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Genotypic response for stomatal conductance due to terminal heat stress under late sown condition in wheat (*Triticum aestivum* L.)

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Abstract

Stomatal conductance (SC) was evaluated for thirty-six wheat (*Triticum aestivum* L.) genotypes sown during 2013-14 and 2014-15 under two dates of sowing. Steady state SC-1 leaf porometer was used to record SC on adaxial leaf surface at late boot (Z 49-50), early milk (Z 73) and late milk (Z 77) growth stages. In late sown conditions, the temperature was high (25.7 to 30.3°C) in boot stage itself and further increased during later stages (32 to 37.4°C). Under these conditions, the genotypes need to maintain an optimum range of stomatal conductance to maintain transpirational cooling without losing too much of water through transpiration. Heat susceptibility index ranged from 0.27 to 1.54 indicating levels of stress tolerance among the genotypes. Heat tolerant genotypes maintained relatively higher SC under heat stress over normal condition. Significant association between high SC with CT and grain yield was observed in all the three growth stages. The study was useful in detailed evaluation of SC and identification of genotypes with stable SC under heat stress. Genotypes KAUZ/AA//KAUZ, BERKUT and RAC 875, maintained high SC over the growth stages. Indian genotypes HD 2932, HD 2987, FLW-18, HW 2004 and RAJ 3765 were equally potential.

Key words: Adaxial, conductance, genotypes, heat, stomatal

Introduction

Wheat is the most widely grown cereal crop in temperate environment. Wheat is grown as winter crop in tropical and sub-tropical countries; high temperature stress is one of the most disadvantageous factors for the production and productivity. In India, a substantial wheat area is under delayed planting facing late heat stress, which is caused mostly by cultivation of long duration paddy varieties. (Joshi et al. 2007). Yield penalties are associated with both chronically high temperatures (mean temperature of the growth cycle being 18°C-25°C, and maximum day temperatures up to 32°C during grain filling) as well as heat shocks, where temperatures are greater than 32°C occuring during mid or late reproductive wheat stages, including grain filling (Wardlaw and Moncur 1995). The crop species have adapted several morphological, biochemical, physiological and genetic mechanisms to overcome high temperature stress. Plants are equipped with specialised epidermal cells called guard cells surrounding minute pores called stomata, which are found in leaves and stems of the plant. The major mechanism adopted by the plants to keep themselves cool is by keeping the stomata open. This anatomical adaptation permit gas exchange (water loss, carbon dioxide uptake, oxygen release or uptake) between atmosphere and interior part of the leaf. The function of gas exchange is called stomatal conductance (Taiz and Zeiger 1991). When ambient temperature is more than plant temperature, stomatal conductance is enhanced which promotes evaporative cooling of leaves to thereby reduce heat stress (Radin et al. 1994). There is a strong relationship between stomatal conductance (SC) and canopy temperature (CT) since stomatal conductance has a direct effect on transpirational cooling (Amani et al. 1996; Fischer et al. 1998). The differences in the vapour pressure deficit between canopy and atmosphere as well as chemical signals synthesized in dehydrating roots, regulate stomatal aperture and therefore water flux to the atmosphere. Consequently, a plant is able to tolerate heat stress to some extent by lowering tissue temperature and frequently by creating signals for changing metabolism (Hasanuzzaman et al. 2013).

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Positive relationships between grain yield and SC were observed under irrigated environments for cotton, wheat and other crops (Radin et al. 1994; Reynolds et al. 1994; Morrison et al. 1999). Stomatal conductance has been proposed as a selection tool, when measured on multiple plants in a canopy, is equally effective as carbon isotope discrimination or canopy temperature (Condon et al. 2007). The heritability of stomatal conductance is reasonably high, with reported values typically in the range of 0.5 to 0.8 (Rebetzke 2003). In a way, we can select individual plants of segregating generations for cooler canopies in later generation. Considering the importance of SC and its relation with yield under heat stress, the objective of the present study was to evaluate wheat genotypes for stomatal conductance at different temperature under Indian field conditions and to assess the relationship between SC and canopy temperature. This study will help in identifying genotypes with high stomatal conductance and cooler canopies.

