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BIOCHEMICAL, PHYSIOLOGICAL AND AGRONOMIC RESPONSE OF WHEAT TO CHANGING CLIMATE OF RAINFED AREAS OF PAKISTAN

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Abstract

Designing the adaptation strategies by studying biochemical, physiological and agronomic response of wheat to climate change will be important for ensuring yield stability and economic sustainability in future. The current study was conducted under three variable climatic sites of rainfed field Pothwar viz. Islamabad (Optimum climatic conditions), URF-Koont Chakwal (Moderate temperature and water stress) and Talagang (high temperature and water stress) under four sowing dates (SD_1 = 21-30 Oct, SD_2 = 11-20 Nov, SD_3 = 01-10 Dec, and SD_4 = 21-30 Dec during 2013-14 and 2014-15) and five wheat genotypes. The study quantified the biochemical, physiological and agronomic response of wheat under different treatments. Stress in the form of drought and unfavorable temperature resulted in increase of total soluble sugar content (TSSC), total soluble protein content (TSPC) proline and leaf membrane stability index (LMSI), while decrease in relative water content (RWC), leaf area (LA), plant height (PH), biological yield (BY) and grain yield (GY). Leaf gaseous exchange parameters i.e. net photosynthesis (A_n), intercellular CO_2 concentration (C_i), transpiration rate (Tr) and stomatal conductance (g_s) remained highest under optimum conditions as compared to stress while opposite trend was observed for stomatal resistance (r_s). Correlation analysis among biochemical, physiological and agronomic traits showed that grain yield was positively correlated with RWC, LA, A_n, C_i, E, g_s, SPAD, PH and BY while negatively correlated with all other parameters. These results suggested to change sowing dates based on prevailing climatic conditions and use of tolerant cultivar to have higher sustainable crop productivity.

Key words: Agronomic, Biochemical, Climate change, Physiological, Wheat.

Introduction

Climate change has emerged as key environmental and economical concern and it is mainly due to increased emissions of anthropogenic greenhouse gases (GHG). Changes in the cyclic pattern of weather condition due to rise in temperature is one impact of higher concentration of GHG. The other impacts include occurrence of extreme weather events (Ahsan *et al.*, 2011). The Intergovernmental Panel on Climate Change (IPCC) reported about 1°C rise in temperature at the end of this century. Meanwhile, this trend of rise in temperature might further intensify to 3°C due to doubling of CO_2 (Harley *et al.*, 2006). Wang *et al*., (2015) reported increased global average surface temperature with warming trend of 0.18°C per decade in the last 50 years. There has been increased intensity and frequency of hot days in the future (Irving *et al.*, 2012). Variation in rainfall is another critical factor which determines the overall impacts of climate change. The summer 2010 floods hitting the Pakistan was one event of climate change while on the other side Europe and Russia experienced severe heatwave (van der Schrier *et al*., 2018).

Crops are sensitive to climate change (temperature, precipitation and elevated CO_2) but temperature has shown more negative impacts on crop yield as compared to other variables (Porter & Gawith, 1999; Ottman *et al.*, 2012; Wheeler & von Braun, 2013; Rosenzweig *et al.*, 2014). Climatic change directly or indirectly affects the crop productivity and this impact is worse on agrarian countries like Pakistan. It has been reported that 1°C rise in mean temperature could lead to 4.1-6.4% decrease in wheat yield (Aslam *et al*., 2017). Similarly, Zhao *et al*., (2017) showed negative of impacts temperature on crop yield at the global scale. They depicted that without CO_2 fertilization each 1°C rise in temperature would reduce wheat yield by 6.0%. Furthermore, increase in temperature by 1°C during cultivation could reduce the wheat yield by 3-10% (You *et al.*, 2009).

Crop growth and development have been affected by global climate change. Changes in the crop phenology are one of the important crop responses to climate change. Meanwhile, changes in biochemical parameters have been observed due to rise in temperature and drought stress. Particularly, disruption in photosynthesis and translocation of carbohydrates into grains under stress resulted in the reduction of grain number and weight as reported by Richards *et al*., (2011). Furthermore, stress resulted to the remobilization of assimilates in wheat and early senescence and grain filling (Yang *et al.*, 2001). Photosynthesis is yield limiting factor which is particularly sensitive to abiotic stress (water and temperature). Decreased in photosynthesis through metabolic impairment have been reported by Cornic, (2000). The photosynthesis response to temperature is linked with its pathway (C_3/C_4). The C_3 plants are mainly active between temperature of 0°C to 30°C (Larcher, 2003), while C_4 plants are efficient between 7°C to 40°C (Sage & Kubien, 2007; Fatima *et al*., 2018). Generally, with the increase in temperature from base to optimum, photosynthesis rate increases linearly but after optimal temperature it declines sharply. The decline is associated with reduced light harvesting in photosystem II (PSII), thylakoid membrane instability and limitations in ribulose-1,5-bisphosphate carboxylase/ oxygenase (RUBISCO)

(Crafts-Brandner & Law, 2000). Murata *et al*., (2007) reported that PSII could be inhibited by low level of heat stress as it is the most heat sensitive protein complex. Stimulation of reactive oxygen species (ROS) due to high temperature could cause membrane electron leakage (Xu *et al.,* 2006) and decline in RUBISCO activity. Respiration rate measurement under heat stress could be another indicator as it increases more as compared to photosynthesis rate. Peng *et al*., (2004) reported increased maintenance respiration under heat stress. Heat stress impacts in plant is primarily mediated by transpiration (Zhao *et al.,* 2013). It is most active and common method of cooling crop tissues but (Wang *et al.,* 2014) reported limitation of increased transpiration under various intensities of heat stress. Series of physiological and biochemical responses (suppression of cell growth and photosynthesis, decreased stomatal conductance, increased stomatal resistance and respiration) in plants occurred due to heat and water stress. At cellular and molecular level plants accumulates reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl ion, superoxide and singlet oxygen and proteins in response to these stresses (Suzuki *et al.,* 2012). However, plant have natural scavenging mechanisms for these ROS by generating enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) in the ascorbate–glutathione (AsA–GSH) cycle. Increased concentration of abscisic acid (ABA) in guard cell resulted to the closure of stomata and decreased water loss and the ultimate impact of this is the suppression of cell growth, net photosynthesis and respirations (Saradadevi *et al.,* 2014). Kousar *et al*., (2018) reported exogenous application of chemicals like salicyclic acid to coup heat stress.

Wheat (*Triticum aestivum* L.) is the most widely grown cereal staple crop and sensitive to high temperature stress. This stress prevails during grain filling period in major wheat growing areas and results in great yield loss (Li *et al*., 2018; Lesk *et al.,* 2016; Farooq *et al*., 2011). Matthew *et al*., (2017) stated 3.5 to 12.9% yield loss in wheat due to climate change. Semenov *et al., (*2008) reported more frequent and severe heat stress in future which will be great risk to the wheat products. The growing temperature of wheat range from -3 to 23°C with sunshine requirements of 4-6 hours per day. Generally, its growing cycle is from 120 to 180 days. The nutrient requirement of wheat for optimum yields are N (70–200 kg ha^{-1}), P (20–40 kg ha^{-1}), and K (80–100 kg ha^{-1}) (Acevedo *et al.*, 2002; FAOSTAT, 2018). Global warming potential (GWP) of wheat (3968 kg CO_2 eq. ha^{-1}) as well as carbon emission (1042 kg C ha^{-1}) is higher as compared to other crops (Wang *et al.*, 2018). Therfore, it is necessary to manage the wheat on sustainable basis to mitigate the impacts of climate change. Change in sowing date is one of the best options to minimize the impacts of climate change on crop. Sowing window of wheat in Pakistan is from Mid-October to end of December. Based upon above scenarios present study was conducted during 2013-2014 and 2014-15 with the objectives (i) To see the impacts of sowing dates on wheat biochemical, physiological and yield traits and (ii) to identify suitable genotype for rainfed regions of Pakistan.

