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AGRONOMICAL AND PHYSIOLOGICAL PERSPECTIVES FOR IDENTIFICATION OF WHEAT GENOTYPES FOR HIGH TEMPERATURE TOLERANCE

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Abstract

High temperature is one of the major limiting factors in wheat (*Triticum aestivum* L.) production in arid and semiarid regions of world. The crop experiences moderate to severe high temperature at most of its growth and physiological stages of growth. A glass house experiment was conducted to investigate the impact of high temperature on growth and productivity of wheat crop under different sowing regimes. Seventeen wheat genotypes were sown under two sowing dates with interval of thirty days in cemented bed filled with river sand. Results showed that high temperature stress due to late sowing has negatively influenced plant growth and yield by modulating physiological and biochemical attributes. Among the genotypes, NIA-AS-14-9, NIA-Sunder, Raskoh, TJ-83, Dani, Saher-2006 and SH-Salt-5B produced high plant biomass, productive tillers, number of grains and grain yield per plant under both sowing conditions. However, maximum relative water contents were observed in LU-26s while NIA-AS-14-10 showed better membrane stability and chlorophyll contents followed by NIA-Sarang, Kiran-95 and Dani under late sown conditions. NIA-Sunder, NIA-AS-14-10 and SRN-09111 had accumulated substantial proline contents but failed to produce significant amount of soluble sugars and glycine betaine (GB). These parameters showed higher accumulation for Dani, Saher-2006, Raskoh, Bathoor, TD-1 and Kiran-95 under high temperature stress. Overall results showed that genotypes SH-Salt-6, NRL-1236, NIA-Sarang, LU-26s and Dani were failed to cope effectively under high temperature stress conditions and illustrated poor performance.

Key words: Wheat; Proline; Cell membrane thermostability; Chlorophyll; High temperature stress, Late sowing

Introduction

Wheat is the world's most important food crop and covers more cultivated land at the global level than any other crop (Muhammady, 2007). In Pakistan it ranks first in terms of production and acreage by covering an area of about 9 million hectares of land and producing 25.5 million tons of grains (Anon., 2017). But the actual yield (about 2 t/ha) is much lower than its yield potential. This is due to many limiting factors, of which high temperature stress is the important physiological factor (Vijayalakshmi & Kolluru, 2007).

Changing climate being an uncontrollable entity is serious threat for the future crop production (IPCC, 2014). The rapid increase in temperature caused by global warming had adverse effects on food production, particularly on bread wheat, an essential food crop that constitutes a significant part of diet to 36% of the world's population (Cossani *et al*., 2012; Prerna *et al*., 2013; Talukder *et al*., 2014).The productivity of wheat has been reduced by up to 5% due to the consequence of increasing temperature (Lobell *et al*., 2011), and for South Asia it has been estimated that by 2050, an increase of 0.3ºC per decade in mean global temperature will have reduced wheat yield by up to 50% (IFPRI, 2017). For every 1°C rise in temperature, the yield of wheat grain is reduced by 3-5% (Gibson, 1999).

High temperature (>30°C) at the time of grain filling is one of the major constraints in increasing productivity of wheat in tropical and sub-tropical countries (Rane & Nagarajan, 2004). Late sown wheat is usually subjected to extreme low temperature at germination stage resulting in poor germination as well as very poor tillering (Farooq *et al*., 2008). Later at the grain filing stage high temperature reduces the kernel weight and grain yield (Mahboob *et al*., 2018). Moreover, ambient temperature surpasses 11.9°C, cause a significant reduction in number of spikelet per spike in wheat crop (Johnson and Kanemasu, 1983). According to Prasad & Djanaguiraman (2014), the rise preanthesis mean daily temperatures >24ºC imposed at start of heading quadratically decreased floret fertility, with the values reaching close to 0% around mean daily temperature of 35ºC; and floret fertility and individual grain weight decreased linearly with increasing duration (in the range from 2 to 30 days). While high temperature at post-anthesis stage significantly affects spike and grain weights which ultimately results in low yield (Mohammadi *et al*., 2006; Din *et al*., 2010; Mahboob *et al*., 2018). Likewise, reduction in grain yield of wheat genotypes was also reported for canopy temperature depression in seed index (i.e. 1000-grain weight) by 8.5%, grain filling period (7.6%) and membrane injury (5.6%) at grain filling stage in response to heat stress (Amandeep *et al*., 2007). Singh *et al*., (2007) also observed a progressive and significant decrease in yield and yield attributing traits in wheat with increasing heat stress intensity.

