 million hectares of wheat is affected by heat stress since early stages of crop growth and 40% of the irrigated wheat growing areas in the faces heat stress at terminal stages of crop growth [1]. In South-Asia, high temperature at reproductive stages of crop growth is causing significant yield loss [2]. High temperature not only hastens the phenological stages of crop development but also reduces grain filling duration [3] which ultimately results in yield reduction. High temperature of 37.6°C and 37.2°C at anthesis and booting stages respectively decreased grain yield by 50% under well watered conditions [4].

Early sowing may be adopted to avoid terminal heat stress [5,6]; however, due to intensive rice-wheat cropping system, wheat is sown delayed in most of the areas. An estimated yield decline @ 1.3% per day occurs when wheat sown delayed beyond optimum time [7,8]. The genotypic response of wheat to planting dates varies for yield and contributing characters. Performance under stress condition depends upon the biochemical and physiological adjustment of the variety.

Malondialdehyde (MDA), a product of fatty acid peroxidation, in plant accumulates due to cellular membrane lipid peroxidation under oxidative stress [9]. The maintenance of stable water content is a prerequisite for the normal growth of plants. Under stress, plants accumulate certain osmoprotective compounds to protect its cell membrane and biochemical compounds. Canopy temperature is an important determinant under high temperature stress. Positive significant correlation of canopy temperature depression (CTD) with grain yield was recorded [10]. Chlorophyll content in the leaves is affected under heat stress. Rapid decline in the

chlorophyll status in leaves under terminal heat stress causes drying of the crops which ultimately reduces the grain yield. Variation among the cultivated wheat varieties for staygreen traits under heat stress was recorded [11]. The study on the changes in chlorophyll and other biochemical traits in seedling and reproductive stages under normal and stress condition is limited. Besides, limited informations are available on how the changes of important physiological and biochemical traits in seedling to reproductive stages under heat stress are related with grain yield? Therefore, the present study has been formulated to understand the effect of heat stress on morpho-physiological and biochemical traits upon exposure to terminal heat stress.

2. MATERIALS AND METHODS

The present investigation was carried out in university research farm of Bihar Agricultural University, Sabour, Bhagalpur, India with eight selected wheat genotypes DBW-14, Sonalika, BRW-3708, C-306, HUW-468 and WR-544. The experiment was laid out in randomised block design with three replications at three date of sowing i.e. 15th November (timely sown), 5th December (late sown), 30th December (very late sown) in 2018-19. The observations were recorded for days to booting, days to milking, days to physiological maturity, flag Leaf area (cm²plant⁻¹), canopy temperature (°C), membrane stability index at seedling stage and reproductive stage (%), chlorophyll a (mg-1 FW) at seedling stage and reproductive stage, chlorophyll b (mg-1 FW) at seedling stage and reproductive stage, relative water content (%) at seedling stage and reproductive stage (%), malondialdehyde (ug-1 g dry weight) at seedling and reproductive stage and grain yield (q/ha). Randomly selected 5 plants of each plot have been recorded at seedlings and reproductive stages of crop growth. The procedure of measurements of important physiological and biochemical trait is given below.

Relative water content (RWC) of leaves were determined by weighing fresh leaves of 0.5 g (w₁=Fresh weight) and soaked in double distilled

water at 25°C for 4 hours. Then the leaves were weighed again (w_2 = Turgid weight) and placed in oven at 65°C for 48 hours. The dried leaves were weighed (w_3 =Dry weight). The RWC were calculated by using following [12].

$$\text{RWC}(\%) = \{(FW-DW)/(TW-DW)\} \times 100$$

Membrane stability index (MSI) of leaf was calculated by using Premachandra et al. [13] approach. Leaf samples were divided into two equal parts of 0.1 g and soaked in 10 ml double distilled water. One part was heated at 40°C for 30 minutes and conductivity (C_1) was determined by conductivity meter. Conductivity (C_2) was determined by heating second part at 100°C for 10 min.