Materials and Methods

The experiment material in present study comprised of five wheat genotypes namely Dharabi, NARC-2009, Pak-13, Chakwal-50 and AUR-809. All the genotypes were sown at variable climatic sites of Pothwar viz. Islamabad, Pir Mehr Ali Shah Arid Agriculture University Research Farm (URF) Koont Chakwal and farmer field Talagang during 2013-14 and 2014-15 under four sowing dates (SD_1 = 21-30 Oct, SD_2 = 11-20 Nov, SD_3 = 01-10 Dec, and SD_4 = 21-30 Dec). Long term historical meteorological data reveal that Islamabad comes under optimum temperature and high rainfall while URF Koont Chakwal comes under high temperature and medium rainfall and Talagang bears high temperature and very low rainfall. Experiment was arranged in randomized complete block design (RCBD), replicated three times with plant to plant distance of 10 cm and row to row distance of 22 cm. Row length was kept five meters and one-meter distance was maintained between replications. The information about Weather variables have been presented in Fig. 1. Pre-sowing and post-harvest soil physiochemical properties have been presented in Table 1. Dubois *et al*., (1951) approach was used to determine total soluble sugar content (TSSC) while total soluble protein content (TSPC) was determined by Lowry *et al*., (1951). Leaf membrane stability index (LMSI) was calculated by using Premachandra *et al*., (2009) 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. Following formula was used to calculate LMSI:

$$\text{Leaf membrane stability index (LMSI)} = \left[1 - \frac{C_1}{C_2}\right] \times 100$$

Relative water content (RWC) of leaves were determined by weighing fresh leaves of 0.5 g (w_1=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 formula:

$$RWC = \frac{w_1 - w_3}{w_2 - w_3} \times 100$$

Leaf area (LA) was measured by using leaf area meter (Delta-T Devices Ltd., Burwell Cambs, UK) when 50% of the crop reaches anthesis. Leaf gas-exchange parameters such as Net Photosynthesis (A_n), Intercellular CO_2 concentrations (C_i), Transpiration rate (E), Stomatal conductance (g_s) and Stomatal resistance (r_s) were determined by using portable photosynthesis instrument (LI-6400XT, LI-COR Biosciences). Plant height (PH), Biological yield (BY) and Grain yield (GY) were determined by harvesting crop at maturity. The data collected for various characteristics were subjected to analysis of variance (ANOVA) and the means obtained were compared by LSD at 5% level of significance (Steel & Torrie, 1986). Standard error of difference between means and correlation among biochemical, physiological traits, plant height, biological yield and grain yield were calculated.

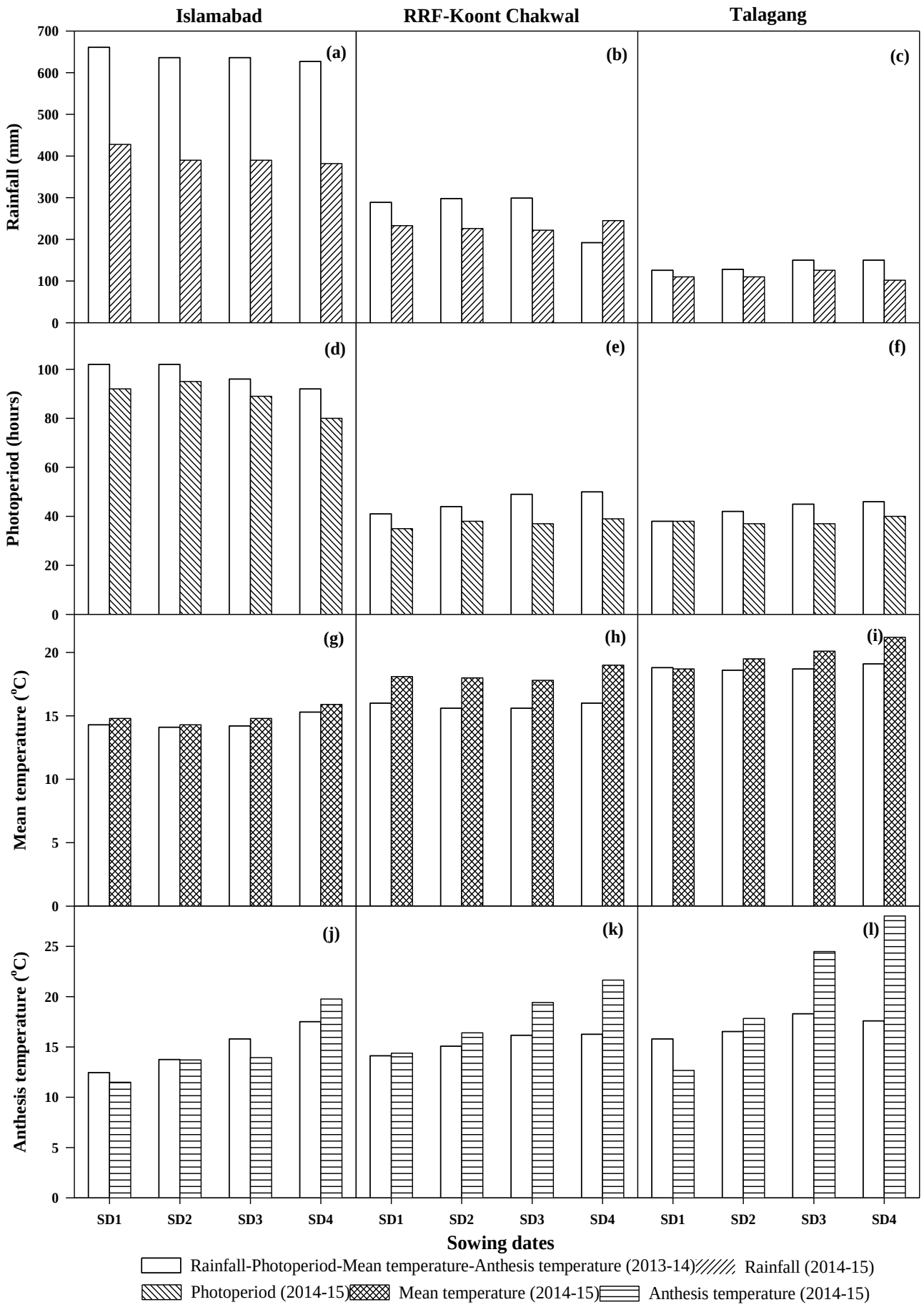

Fig. 1. Weather variables; Rainfall (a, b, and c), photoperiod (d, e, and f), mean temperature (g, h, and i), and anthesis temperature (j, k, l) at Islamabad, URF-Koont Chakwal, and Talagang during years 2013-14 and 2014-15.

Table 1. Pre-harvest and post-harvest soil physiochemical properties.

Soil parameters	Islamabad				URF-Koont Chakwal				Talagang			
	Pre-sowing		Post-harvest		Pre-sowing		Post-harvest		Pre-sowing		Post-harvest	
	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
pH	7.5	7.6	7.4	7.6	8.1	8.1	7.7	7.6	7.9	7.9	7.7	7.6
EC (dsm^{-1})	0.24	0.2	0.24	0.2	0.32	0.3	0.3	0.3	0.2	0.21	0.2	0.2
N (%)	0.046	0.044	0.030	0.274	0.04	0.03	0.020	0.015	0.030	0.032	0.017	0.014
Nitrate-N (mg kg^{-1})	3.64	3.39	2.60	2.70	3.04	2.83	2.40	1.50	2.25	2.40	1.70	1.60
Available P (mg kg^{-1})	7.86	7.28	5.13	5	2.80	2.61	1.79	1.89	2.13	2	1.8	1.5
K (mg kg^{-1})	160	180	150	170	114	129	103	110	130	120	114	107
Organic C (%)	0.91	0.87	0.8	0.7	0.72	0.65	0.63	0.54	0.6	0.64	0.5	0.52
Silt (%)	33	33	33	33	23	23	23	23	26	26	26	26
Sand (%)	35	35	35	35	56	56	56	56	58	58	58	58
Clay (%)	32	32	32	32	21	21	21	21	16	16	16	16
Soil texture	loam	loam	loam	loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Bulk density (g cm^{-3})	1.24	1.42	1.24	1.42	1.29	1.45	1.29	1.45	1.35	1.51	1.35	1.51
SLL ($mmmm^{-1}$)	0.07	0.09	0.07	0.09	0.061	0.080	0.061	0.080	0.06	0.08	0.06	0.08
SDUL ($mmmm^{-1}$)	0.34	0.30	0.25	0.24	0.25	0.18	0.17	0.13	0.15	0.16	0.11	0.13
Saturated SW ($mmmm^{-1}$)	0.48	0.39	0.30	0.30	0.48	0.38	0.32	0.25	0.41	0.36	0.21	0.25

Table 2. Biochemical, physiological and yield traits for five wheat genotypes under four sowing dates for two years at three study sites.