Heat tolerance is a complex phenomenon which is difficult to assess, however, resistant wheat varieties have evolved special defense mechanisms to withstand high temperature like enhanced accumulation of phenolics, glycine betaine and proline (Mahboob *et al*., 2018), activation of antioxidant systems and the maintenance of stay-green character are among the most prominent mechanisms which help the plants to withstand with drought and heat stresses (Farooq *et al*., 2017). Many other selection criteria based on morpho-physiological traits were also reported to be associated with heat tolerance in wheat like tillering capacity, spike fertility, spike number, grains per spike, biomass, 1000-grain weight and grain yield (Khan *et al*., 2014).

Genetic variation may exist within the wheat genotypes for heat tolerance, thus evaluation of local and exotic germplasm for heat tolerance is important. The study area is characterized by short winter environments resulting high temperature during reproductive stage of wheat which is most vulnerable period in response to heat. Hence, there is an urgent need to evolve heat tolerant wheat genotypes and to assess mechanism of heat tolerance. Therefore, the present study was planned to screen out some newly developed wheat genotypes for heat tolerance on the basis of morpho-physiological and biochemical attributes responsible for their tolerance under high temperature stress induced by late sowings.

Materials and Methods

Planting material and experimental details: Sixteen wheat genotypes collected from diversified climatic zones of Pakistan were tested for high temperature tolerance in glass house. Seeds of eight wheat genotypes (LU- 26s, Dani, NIA-Sarang, NIA-Sunder, Saher-2006, Kiran-95, NIA-AS-14-9 and NIA-AS-14-10) were collected from Plant Breeding and genetics Division, Nuclear Institute of Agriculture (NIA), Tandojam, three genotypes (SRN-09111, NRL-1236 and Bathor-08), from Nuclear Institute of Food and Agriculture (NIFA), Peshawar, KPK, two genotypes (SH-Salt-5B & SH-Salt-6) from Pakistan Agriculture Research Council, Islamabad and two genotypes (TD1 & TJ-83) Agriculture Research Institute (ARI) Tandojam, Sindh and one (Raskoh) from Agriculture Research Institute (ARI), Quetta, Balochistan.

The experiment was conducted in completely randomized design (CRD) with factorial arrangement using three replicates. Healthy and uniform sized seeds of selected genotypes were surface sterilized with 1% (v/v) sodium hypochlorite (H_2O_2) for 3 min, rinsed thoroughly with distilled water and air-dried at room temperature (25°C) for 60 minutes. The sterilized seeds of different wheat genotypes were sown in cemented beds (30×12 ft^2) filled with gravels with thin layer (6 cm) of river sand under two planting dates i.e. 15th Nov and 15th December at 30 days interval. Temperature data throughout whole wheat growing season was recorded regularly (Fig. 1). After completion of seedlings emergence, plants were thinned to fifteen plants per replicate, maintained in a row with recommended plant to plant distance (10 cm).

Measurement of growth attributes and yield related traits: The growth and yield attributes including plant height, productive tillers, biomass and grain yield per plant were recorded at the time of maturity. Five uniform plants from each treatment were tagged and mean to record growth and yield attributes. Plant height was assessed with the help of measuring rod and productive tillers were computed manually, plant biomass of whole plant (above ground) was determined through electronic weighing balance (AND-3000; Japan). All spikes per plants were threshed manually and total numbers of counted and grains weight per plant was recorded.

Physiological and biochemical attributes: Physiological and biochemical parameters were determined at booting stage (Mahboob *et al*., 2017). Ten fully matured fresh leaves were harvested and brought to the laboratory for further processing.

Estimation of chlorophyll: Total Chlorophyll contents (a + b) were determined by following the method described by Arnon (1949). Fresh leaf samples were extracted with 10 ml of 80% acetone and the optical density (absorption) was read at wave length of 663 and 645 nm using spectrophotometer (Hitachi-150-20, Japan). Total chlorophyll was calculated by using the following formulae:

$$\text{Total Chl. } (\mu g.\ g^{-1}\ F.\ Wt.) = ((20.2\ (O\ D\ 645) + 8.02\ (O\ D\ 663) \times Vol.) / (1000 \times \text{sample Wt.})$$

Quantification of compatible solutes: Free proline, glycine betaine, total soluble sugars were measured on fresh weight basis by following the methods proposed by Bates *et al*., (1973), Grieve & Gartan (1983) and Riazi *et al*., (1985), respectively.