$$\text{Membrane stability index (MSI)} = [1 - C_1/C_2] \times 100$$

Canopy temperature was recorded with the help of infrared thermometer at reproductive stage of crop growth particularly after anthesis. Flag leaf area ($\text{cm}^2 \text{ plant}^{-1}$) has been taken randomly collected 5 leaves in each plot and calculated the leaf area manually on graph paper. Chlorophyll extraction was performed from 0.1 g of matter fresh leaf (median third of younger leaves) finely ground by cold mortar in the presence of a few milligrams of sand, magnesium carbonate and anhydrous sodium sulfate. The obtained homogenate is supplemented with 5 ml of pure acetone and the supernatant is filtered into a volumetric flask of 25 ml. The acetone operation extraction is renewed on the residue remaining in the mortar until the plant material appears devoid of all traces of pigments and the filtrate is collected in the flask of filtration whose volume is adjusted with acetone up to the mark. From the extraction solution, it prepares a sample (8 ml of the acetone extract 2 ml of distilled water). In parallel, we prepared a reference standard to 80% of acetone. The optical density (OD) is then measured at 645 nm and 663 nm by using a spectrophotometer. The contents of chlorophylls were calculated using the equations:

$$\text{Chlorophyll a (mg/l)} = 12.7 \times \text{OD}_{663} - 2.63 \times \text{OD}_{645}$$

$$\text{Chlorophyll b (mg/l)} = 22.9 \times \text{OD}_{645} - 4.68 \times \text{OD}_{663}$$

$$\text{Total chlorophyll (mg/l)} = (20.2 \times \text{OD}_{645}) + (8.02 \times \text{OD}_{663})$$

The level of lipid peroxidation was determined by measuring the amount of MDA produced by the

thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). A fresh leaf sample (0.5 g) was homogenized in 10 mL of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at $15000 \times g$ for 10 min. To 2 mL aliquot of the supernatant, 4 mL of 0.5% thiobarbituric acid in 20% TCA were added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath and centrifuged at $10,000 \times g$ for 10 min, the absorbance of supernatant was recorded at 532 and 600 nm.

3. RESULTS

Most of the characters showed significant genetic variation under timely sown, late sown and very late sown conditions. Temperature and rainfall variation during crop season is depicted in the Fig. 1. It reveals the presence of genetic variation among the genotypes for the traits under consideration. The observations for analysis of variance have been depicted in the Table 1 for timely sown, late sown and very late sown respectively. In timely sown condition the days to 50% booting and canopy temperature was found to be non significant. Besides, the traits like RWC, MDA, MSI, Chlorophyll a and Chlorophyll b were found to be significant at seedling and reproductive stages. It can be revealed that the response of the genotypes under seedling stage and reproductive stage was different. Unlike timely sown condition, days to 50% booting was found to be significant in late sown condition. All the traits except canopy temperature were significant in late sown condition. Similarly, in very late sown condition all the traits but canopy temperature has shown significant variation. Genetic potentiality of for the traits likes chlorophyll a and b; MSI, RWC, and MDA were observed under timely sown, late sown and very late sown conditions.

Yield is a complex trait that determined by several independent associated traits. In the present experiment character interrelationship of component traits with grain yield was varied under normal and heat stress condition. Coefficient of correlation among the traits evaluated under timely sown condition has been given in the Table 2. Days to 50% booting, days to ear emergence, and days to physiological maturity have shown significantly positive correlation with the grain yield when crop sown mid November. Beside, RWC and MDA at seedling stage were found to be positively and significantly correlated with the grain yield. However, RWC at reproductive stage was found

to be significant but negatively correlated with grain yield. While MDA at reproductive stage has shown negative but non-significant correlation with the grain yield. Whereas, chl a at seedling stage and chl b at reproductive stage was found to be negatively and significant correlation with grain yield. MDA showed to be positively correlated with RWC and negatively correlated with canopy temperature. Relative water content at seedling stage was found to be significantly positive correlation with days to 50% booting, days to 50% ear emergence, days to 50% percent milking stage. Whereas RWC at reproductive stage showed negative correlation with days to 50% booting, days to 50% ear emergence, days to 50% percent milking stage, temperature played important role in chlorophyll concentration and its stability in leaves. Chlorophyll a at seedling and reproductive stage showed positive correlation with MDA and MSI at both the stage of evaluation. But they were found to be negatively associated with canopy temperature; canopy temperature also showed negative effect on MDA concentration and MSI at seedling and reproductive stage.