	TSSC (mg g^{-1})	TSPC (mg g^{-1})	LMSI (%)	Proline (µg g^{-1})	RWC (%)	LA (cm^2)	PH (cm)	BY (t ha^{-1})	GY (t ha^{-1})
Years									
2013-14	1.48 ± 0.03^{B}	0.79 ± 0.03^{B}	80 ± 1.95^{B}	27.77 ± 2.55^{B}	85.71 ± 1.22^{A}	26.7 ± 0.89^{A}	80.74 ± 1.23^{A}	8.10 ± 2.79^{A}	2.39±0.88A
2014-15	1.8 ± 0.04^{A}	0.87 ± 0.04^{A}	85 ± 2.07^{A}	36.25 ± 3.01^{A}	80.2 ± 0.89^{B}	25.4 ± 0.78^{B}	73.55 ± 0.88^{B}	6.66 ± 2.55^{B}	2.19 ± 1.05^{B}
Locations									
Islamabad	1.65 ± 0.03^{C}	0.74 ± 0.01^{C}	79 ± 1.76^{C}	24.57 ± 2.08^{C}	90.15 ± 2.55^{A}	28.1 ± 1.33^{A}	84.59 ± 1.88^{A}	7.99 ± 1.32^{A}	2.73 ± 1.01^{A}
URF-Koont	1.79 ± 0.05^{B}	0.89 ± 0.02^{B}	82 ± 1.95^{B}	32.363 ± 3.42^{B}	86.35 ± 1.50^{B}	27 ± 1.11^{B}	76.67 ± 1.50^{B}	7.42 ± 1.66^{B}	2.29 ± 0.87^{B}
Talagang	1.94 ± 0.06^{A}	0.95 ± 0.03^{A}	85 ± 2.15^{A}	39.127 ± 5.25^{A}	82.17 ± 3.20^{C}	25.1 ± 1.26^{C}	70.19 ± 1.33^{C}	6.73 ± 1.88^{C}	1.85 ± 0.59^{C}
Sowing Dates									
SD_1	1.42 ± 0.03^{D}	0.81 ± 0.03^{C}	78 ± 1.76^{C}	30.745 ± 2.79^{C}	87.3 ± 1.14^{B}	28 ± 0.88^{B}	83.30 ± 1.10^{B}	8.77 ± 1.86^{A}	2.89 ± 0.95^{B}
SD_2	1.49 ± 0.02^{C}	0.79 ± 0.03^{D}	76 ± 1.88^{D}	27.977 ± 1.85^{D}	89.19 ± 2.88^{A}	29.1 ± 1.11^{A}	94.44 ± 1.03^{A}	8.99 ± 2.15^{A}	3.04 ± 1.20^{A}
SD_3	1.53 ± 0.06^{B}	0.85 ± 0.05^{B}	80 ± 2.00^{B}	32.099 ± 2.00^{B}	84.2 ± 3.12^{C}	25.1 ± 0.78^{C}	74.96 ± 0.88^{C}	6.25 ± 1.88^{B}	1.79 ± 1.07^{C}
SD_4	1.9 ± 0.07^{A}	0.9 ± 0.06^{A}	83 ± 2.10^{A}	33.82 ± 2.15^{A}	80.15 ± 3.68^{D}	24 ± 1.22^{D}	55.88 ± 0.95^{D}	5.52 ± 1.17^{C}	1.44 ± 0.88^{D}
Genotypes									
NARC-2009	1.43 ± 0.03^{E}	0.81 ± 0.03^{C}	76 ± 1.76^{D}	30.5 ± 1.27^{C}	84.13 ± 2.55^{C}	26 ± 1.52^{B}	82.71 ± 0.55^{A}	7.65 ± 11.33^{A}	2.39 ± 0.96^{C}
AUR-809	1.48 ± 0.05^{C}	0.8 ± 0.02^{C}	78 ± 2.10^{C}	29.83 ± 1.13^{D}	87.77 ± 1.78^{B}	27.3 ± 1.13^{A}	82.00 ± 1.13^{A}	7.75 ± 1.47^{A}	2.50 ± 0.69^{B}
Pak-13	1.46 ± 0.04^{D}	0.75 ± 0.03^{D}	80 ± 1.83^{B}	28.23 ± 1.32^{E}	90.36 ± 3.22^{A}	27.6 ± 0.89^{A}	86.26 ± 1.06^{A}	7.89 ± 1.01^{A}	2.63 ± 1.03^{A}
Dhurabi	1.55 ± 0.06^{A}	0.85 ± 0.05^{A}	83 ± 2.09^{A}	34.1 ± 1.33^{A}	81.71 ± 1.36^{E}	25 ± 0.99^{C}	68.04 ± 0.88^{B}	6.65 ± 1.22^{B}	1.88 ± 0.76^{E}
Chakwal-50	1.51 ± 0.04^{B}	0.83 ± 0.03^{B}	82 ± 2.01^{A}	33.6 ± 1.35^{B}	83.5 ± 1.55^{D}	25.4 ± 0.89^{D}	66.71 ± 0.97^{B}	6.96 ± 1.63^{B}	2.03 ± 0.88^{D}

Where TSSC = Total soluble sugar content, TSPC = Total soluble protein content, LMSI = Leaf membrane stability index, RWC = Relative water content, LA = Leaf area, An = Net Photosynthesis, Ci = Intercellular CO_2 concentrations, E = Transpiration rate, gs = Stomatal conductance, rs = Stomatal resistance, PH = Plant height, BY = Biological yield, GY = Grain yield

Results

Total soluble sugar content (TSSC) remained significantly different under all treatments. Maximum TSSC (Table 2) was observed during 2014-15 (1.8 mg g^{-1}) as compared to 2013-14 (1.48 mg g^{-1}). Among locations maximum TSSC was observed at Talagang (1.94 mg g^{-1}) followed by URF-Koont (1.79 mg g^{-1}) and Islamabad (1.65 mg g^{-1}). Maximum TSSC was observed for SD_4 (1.9 mg g^{-1}) while, it remained minimum under SD_1 (1.42 mg g^{-1}). TSSC remained highest for genotype Dhurabi (1.55 mg g^{-1}) while, it remained lowest for NARC-2009 (1.43 mg g^{-1}).

Total soluble protein content (TSPC) was the highest during 2014-15 as compared to 2013-14. Among sites the highest TSPC (0.87 mg g^{-1}) was recorded at Talagang the area was having lower rainfall and higher temperature as compared to other two sites. The TSPC remained maximum under SD_4 (0.87 mg g^{-1}) followed by SD_3 (0.85 mg g^{-1}), SD_1 (0.81 mg g^{-1}) while it remained minimum under SD_2 (0.79 mg g^{-1}). The TSPC remained significantly different for genotypes. The highest TSPC was observed for Dhurabi (0.85 mg g^{-1}) while lowest was depicted by AUR-809 (0.80 mg g^{-1}) which was at par with NARC-2009 (0.81 mg g^{-1}) (Table 2).

Proline accumulation remained significantly different under all treatments. During 2014-15 highest (36.25 µg g^{-1}) proline accumulation was recorded while it remained lowest during 2013-14 (27.77 µg g^{-1}). Higher proline accumulation was observed under stress conditions i.e. Talagang (39.13 µg g^{-1}) followed by URF-Koont (32.36 µg g^{-1}) and Islamabad (24.57 µg g^{-1}) where environmental conditions prevailed during crop life cycle remained normal. Proline accumulation under different sowing dates showed significant variability. Maximum proline accumulation was observed under SD_4 (33.82 µg g^{-1}) followed by SD_3 (32.10 µg g^{-1}) and SD_1 (30.75 µg g^{-1}) while it remained minimum under SD_2 (27.98 µg g^{-1}). The highest proline accumulation was observed for genotype Dhurabi and lowest recorded for Pak-13. Proline content of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes have been presented in Fig. 2.

Decreased LMSI was observed during 2014-15 as compared to 2013-14. Similarly, among locations lowest LMSI was observed for the site (Talagang) where limited water in the form of rain was available during crop life cycle and temperature remained highest. However, at Islamabad LMSI remained highest (Table 2). LMSI remained maximum under SD_2 followed by SD_3, SD_1 and SD_4. Tolerant wheat genotypes i.e. Dhurabi and Chakwal-50 maintained significant higher LMSI as compared to sensitive genotypes.

Relative water content (RWC) is good criteria to measure leaf water status. The highest RWC remained during 2013-14 than 2014-15. Similarly, among locations the highest RWC was observed for normal environmental site (Islamabad) as compared to stress site (Talagang) where it remained lowest (Table 2). Variable RWC was observed under sowing dates. Maximum RWC was recorded under SD_2 followed by SD_1, SD_3 while it remained minimum under SD_4. Significant variability was observed for RWC among genotypes. Genotype, Dhurabi depicted maximum RWC which was at par with Chakwal-50 while minimum RWC was observed for NARC-2009.