Cell Membrane stability and relative water contents: Leaf segments of approximately same size were obtained from stock plants and were evaluated for cell membrane thermostability by following the procedures described by Blum & Ebercon (1981). For relative water contents, fresh leaves samples of uniform size and weight (approximately 0.5 gm frsh weight) was taken as (Wf) were dipped in distilled water in till the leaves achieve constant weight i.e. 24 hours. After gaining turgidity leaves were weighted again for a second time (Ws) and after that kept for drying for 72 hours at 70°C to estimate dry weight (Wd). Relative water contents were calculated by following formula:

$$\text{RWC } \% = \frac{(Wf - Wd)}{(Ws - Wd)} \times 100$$

Statistical analysis

Standard statistical procedures were employed to find out the significant difference among the treatment means using Statictix 8.1 computer program. To present graphical demonstration of data and to estimate standard errors meant for treatments comparison Microsoft Excel (Microsoft Corporation, Los Angeles, and CA, USA) was used.

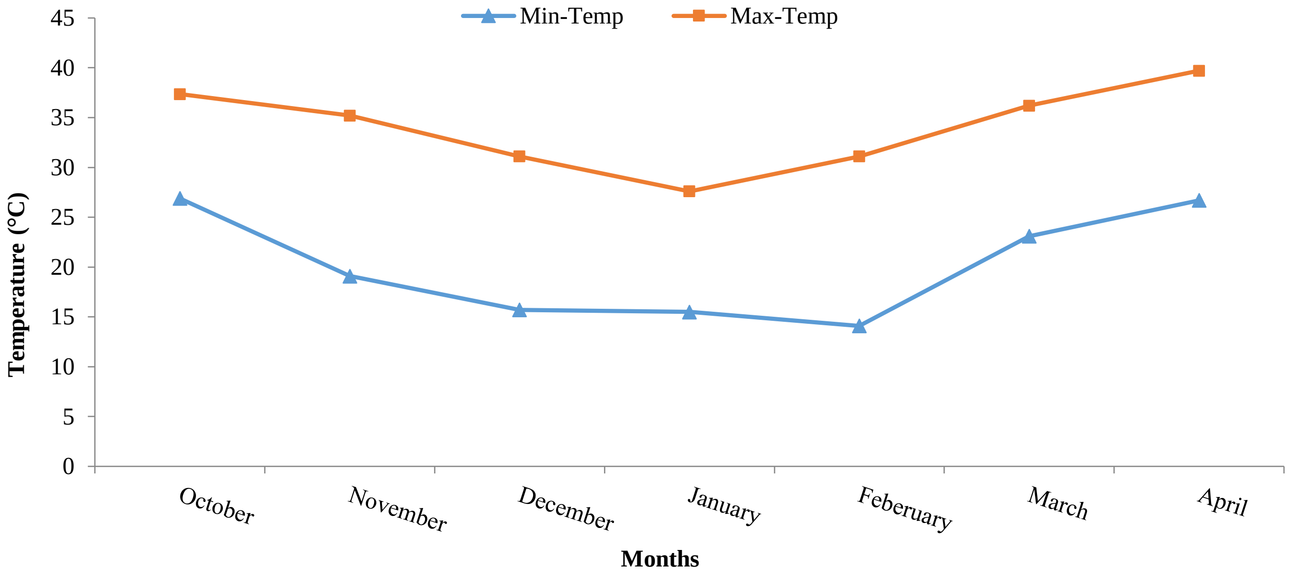

Fig. 1. Temporal variations in air temperature during crop season from October-April.

Results

Wheat crop under high temperature stress induced by late sowing showed drastic changes in its growth and yield contributing traits (Tables 1 and 2). Plant biomass reduced in all wheat genotypes under high temperature stress expose by late sowing. Among genotypes NIA-AS-14-9, Raskoh, NIA-Sarang and TJ-83 produced higher plant biomass, while NIA-AS-14-10 and NIA-Sunder were not effective to give significant results as compared to other tested genotypes. On other hand, Kiran-95, NIA-AS-14-9 and SH-Salt-5B showed utmost productive tillers as compared to other genotypes. NIA-Sarang revealed minimum productive tillers and statistically at par with NIA-Sundar and LU-26s (Table 1). Furthermore, maximum number of grains per plant was produced by NIA-Sundar that was statistically at par with NIA-AS-14-9, NIA-AS-14-10, TJ-83 and TD1 and behaved alike. Contrary, LU-26s revealed minimum number of grains per plant followed by SRN-09111, NIA-Sarang and Dani while rest of the genotypes performed moderately in terms of grains per plant. On exposure to high temperature under late sowing, a drastic reduction in wheat genotypes was observed (Table 2). Comparison of grand means revealed that NIA-AS-14-9 had given maximum grain weight per plant followed by Bathor-08, NRL-1236 and NIA-Sunder which showed their potential to withstand against high temperature under late sown conditions. Among genotypes, LU-26s and Dani produced minimum average grain yield in response to optimal and high temperature stress.