Correlation coefficient analysis at late sown condition found that the physiological traits directly associated with grain yield (Table 3). Only two physiological traits MDA and Chl a at seedling stage were positively associated with grain yield. Besides, the maturity duration of the crops under late sown condition were found to be positive but non significant. However, Chl b at

reproductive stage was found to be negatively associated with grain yield. The relationship of chlorophyll content at seedling and reproductive stage with RWC has shown similar results like timely sown condition. Negative correlation of RWC with chlorophyll A and B was recorded in late sown condition. While MSI at seedling stage showed positive correlation with days to 50% booting, days to 50% ear emergence, days to physiological maturity RWC and canopy temperature. While, positive association of MDA has shown with days to milking and RWC at reproductive stage. Canopy temperature (CT) is considered to be an important trait for the selection of genotypes under heat stress. The relationship of canopy temperature with yield and other associated traits is important to use the traits in breeding programme. Under late sown condition CT was found to be negatively associated with RWC at seedling stage and days to 50% milking.

The character association analysis of different traits under very late sown condition showed that grain yield was positively associated with days to 50% booting, days to ear emergence, days to 50% milking (Table 4). MDA and RWC at seedling stage were also found to be associated positively with grain yield per plant. Similar to timely sown condition the chlorophyll b at reproductive stage also negatively associated with grain yield. However, Chlorophyll a content at seedling and reproductive stage both was found to be associated negatively with grain yield.

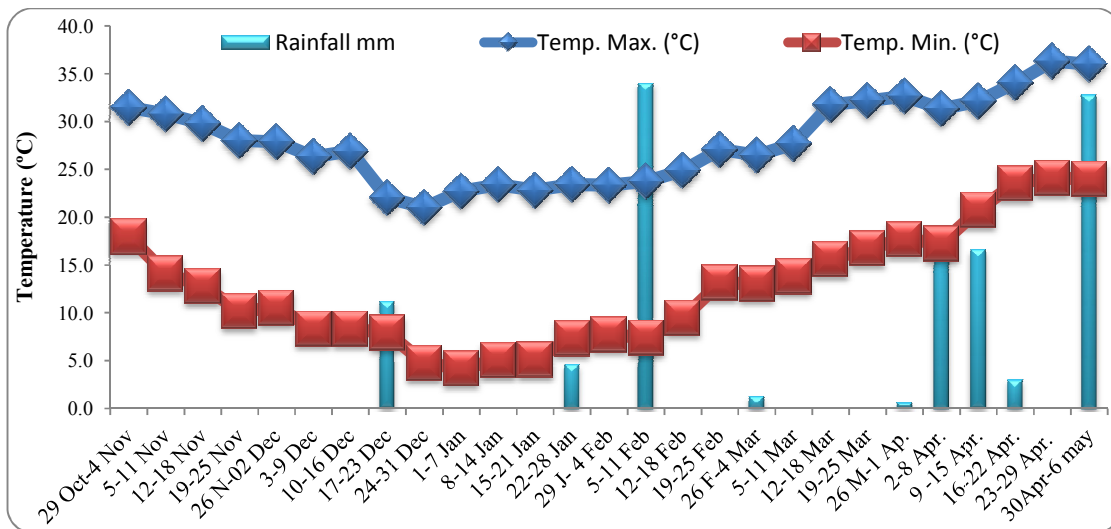


Fig. 1. Weekly rainfall, maximum and minimum temperature data during growing season of the crop

Table 1. Analysis of variance for the traits evaluated under timely (TS), Late (LS) and very late sown (VLS) conditions

Characters	Days to booting			Days to ear emergence			Days to milking			Days to physiological maturity			Flag Leaf area (cm ² plant ⁻¹)		
	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS
Mean	71.25	65.37	59.0	81.87	73.37	66.25	94.5	85.5	72.25	120.12	107.3	91.87	27.24	25.93	23.09
SE m(±)	2.59	1.47	1.58	0.95	1.15	0.77	3.37	3.40	4.78	1.40	1.24	1.55	1.44	1.20	1.66
CD _{0.05}	7.86	4.46	4.79	2.90	3.51	2.34	NS	10.32	14.52	4.25	3.78	4.70	4.37	3.65	5.04
CV(%)	6.11	5.63	5.26	2.08	4.15	2.28	8.12	8.94	10.07	2.25	2.05	2.4	9.16	8.05	12.47