The SPAD Chlorophyll contents are presented in Fig. 3. The interactive effect of locations x years depicted that highest SPAD values recorded at Islamabad during 2013-14, while it remained lowest at Talagang during 2014-15. Similarly, sowing dates x years interactive effect revealed that lowest SPAD value remained during 2014-15 as compared to 2013-14 under SD_4 while it remained highest under SD_2 during both years. Sowing date x locations interaction depicted that SPAD value remained highest under SD_2 at Islamabad while it remained lowest at Talagang under SD_4. SPAD value for genotypes x years interaction remained highest during 2013-14 as compared to 2014-15 for all genotypes. Locations x genotypes interaction showed that it remained highest at Islamabad followed by URF-Koont and Talagang. Sowing dates x genotypes interactive effect on SPAD revealed that it remained maximum under SD_2 while minimum under SD_4 for all genotypes.

Leaf gaseous exchange parameters such as net photosynthesis (A_n), intercellular CO_2 concentration (C_i), transpiration rate (Tr), stomatal conductance (g_s) and stomatal resistance (r_s) are presented in Figs. 4 to 8. The results showed that for locations x years interaction, the highest A_n was observed at Islamabad during 2013-14 while it remained lowest at Talagang during 2014-15. Among sowing dates x years interaction, the A_n remained maximum during 2013-14 as compared to second year. The graphical trend of net photosynthesis under all interactions showed that it remained highest under optimum conditions as compared to stress (Fig. 4). However, genotypic interactive effect revealed significant variability for A_n. Similar results were obtained for Intercellular CO_2 concentration (C_i), Transpiration rate (Tr) and Stomatal conductance (g_s) under all interactions (Figs. 5-7). However, opposite results were obtained for stomatal resistance (r_s) (Fig. 8).

Leaf area (LA) of crop remained significantly different under all treatments. The highest LA was observed during 2013-14 (26.7 cm^2) while it was lowest in 2014-15 (25.4 cm^2). Among sites LA remained maximum at Islamabad followed by URF-Koont and Talagang. Leaf area was highest under sowing date (SD_2) while it was lowest under SD4. Genotypes showed significant variation for LA and maximum LA was recoded for Pak-13 which was at par with AUR-809. However, it was minimum for Dhurabi and Chakwal-50.

Plant height (PH) is an important growth parameter. The results showed that PH remained highest during 2013-14, while it was lowest in 2014-15. The PH was maximum at Islamabad followed by URF-Koont and Talagang. The effect of sowing date on PH revealed that it remained maximum for SD_2 while minimum PH was observed for late sowing i.e. SD_4. Highest PH was recorded for genotype Pak-13 which was at par with AUR-809 and NARC-2009 while lowest PH was depicted by Dhurabi and Chawal-50 genotypes (Table 2).

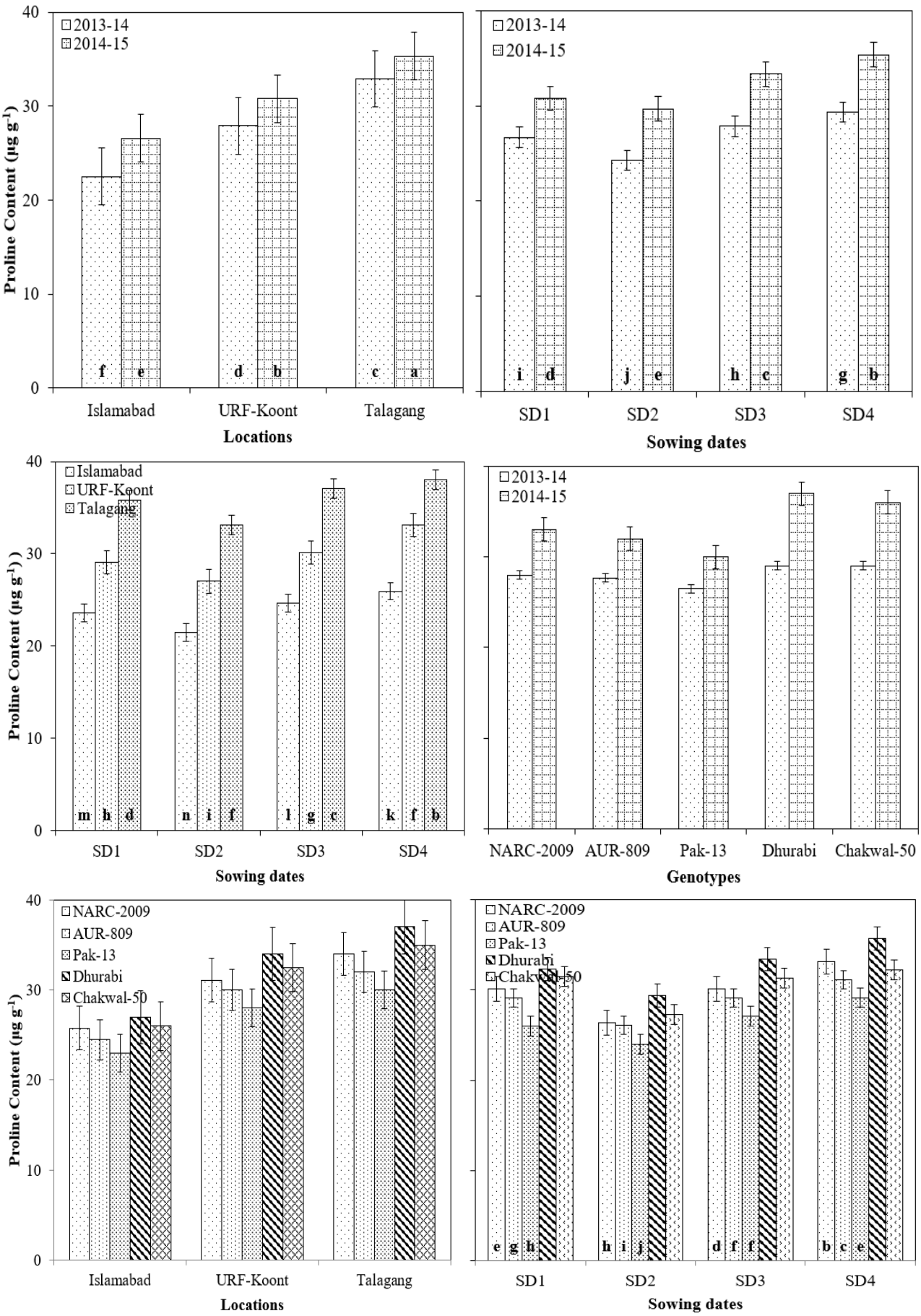

Fig. 2. Proline content of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes.