Maintaining high water contents under a-biotic stresses conditions is pre- requisite. The relative water contents (RWC) values under normal sowing condition were higher in all the tested wheat genotypes i.e. > 95%. The genotype Salt-6 had maximum RWC values i.e. 99.36% followed by LU-26s (98.81%). Relative water contents in all the wheat genotypes reduced under late sowing treatment due to heat stress at the time of flowering (Fig. 2a),. Under late sowing treatment RWC values were ranged between 80 to 93%. The genotype TJ-83 had maximum RWC under late sowing treatment (92.83%) followed by SRN-09111 and KIRAN-95, NRL-1236 with RWC values 92.39 and 92.28%, respectively. Minimum RWC values under late sowing treatment were recorded in genotype NIA-AS-14-10 (82.8%). High temperature stress due to late sowing (approximately 2 to 3 °C higher as compare to normal sowing), resulted in significant decrease in cell membrane thermo stability index (CMTI) in tested wheat genotypes (Fig. 2b). Under optimal sowing condition CMTI was` ranged from 29 to 70.6 percent. Maximum CMTI values under optimal sowing condition were recorded in genotype SRN-09111 (70.6%) followed by Dani (63.7%). Both the genotype in spite of higher values for CMTI under optimal sowing conditions could not maintain it under late sowing treatment, showed 87.11% relative decrease. The CMTI values recorded under stress condition were only 9.1%. Similarly the genotype Dani also had low CMTI value i.e. 15.8 % (78.34% rel. dec.). The only genotype which had quite satisfactorily maintained it CMTI was NIA-AS-14-10 with only 37% reduction. The CMTI values in case of NIA-AS-14-10 under optimal and late sowing treatment were 46.7 and 26.9%, respectively. Minimum CMTI values under late sowing treatment were recorded in genotype NIA-Sundar (8.8%). The data with respect to total chlorophyll contents showed a significant decrease under late sowing treatment (Fig. 2c). Total chlorophyll contents ranged between 0.48 to 1.34 mg. g^{-1} F. wt., with maximum value for genotype NIA-AS-14-10 (1.34 mg/g FW) followed by Dani (1.16 mg. g^{-1} F. wt). On the other hand, chlorophyll content under optimal sowing was significantly high in genotype NIA-Sundar (3.11 mg. g^{-1} F. wt) and Sehar- 2006 (3.06 mg. g^{-1} F. wt). Minimum values for total chlorophyll were noted for NIA-Sunder followed by SRN-09111 under both treatments.