TS, timely sown; LS, Late sown and VLS, very late sown

Characters	Canopy temperature (°C)			Membrane stability index at seedling stage (%)			Membrane stability index at reproductive stage (%)			Chlorophyll a at seedling stage (mg-1 FW)			Chlorophyll a at reproductive stage (mg-1 FW)			Chlorophyll b at seedling stage (mg-1 FW)		
	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS
Mean	24.22	25.96	28.63	52.68	50.56	40.68	75.71	66.05	57.19	19.86	7.01	5.17	7.45	6.66	5.05	18.13	7.4	4.9
SE m(±)	0.68	0.88	0.58	1.79	1.0334	0.86	2.13	0.77	1.05	0.86	0.29	0.30	0.36	0.32	0.3	0.85	0.38	0.26
CD _{0.05}	NS	NS	NS	5.44	3.13	2.61	6.46	2.33	3.20	2.61	0.89	0.91	1.09	0.98	0.97	2.59	1.17	0.79
CV (%)	4.89	6.76	4.44	5.9	2.16	2.52	5.21	2.06	2.6	7.51	7.28	10.06	8.39	8.4	11.06	8.17	9.04	9.22

TS, timely sown; LS, Late sown and VLS, very late sown

Characters	Chlorophyll b at reproductive stage (mg-1 FW)			Relative water content at seedling stage (%)			Relative water content at reproductive stage (%)			Malondialdehyde at seedling stage ug-1 g dry weight)			Malondialdehyde at reproductive stage (ug-1 g dry weight)			Grain yield (q/ha)		
	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS	TS	LS	VLS
Mean	6.33	4.33	3.32	83.27	80.55	78.69	89.47	87.29	81.34	20.76	20.51	19.99	21.12	54.38	71.63	48	44.06	23.79
SE m(±)	0.25	0.3	0.22	0.18	0.19	0.2	0.6	0.4	0.34	0.76	0.81	0.66	2.22	0.98	2.32	1.92	2.19	2.22
CD _{0.05}	0.77	0.91	0.68	0.4	0.6	0.59	0.91	0.24	0.11	1.32	1.46	1.02	2.98	6.73	7.06	1.71	1.03	0.69
CV(%)	6.96	8.85	9.42	9.84	9.84	9.84	9.66	7.6	8.86	6.41	6.87	5.79	7.07	8.06	5.16	8.7	9.56	8.63

TS, timely sown; LS, Late sown and VLS, very late sown

Table 2. Pearson’s correlation coefficient among the traits evaluated under timely sown condition

Parameters	FLA (cm ² plant ⁻¹)	DTB	DEE	DTMK	DPM	RWC at SS (%)	RWC at RS (%)	CT (°C)	MDA at SS (ug ⁻¹ g dry weight)	MDA at RS (ug ⁻¹ g dry weight)	MSI at SS (%)	MSI at RS (%)	CHL a at SS (mg ⁻¹ FW)	CHL b SS (mg ⁻¹ FW)	CHL a at RS (mg ⁻¹ FW)	CHL b at RS (mg ⁻¹ FW)	Grain Yield (q/ha)
FLA (cm ² plant ⁻¹)	1.00	-0.14	-0.26	-0.67**	0.02	-0.37	0.39	-0.20	-0.30	-0.01	0.517**	0.496*	0.20	-0.03	0.57**	0.46*	-0.58**
DTB		1.00	0.92**	-0.02	0.43*	0.41*	-0.53**	0.02	0.29	0.00	-0.18	-0.11	-0.69**	0.47*	-0.41*	-0.41*	0.70**
DEE			1.00	0.11	0.33	0.59**	-0.39	0.25	0.27	-0.26	-0.17	-0.35	-0.85**	0.30	-0.47*	-0.35	0.79**
DTMK				1.00	-0.69**	0.47*	-0.06	-0.10	0.49*	0.06	-0.08	-0.03	-0.03	0.13	-0.66**	-0.59**	0.59**
DPM					1.00	-0.33	-0.45*	0.36	-0.46*	-0.14	-0.50*	-0.22	-0.34	-0.09	0.35	0.25	-0.10
RWC at SS (%)						1.00	0.21	-0.08	0.78**	0.00	0.41*	-0.61**	-0.50*	0.45*	-0.76**	-0.31	0.72**
RWC at RS (%)							1.00	-0.26	0.19	0.09	0.78**	-0.18	0.22	0.09	0.14	0.58**	-0.46*
CT (°c)								1.00	-0.47*	-0.93**	-0.57**	-0.41*	-0.27	-0.82**	0.04	-0.06	0.04
MDA at SS (ug ⁻¹ g dry weight)									1.00	0.43*	0.46*	-0.29	0.02	0.67**	-0.85**	-0.51*	0.50*
MDA at RS (ug ⁻¹ g dry weight)										1.00	0.35	0.28	0.30	0.80**	-0.04	0.05	-0.05
MSI at SS (%)											1.00	-0.01	0.08	0.46*	-0.02	0.34	-0.14
MSI at RS (%)												1.00	0.37	0.05	0.39	-0.07	-0.27
CHL a at SS (mg ⁻¹ FW)													1.00	-0.18	0.11	-0.05	-0.66**
CHL b SS (mg ⁻¹ FW)														1.00	-0.34	-0.09	0.36
CHL a at RS (mg ⁻¹ FW)															1.00	0.79**	-0.71**
CHL b at RS (mg ⁻¹ FW)																1.00	-0.63**