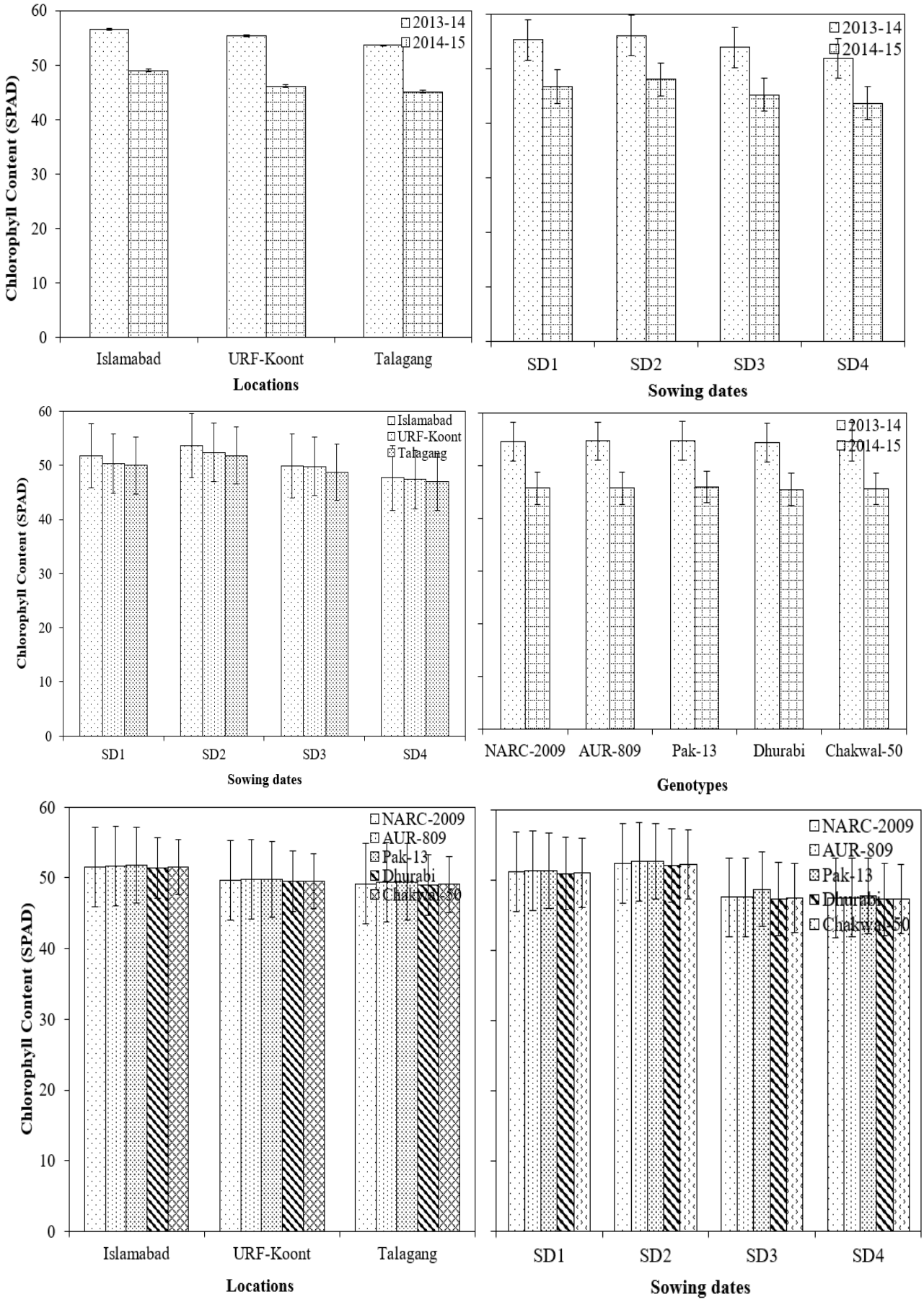

Fig. 3. Chlorophyll content (SPAD) of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes.

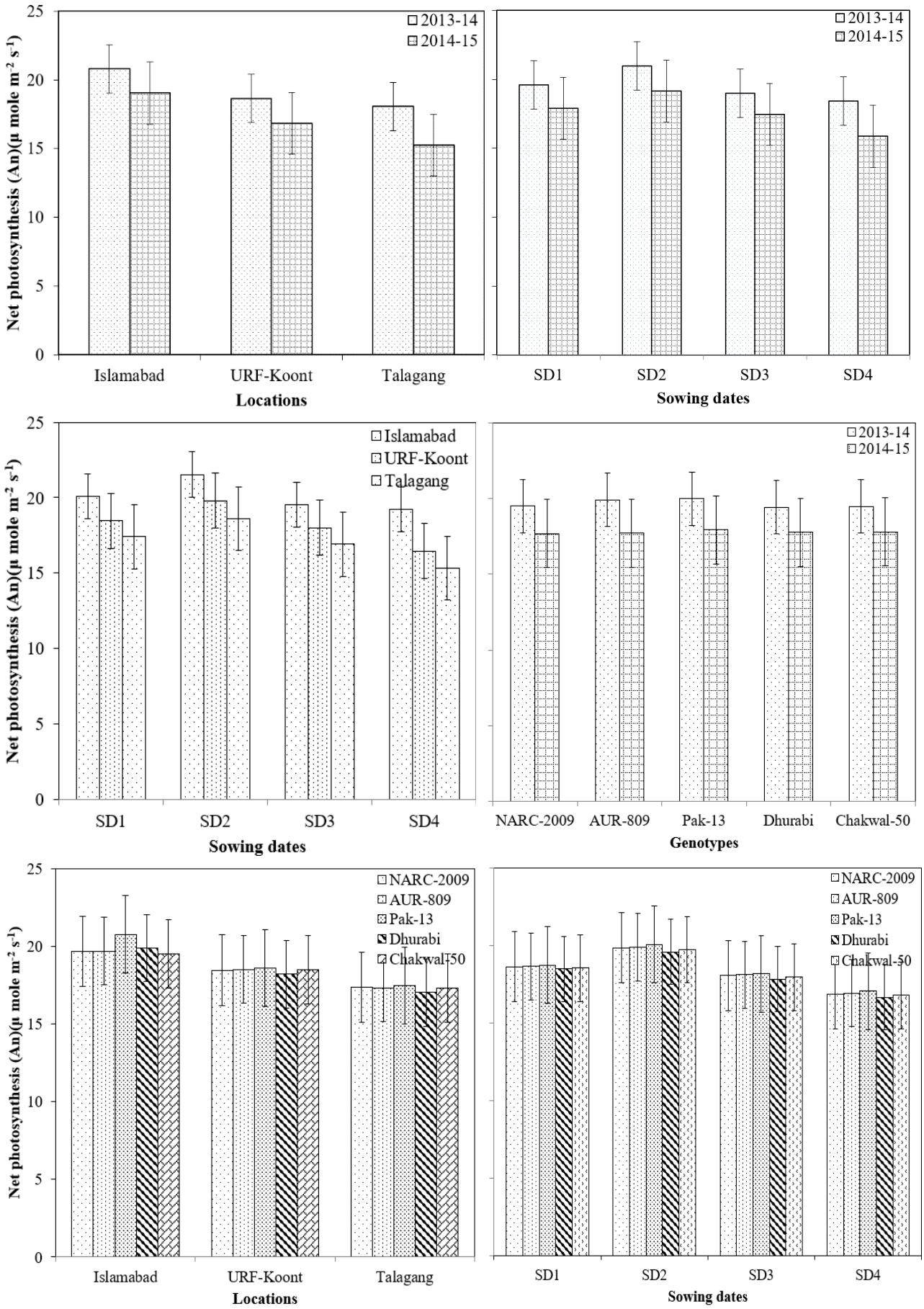

Fig. 4. Net photosynthesis of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes.