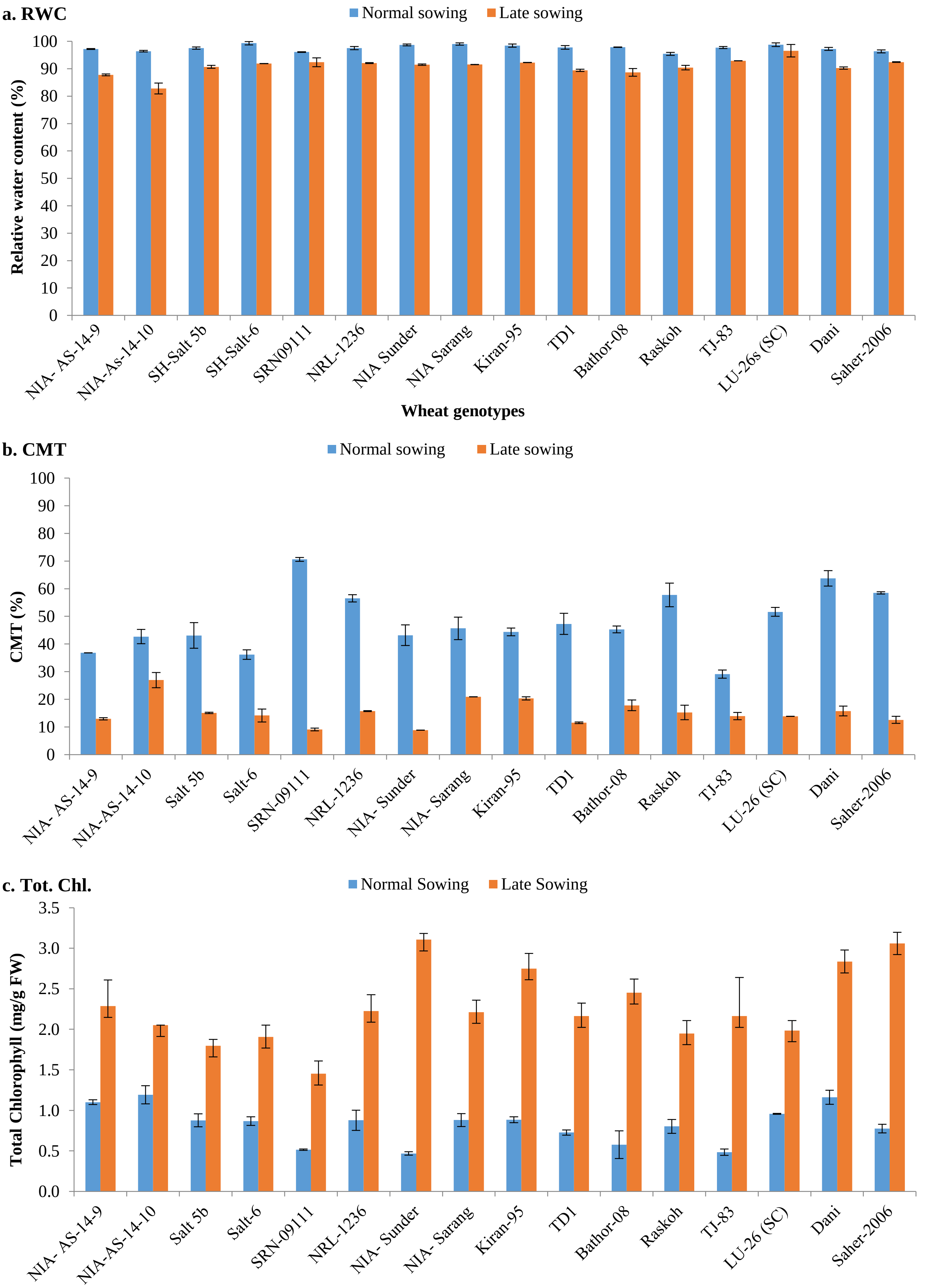

Fig. 2. Effect of high temperature on relative water content and cell membrane thermostability and Total Chlorophyll in wheat genotypes under normal and late sown condition.