Abbreviations: FLA: Flag leaf area, DTB: Days to booting, DEE: Days to Ear emergence, DTMK: Days to milking, DPM: Days to physiological maturity, RWC : Relative water content, MSI: Membrane stability index, SS: Seedling stage, RS: Reproductive stage, MDA: Malondialdehyde, CHL: Chlorophyll, CT: Canopy Temperature

*&** indicates 5% and 1% level of significance respectively

Table 3. Pearson's correlation coefficient among the traits evaluated under late sown condition

Parameters	FLA (cm ² plant ⁻¹)	DTB	DEE	DTMK	DPM	RWC at SS (%)	RWC at RS (%)	CT (°C)	MDA at SS (ug ⁻¹ g dry weight)	MDA at RS (ug ⁻¹ g dry weight)	MSI at SS (%)	MSI at RS (%)	CHL a at SS (mg ⁻¹ FW)	CHL b SS (mg ⁻¹ FW)	CHL a at RS (mg ⁻¹ FW)	CHL b at RS (mg ⁻¹ FW)	Grain Yield (q/ha)
FLA (cm ² plant ⁻¹)	1.00	-0.01	-0.06	-0.16	-0.03	0.33	0.24	0.03	-0.04	-0.23	-0.11	-0.09	0.133	-0.07	0.39	-0.33	0.00
DTB		1.00	0.93**	-0.05	0.27	0.32	-0.58**	-0.02	-0.25	0.14	0.42*	0.19	0.10	0.40*	-0.11	-0.29	0.33
DEE			1.00	-0.26	0.46*	0.07	-0.56**	0.32	-0.15	0.21	0.51**	0.23	0.29	0.51**	-0.13	-0.34	0.34
DTMK				1.00	-0.53**	0.13	0.29	-0.50*	-0.25	0.45*	-0.46*	-0.24	-0.75**	-0.46*	0.40*	0.04	0.39
DPM					1.00	0.17	-0.38	0.65**	0.29	-0.1	0.89**	0.06	0.26	0.56**	-0.39	-0.51	0.38
RWC at SS (%)						1.00	0.008	-0.45*	-0.3	-0.13	0.43*	0.31	-0.28	0.02	0.14	-0.48*	0.37
RWC at RS (%)							1.00	0.07	-0.04	0.52**	-0.28	0.26	-0.40*	-0.71**	0.78**	0.00	0.26
CT (°c)								1.00	0.37	0.29	0.44*	0.04	0.32	0.22	0.02	-0.26	0.29
MDA at SS (ug ⁻¹ g dry weight)									1.00	0	0.19	-0.63**	-0.28	-0.37	-0.50*	0.55**	0.40*
MDA at RS (ug ⁻¹ g dry weight)										1.00	0.04	0.22	-0.53**	-0.502*	0.58**	-0.02	0.72**
MSI at SS (%)											1.00	0.32	0.085	0.37	-0.29	-0.45*	0.49*
MSI at RS (%)												1.00	0.3	0.19	0.45*	-0.45*	0.11
CHL a at SS (mg ⁻¹ FW)													1.00	0.764**	-0.21	-0.25	0.58**
CHL b SS (mg ⁻¹ FW)														1.00	-0.38	-0.55**	0.20
CHL a at RS (mg ⁻¹ FW)															1.00	-0.37	0.38
CHL b at RS (mg ⁻¹ FW)																1.00	-0.46*