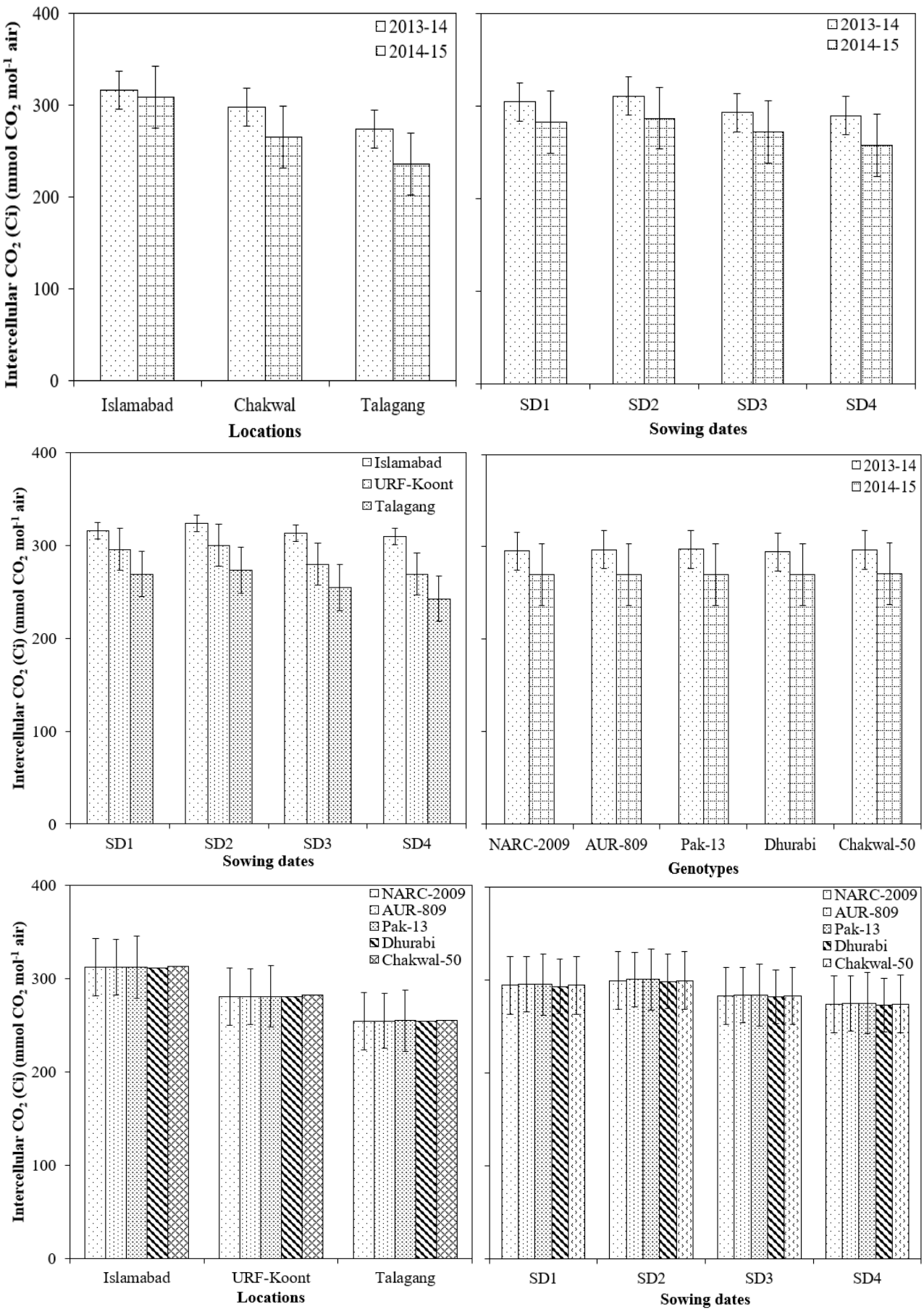

Fig. 5. Intercellular CO_2 concentration of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes.

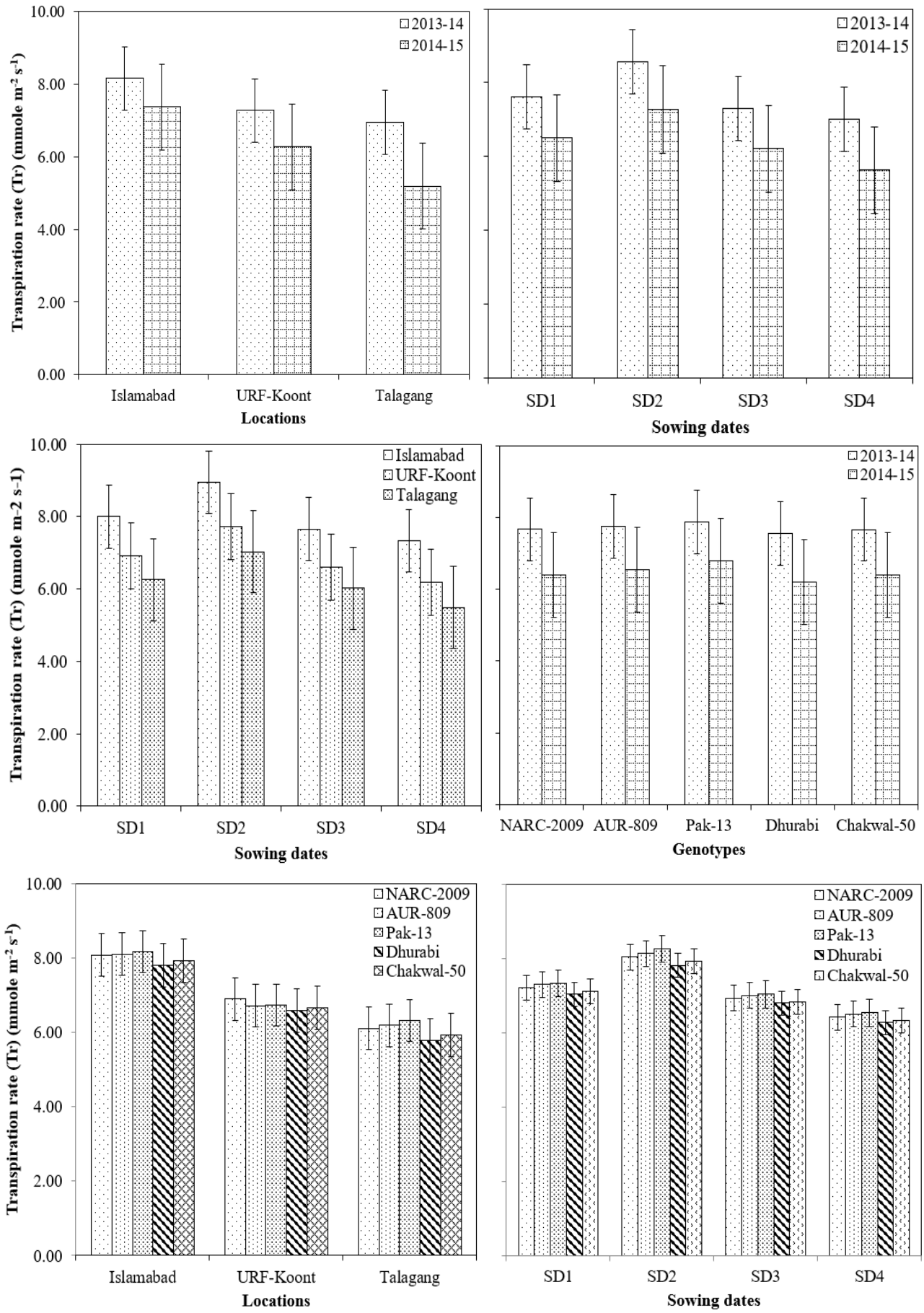

Fig. 6. Transpiration rate of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes.

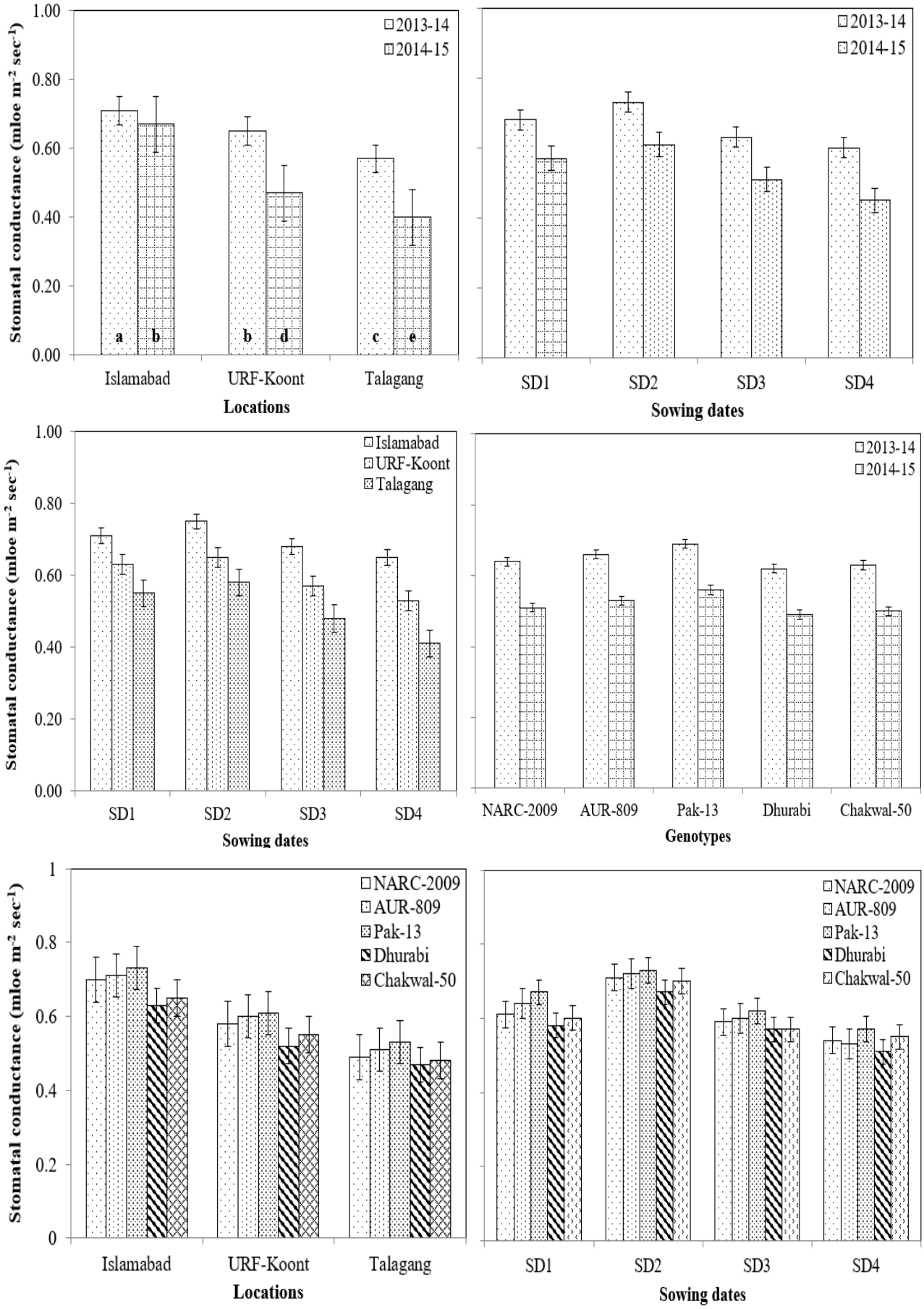

Fig. 7. Stomatal conductance of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes.