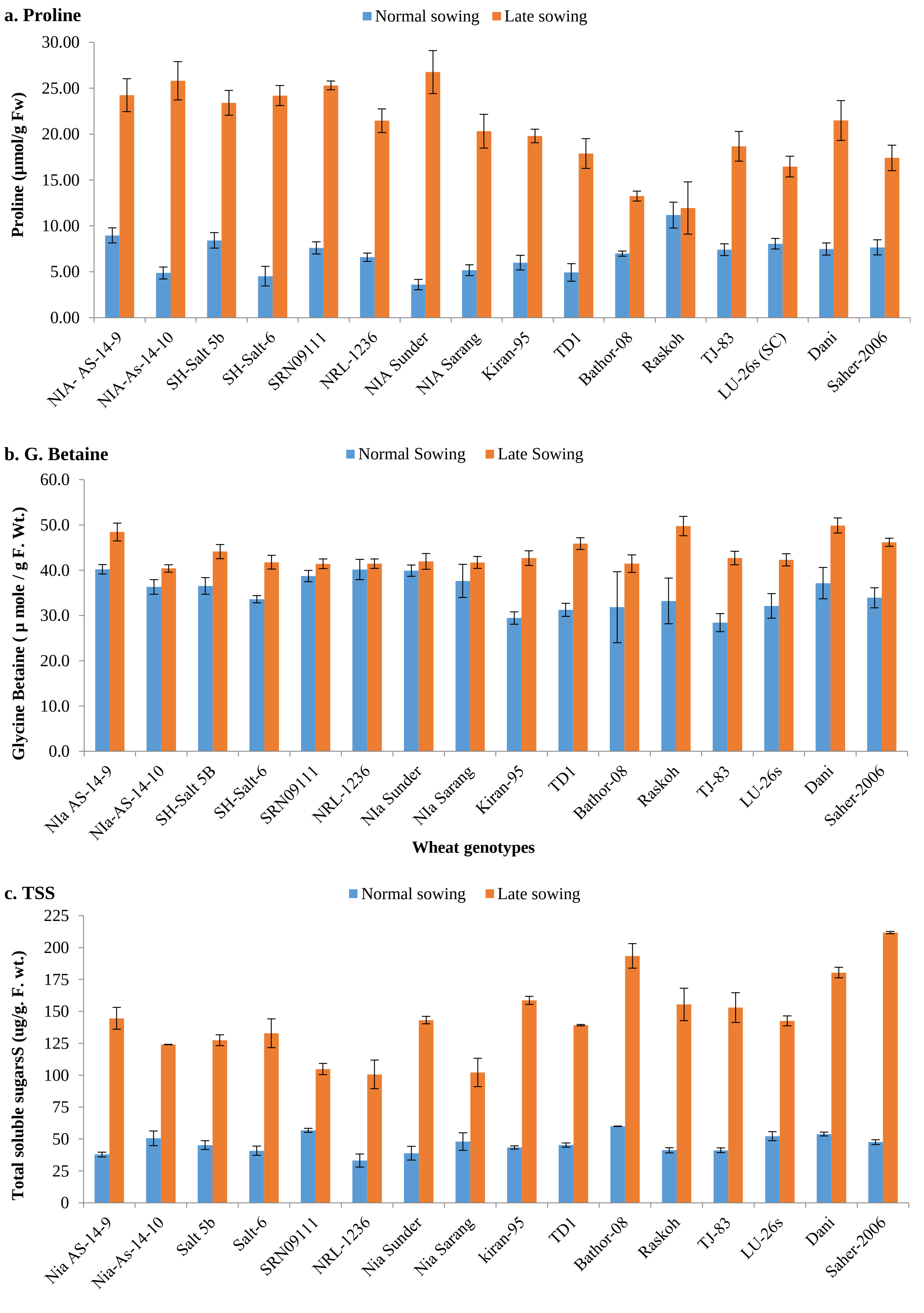

Fig. 3. Effect of high temperature on accumulation of compatible solutes in wheat genotypes under normal and late sown condition.

Table 1. Plant biomass and productive tillers affected under optimal and late sown conditions.

Genotype	Plant biomass (g)			Productive tillers		
	Normal sowing	Late sowing	Mean	Normal sowing	Late sowing	Mean
NIA- AS-14-9	10.88	10.84	10.86 a	3.50	2.50	3.00 abc
NIA-AS-14-10	6.67	6.67	6.67 g	3.53	2.25	2.89 bcde
SH-Salt 5B	8.50	8.50	8.50 def	3.56	2.53	3.00 abc
SH-Salt-6	7.89	8.62	8.26 ef	3.17	2.00	2.58 fg
SRN-09111	8.69	7.94	8.32 def	2.89	3.00	2.94 abcd
NRL-1236	9.34	7.98	8.66 de	3.00	2.44	2.72 ef
NIA- Sunder	7.74	6.34	7.04 g	2.78	2.11	2.44 gh
NIA- Sarang	10.25	9.91	10.08 bc	2.78	2.00	2.39 h
Kiran-95	7.33	5.57	6.45 gh	3.19	3.04	3.11 a
TD1	7.28	4.65	5.96 gh	3.44	2.33	2.88 bcde
Bathor-08	9.50	7.51	8.51 def	3.56	2.11	2.83 cde
Raskoh	11.31	9.46	10.38 ab	2.89	2.73	2.81 de
TJ-83	10.87	8.31	9.59 c	2.89	2.75	2.82 de
LU-26 (SC)	8.66	7.18	7.92 f	3.00	2.00	2.50 gh
Dani	9.68	8.19	8.94 d	3.00	2.75	2.87 bcde
Saher-2006	9.97	7.79	8.88 de	2.89	2.83	2.86 cde
Mean	**9.03 A**	**7.84 B**		**3.13 A**	**2.46 B**	
LSD ($p \leq 0.05$)	Treat =0.228		Geno =0.645	Treat = 0.061		Geno = 0.173

Table 2. Plant biomass and productive tillers affected under optimal and late sown conditions.