Abbreviations: FLA: Flag leaf area, DTB: Days to booting, DEE: Days to Ear emergence, DTMK: Days to milking, DPM: Days to physiological maturity, RWC : Relative water content, MSI: Membrane stability index, SS: Seedling stage, RS: Reproductive stage, MDA: Malondialdehyde, CHL: Chlorophyll, CT: Canopy Temperature
* & ** indicates 5% and 1% level of significance respectively

Table 4. Pearson’s correlation coefficient among the traits evaluated under very late sown condition

Parameters	FLA (cm ² plant ⁻¹)	DTB	DEE	DTMK	DPM	RWC at SS (%)	RWC at RS (%)	CT (°C)	MDA at SS (ug ⁻¹ g dry weight)	MDA at RS (ug ⁻¹ g dry weight)	MSI at SS (%)	MSI at RS (%)	CHL a at SS (mg ⁻¹ FW)	CHL b SS (mg ⁻¹ FW)	CHL a at RS (mg ⁻¹ FW)	CHL b at RS (mg ⁻¹ FW)	Grain Yield (q/ha)
FLA (cm ² plant ⁻¹)	1.00	-0.14	-0.26	-0.66**	0.02	-0.37	0.39	-0.20	-0.30	-0.01	0.51**	0.49*	0.20	-0.03	0.57**	0.46*	-0.58**
DTB		1.00	0.92**	-0.03	0.43*	0.42*	-0.53**	0.02	0.29	0.00	-0.18	-0.12	-0.69**	0.47*	-0.41*	-0.41*	0.70**
DEE			1.00	0.12	0.34	0.59**	-0.39	0.26	0.27	-0.26	-0.17	-0.35	-0.85**	0.30	-0.47*	-0.36	0.79**
DTMK				1.00	-0.69**	0.48*	-0.07	-0.11	0.49*	0.06	-0.09	-0.04	-0.04	0.13	-0.66**	-0.59**	0.59**
DPM					1.00	-0.33	-0.45*	0.36	-0.46*	-0.14	-0.50*	-0.22	-0.35	-0.10	0.36	0.26	-0.10
RWC at SS (%)						1.00	0.21	-0.08	0.78**	0.00	0.41*	-0.61**	-0.50*	0.45*	-0.76**	-0.31	0.73**
RWC at RS (%)							1.00	-0.27	0.19	0.10	0.78**	-0.19	0.23	0.09	0.14	0.58**	-0.46*
CT (°c)								1.00	-0.47*	-0.93**	-0.57**	-0.41*	-0.28	-0.82**	0.04	-0.07	0.05
MDA at SS (ug ⁻¹ g dry weight)									1.00	0.43*	0.46*	-0.30	0.02	0.67**	-0.85**	-0.51*	0.51*
MDA at RS (ug ⁻¹ g dry weight)										1.00	0.35	0.29	0.30	0.80**	-0.04	0.05	-0.05
MSI at SS (%)											1.00	-0.02	0.09	0.46*	-0.03	0.34	-0.14
MSI at RS (%)												1.00	0.37	0.057	0.39	-0.07	-0.28
CHL a at SS (mg ⁻¹ FW)													1.00	-0.18	0.11	-0.05	-0.66**
CHL b SS (mg ⁻¹ FW)														1.00	-0.35	-0.09	0.36
CHL a at RS(mg ⁻¹ FW)															1.00	0.80**	-0.71
CHL b at RS(mg ⁻¹ FW)																1.00	-0.63**

Abbreviations: FLA: Flag leaf area, DTB: Days to booting, DEE: Days to Ear emergence, DTMK: Days to milking, DPM: Days to physiological maturity, RWC : Relative water content, MSI: Membrane stability index, SS: Seedling stage, RS: Reproductive stage, MDA: Malondialdehyde, CHL: Cholorophyll, CT: Canopy Temperature

* & ** indicates 5% and 1% level of significance respectively

It may be due to higher rate of chlorophyll loss under heat stress. Chlorophyll a at seedling and reproductive stage was also found to be negatively associated with days to 50% booting, days to 50% ear emergence and RWC at seedling stage. Flag leaf area was found to be associated with grain yield per plant. However, chlorophyll b was positively associated with days to 50% booting, RWC at seedling stage, MDA MSI both at seedling and reproductive stage. Canopy temperature was negatively associated with the chlorophyll a and b content at seedling and reproductive stage. MSI at seedling stage was found to be positive association with flag leaf area, RWC at seedling and reproductive stage and MDA at seedling stage. MSI was negatively associated with canopy temperature and days to physiological maturity.