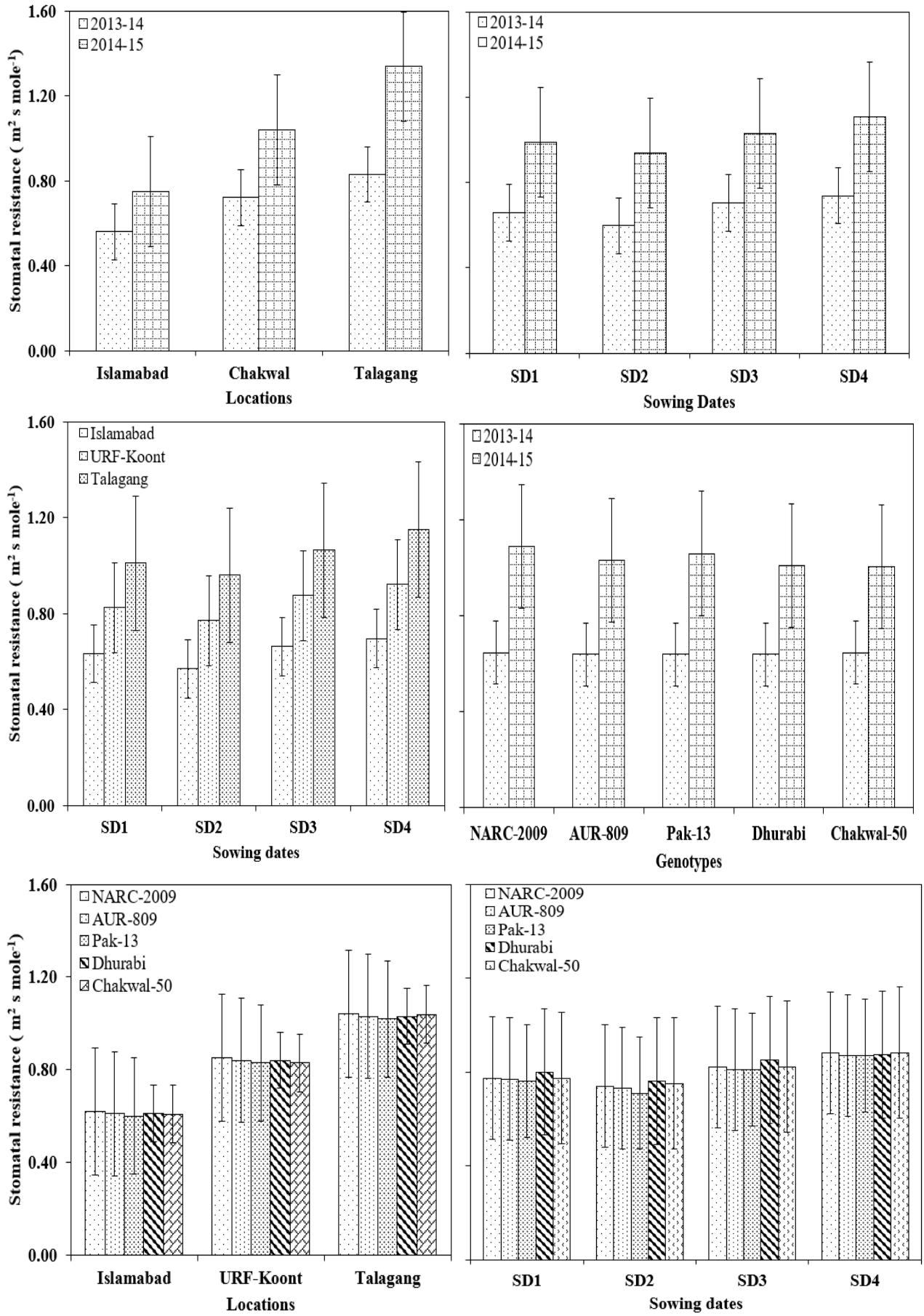

Fig. 8. Stomatal resistance of wheat under interactive effect of locations x years, sowing dates x years, sowing date x locations, genotypes x years, locations x genotypes and sowing dates x genotypes.

Table 3. Biochemical, physiological traits, plant height, biological yield and grain yield correlation for five wheat genotypes under four sowing dates during 2013-14 and 2014-15 at three study sites.

	TSSC	TSPC	LMSI	Proline	RWC	LA	An	C_i	E	g_s	r_s	SPAD	PH	BY	GY
TSSC	1														
TSPC	0.77	1													
LMSI	0.76	0.72	1												
Proline	0.62	0.91	0.77	1											
RWC	-0.54	-0.78	-0.69	-0.83	1										
LA	-0.50	-0.71	-0.69	-0.75	0.91	1									
A_n	-0.76	-0.89	-0.78	-0.89	0.78	0.7	1								
C_i	-0.60	-0.84	-0.69	-0.92	0.72	0.71	0.92	1							
E	-0.70	-0.89	-0.77	-0.92	0.83	0.81	0.98	0.91	1						
g_s	-0.65	-0.90	-0.75	-0.95	0.86	0.84	0.95	0.95	0.96	1					
r_s	0.63	0.81	0.64	0.89	-0.66	-0.58	-0.91	-0.95	-0.89	-0.90	1				
SPAD	-0.49	-0.59	-0.54	-0.73	0.65	0.62	0.74	0.73	0.77	0.78	-0.81	1			
PH	-0.60	-0.71	-0.76	-0.70	0.84	0.91	0.72	0.61	0.76	0.76	-0.48	0.58	1		
BY	-0.62	-0.68	-0.72	-0.67	0.81	0.94	0.74	0.66	0.78	0.79	-0.56	0.69	0.90	1	
GY	-0.57	-0.73	-0.71	-0.70	0.83	0.97	0.73	0.68	0.78	0.79	-0.53	0.56	0.98	0.97	1

Where TSSC = Total soluble sugar content, TSPC = Total soluble protein content, LMSI = Leaf membrane stability index, RWC = Relative water content, LA = Leaf area, A_n = Net Photosynthesis, C_i = Intercellular CO_2 concentrations, E = Transpiration rate, g_s = Stomatal conductance, r_s = Stomatal resistance, PH = Plant height, BY = Biological yield, GY = Grain yield

Biological and grain yield show significant variability under different treatments. Among years the highest biological and grain yield remained during 2013-14, while both remained lowest in 2014-15. Maximum biological and grain yield was observed at Islamabad followed by URF-Koont and Talagang. Grain yield remained highest for SD_2 while it was lowest for SD_4. Furthermore, among genotypes grain yield remained maximum for Pak-13 followed by AUR-809, NARC-2009, Chakwal-50 while minimum grain yield was observed for genotype Dhurabi (Table 2). Correlation analysis among biochemical, physiological traits, plant height, biological yield and grain yield have been presented in Table 3. The results show that TSSC have negative correlation with RWC, LA, A_n, C_i, E, g_s, SPAD, PH, BY and GY. TSPC have only positive correlation with LMSI and proline and with all other parameters it has negative correlation. LMSI depicted positive correlation with proline and r_s while for all other parameters it was negatively correlated. Proline correlation was positive with r_s only. RWC was negatively correlated with r_s but for all others it was positively correlated. LA, A_n, C_i, E and g_s were negatively correlated with r_s only. Correlation of grain yield with other parameters showed that it was positive for RWC, LA, A_n, C_i, E, g_s, SPAD, PH and BY. However, for all other parameters it was negatively correlated.