Genotype	Number of grains per plant			Grain weight per plant (g)		
	Normal sowing	Late sowing	Mean	Normal sowing	Late sowing	Mean
NIA- AS-14-9	96.6	54.3	75.4 ab	3.84	2.03	2.92 a
NIA-AS-14-10	101.0	48.4	74.9 ab	3.57	1.54	2.55 cd
SH-Salt 5B	94.6	50.8	72.4 b	3.28	1.35	2.32 e
SH-Salt-6	74.0	59.2	66.6 cd	2.33	1.51	1.92 hi
SRN-09111	70.3	39.1	54.8 g	2.47	1.49	1.97 fgh
NRL-1236	86.6	48.8	67.6 c	3.85	1.39	2.62 bc
NIA- Sunder	101.1	55.9	78.7 a	3.69	1.48	2.58 bcd
NIA- Sarang	85.3	34.3	59.8 f	2.83	1.06	1.93 gh
Kiran-95	59.4	67.1	63.0 def	2.10	2.15	2.12 f
TD1	87.6	61.3	74.4 ab	2.40	1.77	2.08 fg
Bathor-08	80.0	50.1	65.0 cde	4.05	1.39	2.72 b
Raskoh	85.1	46.6	65.8 cd	3.35	1.56	2.46 de
TJ-83	93.4	57.1	75.2 ab	3.24	1.80	2.52 cd
LU-26 (SC)	73.9	25.3	50.2 h	2.53	0.88	1.71 j
Dani	79.7	42.9	61.3 ef	2.23	1.33	1.77 ij
Saher-2006	80.8	54.8	67.8 c	2.41	1.67	2.06 fgh
Mean	**84.4 A**	**49.7 B**		**3.13 A**	**1.53 B**	
LSD ($p \leq 0.05$)	Treat = 1.84		Geno =4.285	Treat = 0.070		Geno = 0.159

Among the organic solute proline plays an important role for osmotic adjustment under a-biotic stresses. Wheat genotypes showed increasing trend under heat stress exerted due to late sowing (Fig. 3a). Proline contents under normal sowing treatment varied between 3 to 10 µmole. g^{-1} F. wt. While, it raised to 4 to 5 times under late sown wheat genotypes. Maximum increase under late sown treatment was observed in NIA-Sundar followed by NIA-AS-14-10 and SRN-09111. Among the tested genotypes the genotype Raskoh exhibited least proline accumulation under late sowing treatments i.e. 11.18 µmole. g^{-1} F. wt. The relative increase was also least in Raskoh. i.e. 6.9% only. Accumulation of glycine betaine was also increased under late sown wheat genotypes (Fig. 3b). However, the relative increase was comparatively less than proline and TSS. Under optimal sowing condition it ranged from 28-40 µmole. g^{-1} F. wt. Whereas, under late sown treatment it varied from 40 to 50 µmole. g^{-1} F. wt. Maximum betaine under late sowing treatment was recorded in genotype Dani (49.87 µmole/gm F. wt.), followed by Raskoh and NIA-AS-14-9 (49.74 and 48.46 µmole. g^{-1} F. wt, respectively). It was also observed that the genotype Raskoh which exhibited minimum values in case of proline, showed higher accumulation of glycine betaine under late sowing treatment. The relative increase (49.72%) was also high in Raskoh. Moreover, late sowing results in increased total soluble sugars (TSS) in all the wheat genotypes (Fig. 3c). There was 3 to 5 fold increase in TSS in late sown wheat genotypes due to heat stress at the time of flowering. Higher accumulation of TSS under stress is necessary for the maintenance of cell turger through osmotic adjustment. Sugar contents under normal sown wheat genotypes were ranged from 30-50µgm/gm F. wt. Under normal sowing condition maximum sugar accumulation was observed Bathoor-08 (59.98 µgm/gm F. wt) followed by SRN-09111 (56.8 µg g^{-1} F. wt). The genotype Bathoor-08 also also accumulated high sugars under late sowing treatment. *i.e.* 193 µg g^{-1} F. wt. However, maximum sugars contents were recorded in genotype Saher-2006 (212.0 µg g^{-1} F. wt). On the other hand, less values of TSS were recorded in genotype NRL-1236 i.e. 33.12 and 100.64 µg g^{-1} F. wt. both under normal and late sowing treatment, respectively.