4. DISCUSSION

Rising of temperature at reproductive stages of crop growth affected the expression of characters among the genotypes. RWC, MSI, MDA was also found to be highly significant under late and very late sown conditions. Earlier studies also reported that the physiological traits like RWC, MSI, MDA etc were influenced by high temperature [14]. Ram et al. [7] reported that high temperature during reproductive and grain filling phases causes significant yield losses.

Character association analysis determines the relationship of yield on other independent trait. Grain yield is a quantitative trait; many independent traits are directly or indirectly affect the yield. Genetic gain on the basis of selection for yield *per se* is slow. Therefore, correlated improvement of other related traits is necessary. In the present experiment, it has been observed that the character inter-relationship has been changed in the experiments conducted in stress and non-stress conditions. In timely sown experiment, days to booting, days to ear emergence, days to maturity has shown strong positive correlation with grain yield. Kumar et al. (2014) also reported positive correlation of seed yield with days to maturity. Among the physiological traits RWC at seedling stage, MDA at seedling stage has shown positive correlation with grain yield. Significant genetic variation among the genotypes for RWC, MDA was reported earlier under stress environment [15]. Canopy temperature has been reported to be one of the important traits under heat stress. We also observed direct relationship of canopy temperature with yield under heat stress.

Significant positive association of CTD with grain yield was observed [10]. However, canopy temperature has found to have negatively correlated with grain yield under irrigated condition. Chlorophyll status in the flag leaf has direct relationship with grain yield. Chlorophyll plays vital role in production of photosynthates. Unlike previous experiments, Chl a at seedling stage and Chl b at reproductive stage was found to be negatively correlated with grain yield. But positive correlation of chlorophyll content with photosynthetic rate and grain yield was reported earlier [16].

Cell membrane stability has a reasonable relationship grain yield under heat stressed environments and therefore, MSI is considered as selection criterion for grain yield under heat stress (Blum et al. 2001). Many researchers have used cell membrane stability to study the genetics of heat tolerance in wheat (Reynolds et al. 1994; Ibrahim and Quick, 2001; Dhanda and Munjal, 2012). Under late sown condition grain yield was found to be associated with only MDA at reproductive stage, MSI at seedling stage. It indicates, MSI and MDA are important traits to be considered while selecting varieties for late sown condition. Farooq et al. [9] reported the increase of MDA level in leaves under stress. Previously, Rehman et al. [17] also suggested MSI as important trait under heat stress condition. While, negative correlation of days to milking was shown with MSI, canopy temperature, chlorophyll content. Heat stress at milking drastically reduces the grain size. Similarly, days to maturity has shown positive association with MSI and canopy temperature.

Under very late sown experiment, days to heading, days to ear emergence and days to milking were affected drastically than the timely and late sown condition. These traits were found to have strong correlation with grain yield. Traits related to seedling stage also determine grain yield under heat stress. RWC and MDA at seedling stage have shown strong positive correlation with grain yield. However, chlorophyll a at seedling stage and Chlorophyll b at reproductive stage has shown negative correlation with grain yield. Interestingly, it can be found that RWC at seedling stage has shown positive correlation with grain yield while at reproductive stage no significant correlation with grain yield was observed. MSI and canopy temperature are important parameters under heat stress condition. High temperature stress causes disruption of cell membrane, as a result

the cell solutes started leaking out. MSI measures the stability of the cell membrane to a particular stress.

5. CONCLUSION

Heat stress is causing severe yield loss in wheat growing in India. We studied the effect of terminal heat stress on changes of key biochemical and physiological traits in the plants and their relationship with grain yield under stress. Significant genetic variation was recorded among the genotypes under normal, late and very late sown condition. The character interrelationship with grain yield has changed under three growing condition. We recommend days to heading, days to milking, RWC and chlorophyll content at seedling stage, canopy temperature at reproductive stages of crop growth for use in the selection programme.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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