Discussion

Plant adopt themselves under stress by accumulation of soluble sugar which is kind of osmotic adjustments. Since in our findings, the highest TSSC were accumulated under stress conditions i.e. during 2014-15, at Talagang and under SD_4. The results were in line with the findings of Abid *et al*., (2018) who reported increased TSSC under water stress as compared to non-limiting soil water. Furthermore, they suggested that TSSC may aid in stress tolerance by improving osmotic adjustment, reactive oxygen species (ROS) detoxification, cell membrane protection and protein stabilization (Reddy *et al.*, 2004). Similar results were reported by Hammad & Ali, (2014) for total soluble sugars. Singh *et al*., (2012) In their work concluded that variable thermal time due to change in sowing date resulted to significant effect on the amount of TSPC. They reported higher protein content under late sown conditions which was like our findings. Similarly, amount of TSPC remained lowest under conditions where water supply was high and significant variability was also observed among genotypes. Similar to our findings several researchers reported higher TSPC under abiotic stress (Sundaravalli *et al.*, 2005; Nouman *et al.*, 2014; Zhang *et al.*, 2015). Proline is main component of osmotic adjustment and it can play significant role to stabilize cell membrane and prevent oxidative damage (Matysik *et al.*, 2002). Our results were in agreement with the findings of Monreal *et al*., (2007) who reported increased proline accumulation in sugar beet leaves under stress and that of Ahmed *et al*., (2017) who found that under stress, there was progressive increase in free proline in wheat plants. Similar results were also reported by Yi *et al*., (2016) who reported increased proline in cotton plant under drought stress. Synthesis and accumulation of solutes like proline under

stress depicted osmotic adjustment mechanism by wheat in present study to survive under stress (Mahboob *et al*., 2016). Meanwhile, Molinari *et al*., (2007) in their findings reported that plant could alter water relations under stress to maintain cellular functions by synthesizing solutes like proline. Since in our findings higher proline accumulation was observed under stress conditions therefore, it supported earlier conclusion made by Abid *et al*., (2018) and Sánchez *et al*., (1998). Among genotype, the sensitive cultivar depicted lower increase in proline as compared to tolerant genotypes which was in close agreement with the findings of Ouhaddach *et al*., (2018). Seed treatment with proline was used to explore the role of proline on maize under salt stress by Perveen & Nazir, (2018).They concluded that proline helps to increase growth of plants by regulating physiochemical parameters under diverse environmental conditions. Similar findings were reported by Khan *et al*., (2009). Shirazi *et al*., (2018) evaluated tolerance of wheat genotypes under different environmental conditions and recommended three genotypes (NIA-AS-14-2 NIA-AS-14-4 and NIA-AS-14-10) and a local check LU-26s which have the potential to perform economically under medium to high saline soils.

Improved membrane stability is an important stress tolerant mechanism which can help plants to avoid stress damage. In our findings the LMSI remained lowest under stress conditions but tolerant genotypes depicted highest LMSI which was at par with the findings of Abid *et al*., (2018) and Blum & Ebercon, (1981). Petrov *et al*., (2018) investigated membrane stability under stress and concluded that under drought stress modern genotypes maintained better water balance and membrane stability. Plant water parameters respond to stress and it has been observed in previous work that RWC and other plant parameters decreases under stress (Farooq *et al.*, 2009; Petrov *et al.*, 2018). Siddique *et al*., (2000) conclusion was in close agreement with our findings. They concluded that wheat under stress have lower RWC as compared to non-stressed one which was due to increased leaf temperature. Since in our findings the RWC was different among genotypes which could be due to their resistant mechanism under stress as reported by Aziz *et al*., (2018) in cotton plant and Fathi *et al*., (2018) in Almond. Khakwani *et al*., (2012) showed that wheat varieties which maintains higher RWC can survive under stressed environment easily.

The SPAD chlorophyll content under stress was assessed by Thomason *et al*., (2018)missing??? and they concluded that genotype showed significant difference for SPAD value. They further elaborated that this significant difference might be due to stress prevailed during crop life cycle as in our findings.

Reduction in leaf gaseous exchange parameters were observed under stress which might be due to less leaf expansion, impaired photosynthetic machinery, earlier leaf senescence and declined food production (Farooq *et al.*, 2009; Fathi *et al.*, 2018). Like our findings Wahid & Rasul, (2005) reported that stress resulted to stomatal closure (increased stomatal resistance) which limits CO_2 uptake by leaves. This restriction further increase vulnerability to photo-damage (Cornic & Massacci, 1996). Declined net photosynthetic rate (P_n) and stomatal conductance (g_s) was also reported by Abid *et al*., (2018) under stress as compared to non-stressed one. Similarly, they reported that decrease in gaseous exchange parameters was more in sensitive genotypes as compared to non-sensitive one which was at par with our findings. Therfore, it is imperative to bring such genotypes in field which can maintain gaseous exchange parameters and thus have less reduction in yield.

Variability in leaf area index (LAI) due to genotypes and sowing date was reported by earlier researcher in their findings. Ihsan *et al*., (2016) reported that stress had significant impact on leaf area index of different genotypes under different sowing dates. They concluded that stress (75% field capacity and 50% Field capacity) resulted to 26–46% (November sowing), 32–67% (December sowing) and 07–40% (January sowing), reduction in leaf area index respectively. Similar results for leaf area were reported in our findings but with different percentage changes in different years, sites sowing dates and genotypes. Reduced plant height due to change in sowing time was reported by earlier researchers (Din & Singh, 2005). Sial *et al*., (2005) stated that under favorable temperature like Islamabad the genotypes with late heading have increased plant height and more number of internodes. However, under stress conditions PH decreases significantly.

Yield of a crop is affected by different known and unknown factors as reported by Araus *et al*., (2001). Similarly, they stated that sowing date had significant impacts on crop yield and different genotypes behaves differently under different sowing dates. Late sowing resulted to significant reduction in crop yield as reported in our findings. Climate variability in the form of low rainfall and high temperature are serious threat to crop yield under rainfed conditions. Monneveux *et al*., (2006) stated that under stress conditions wheat yield is related to photosynthetic activity and transpiration efficiency. They suggested for the higher grain at maturity we need to have genotypes with higher stomatal conductance and lower transpiration efficiency. Similarly, Shirazi *et al*., (2010) concluded that to minimize the effects of drought and high temperatures, heat and drought tolerant genotypes needed to be evolved in addition to the use of good sowing time simila to our findings. Ahmad *et al*., (2006) stated that wheat plants tolerate stress on the expense of yield. In our work greater grain yield declines was under stress sensitive genotypes which was at par with the findings of Abid *et al*., (2018). Similarly, Khakwani *et al*., (2012) suggested use of stressed resistant genotypes for better yield under stressed environment. However, earlier researchers also suggested phenotyping using physiological traits to accelerate breeding for higher yield potential under stress conditions (Fleury *et al.*, 2010; del Pozo *et al.*, 2016; Ihsan *et al.*, 2016).

Correlation analysis is good technique to see response of different variables and their contribution to grain yield. Similar technique was used by Ahmad *et al*., (2006) to see relationship among different crop variables under stress. Meanwhile, Abid *et al*., (2018) reported negative correlation of proline with leaf water potential which was at par with our findings showing contribution of proline to do osmotic adjustment. Shirazi *et al*., (2010) reported positive correlation with shoot dry weight and carbon isotopes discrimination (CID) under stress

conditions. Correlation analysis between chlorophyll content, grain yield and agronomic traits were conducted by del Pozo *et al*., (2016) and they reported positive association with grain yield, chlorophyll content, kernels per spike (KS) and thousand kernel weight (TKW). Similarly, Ihsan *et al*., (2016) demonstrated strong correlation with growth indices and grain yield.

Conclusions

Wheat crop exhibited different biochemical, physiological and agronomic response to changing climate of rainfed Pakistan. Different biochemical features in terms of total soluble sugar content (TSSC), total soluble protein content (TSPC) and proline under variable climatic conditions, sowing dates and genotypes depicted great variability. These metabolites could help to facilitate osmotic adjustments and help wheat plant to survive under stress. Leaf membrane stability index (LMSI), relative water content (RWC), SPAD Chlorophyll and leaf gaseous exchange parameters were greater under tolerant cultivar which could be due to their ability to adjust osmotically. Moreover, sowing date adjustment could bring sustainability in agronomic traits. Under varying climatic study sites, we were able to identify response of changing sowing dates as adaptation strategies. A shift from optimum sowing time to later sowing significantly reduces wheat yield. Meanwhile, the atmospheric parameters especially rainfall and temperature had their impact on agronomic traits. It is need of the time that adaptation strategies should be adopted to increase wheat yield which will ultimately help in securing of food security for under developing countries like Pakistan. Based upon the current study it is recommended that sowing of wheat should be done according to prevailed climatic conditions to get sustainable yield. Resistant genotypes like Pak-13 and AUR-809 must be planted in rainfed areas of Pothwar Pakistan. Similarly, such genotypes should be developed which will be tolerant to higher temperature and water stress to feed the increasing population of the globe particularly Pakistan.

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