Discussion

Heat stress is an important abiotic stress causing various agronomical, physiological and biochemical changes associated with plant growth and development (Liu & Huang, 2000; Mahboob *et al*., 2018). The productivity of wheat has been reduced up to 5% due to the consequence of increasing temperature (Lobell *et al*., 2011). Similar effects were also observed in present study where high temperature has restricted the plant growth and yield traits in terms of plant biomass, productive tillers, number of grains per plant (Tables 1 and 2). The reduced growth and grain yield might be due to obvious detrimental results of high temperature attributed to impeded pollination and seed set (Farooq *et al.*, 2011), reduced number of ear heads and decreased grains per spike (Nawaz *et al*., 2013). High temperature stress due to late sowing had shorten the period of grain filling, resulting in reduced development of grains ultimately decreasing the grain number and grain weight in wheat crop (Guilioni *et al*., 2003; Sattar *et al*., 2010).

Observations regarding leaf RWC and CMT clearly showed a significant reduction in leaf RWC and CMT on the exposure to high temperature in late sown wheat genotypes (Fig. 2a, b). Normal plants have higher water contents as compared to stressed-plant; hence, leaf relative water content (RWC) may provides a direct measure of cellular water contents (Mahboob *et al*., 2015; 2018). Results revealed that wheat genotypes TJ-83, SRN-09111, KIRAN-95 and NRL-1236 were more effective to maintain high water contents (Fig. 2a); this could be because of better water conductance of the plasma membrane (Azaizeh *et al*., 1992), which consequently ameliorates water uptake. Likewise, cell membrane thermo stability significantly reduced in all tested wheat genotypes exposed to high temperature. However, NIA-AS-14-10, NIA-Sarang and Kiran-95 had significantly maintained membrane stability (Fig. 2b). According to Savchenko *et al*., 2002., the increase in temperature beyond threshold level cause an increase in kinetic energy of molecules across membranes which results in loosening of membranes either because of increase in unsaturated fatty acids or due to protein denaturation. Moreover, cell membrane thermostability was reduced because of change in secondary and tertiary structure due to higher electrolyte losses (Wahid *et al*., 2007).

Under late sowing high temperature showed significant degradation in chlorophyll contents and resulted in lower values as compare to control treatment (Fig. 2c) which confirmed the findings of Nawaz *et al*., (2013). This chlorophyll loss is closely associated with damage to thylakoid membrane, due to enhanced activity of chlorophyllase under high temperature, which ultimately reduces the plant photosynthetic activity (Sharkey & Zhang, 2010). Moreover, high temperature causes oxidative damage to the chloroplast resulting in minimum grain yield (Farooq *et al*., 2011).

The accumulation of osmolytes such as proline, glycine betaine and sugars are well-known osmoprotectents involve actively in adaptive mechanism against abiotic stress conditions including heat tolerance. Since heat-sensitive plants apparently lack the ability to accumulate these substances (Rasheed *et al*., 2011, Mahboob *et al*., 2018). Our results are in agreement with these findings and showed that sensitive wheat genotypes have accumulated low osmoprotectants (Fig. 3). Highest proline accumulation under late sown condition is attributed to increase in γ-glutamyl kinase and decrease in proline oxidase activities as were reported by Khan *et al*., (2013). Moreover, proline and glycine betaine (GB) considerably reduced the H_2O_2 production, improved the accumulation of soluble sugars and protect the developing tissues from heat stress effects (Bavita Asthir, 2015).

High accumulation of GB improved the resistance to high-temperature stress (Sakamoto & Murata, 2002) as GB production protects Rubisco activase near thylakoids which prevents inactivation of Rubisco even at higher temperature (Allakhverdiev *et al*., 2008). It is obvious from data that accumulation of GB is closely associated with the stability of cell membranes. Results showed a significant increase in the concentration of total soluble sugars, when exposed to high temperature (Fig. 3c), which substantiate the reports of Wahid *et al*., (2007). These sugars play a critical role in mitigating the effects of high temperature, either by osmotic adjustment or by protecting cellular structures through maintaining water balance (Farooq *et al*., 2008; Mahboob *et al*., 2018), that might contribute towards better growth and grain yield (Table 2).

Conclusion

Keeping in view all results, we concluded that wheat growth and physiological processes significantly modulate under late sowing. Among the genotypes that showed ameliorated performance in terms of plant growth, improved RWC, CMT, Chlorophyll and high accumulation of osmoprotectants and better yield related traits considered as tolerant. So these genotypes can be successfully grown even at high temperature under delayed planting.

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