Materials and methods

Experimental material and site

The experimental material for the present study comprised of 36 bread wheat genotypes, of which ten genotypes taken from international core set for abiotic stress developed by International Maize and Wheat Improvement Centre (CIMMYT), Mexico and 26 elite Indian genotypes released for different agro-ecological conditions except FLW18 (Singh et al., 2011). The experiments were conducted at experimental farm, ICAR-Indian Agricultural Research Institute, New Delhi, India. The latitude of the research farm is 280 38’23"N, longitude 770 09’27"E and altitude is 228.61 m above mean sea level. The experiment was laid out in a 6×6 simple lattice design with two replications and two dates of sowing with the help of Weintersteiger seed drill. The crop was sown on 15th November (Timely sowing-TS) and 6th January (late sowing-LS) to create temperature effect during growth stages. Standard cultivation practices prescribed for wheat under irrigated conditions were precisely followed.

Stomatal conductance measurement

Stomatal conductance was measured using steady state SC-1 leaf porometer (Decagon Devices, Inc. USA) version 9, 2005. The SC-1 leaf porometer is a battery-operated menu-driven device that measures stomatal conductance of leaves in mmol m^{-2} s^{-1}. Three reproductive stages *viz.,* late boot (Z 49-50), early milk (Z 73) late milk stage (Z 77) were considered for recording SC from the flag leaf. Measurements during heading and anthesis were avoided to overcome the confounding effects of phenology. Three randomly chosen flag leaves from three plants per plot were labelled. Utmost care was taken to select leaves, which were fully exposed to sunlight and border leaves were avoided. The sensor head was calibrated in the experimental plot before taking the measurement as per the company’s protocol. The sensor head was placed on the leaf surface for 30 seconds to record conductance. Measurements were made from upper (adaxial) surface at the middle portion of the flag leaf. Recordings were done for three consecutive days in each stage to add additional replication and to avoid the variation due to differences in genotypes. Infrared thermometer was used to record canopy temperature. All the measurements were made between 11.00 to 14:00 hours in the absence of clouds.

Phenology and grain yield

The number of days to anthesis and maturity were determined from 50% seedling emergence to when 50% of the spikes had reached anthesis and when 50% of the spikes showed complete absence of green respectively. The data for grain yield per plant were recorded as the average of five plants selected randomly including the plants from which SC was measured. Relative per cent change in SC and yield due to late sowing were worked out as change in genotypic mean under late over timely sowing and expressed as per cent of mean of timely sowing. Heat susceptibility index (HSI) of individual genotype was worked out using Fischer and Maurer (1978) formula: HSI= [1-(Y/Yp)]/ [1-(X/Xp)].

Statistical analysis

There was unbalance associated with the different number of observations used to estimate and compare each genotype. This analysis was carried using SAS mixed model procedure with sampling date and genotypes as fixed, and blocks as random effects for each year and sowing dates and pooled analysis was carried to estimate the interaction effects. The mean stomatal conductance under two sowing dates were tested for contrast between the growth stages. Least squares analysis was used to study relation between leaf conductance and grain yield per plant. Statistical analysis was performed with SAS version 9.2 (SAS, 2009, SAS institute Inc., Cary, NC, USA). Student’s *t* test was performed to compare the temperature during measurements in two sowing schedules.

Results and discussion

Stomatal conductance was measured on 36 genotypes during late boot, early milk and late milk stages for two crop years on adaxial leaf surface. Preliminary measurement of SC on abaxial and adaxial surface was done. Adaxial surface contributed 60 to 67 % to total leaf conductance under both the sowing dates, confirming the results of Clarke and Clarke 1996. Therefore SC was recorded on adaxial surface only to save the time. Dates of recording of stomatal conductance during 2013 and 2014 are given in Table 1. The atmospheric temperatures *viz*., maximum, minimum and mean for timely sown and late sown varied significantly during all three growth stages in both the years. Recording data on three leaves per plot for three consecutive days, added extra replication reducing phenological effect of the genotypes on measurements as suggested by Rebetzke (2001). Adjusted mean over three days was used for further analysis. Analysis of variance showed significant variation across the genotypes for SC recorded. Genotypic responses were significantly different in both the sowing dates during 2012-13 and 2013-14 (Table 2). Similarly, there was significant variation in SC across three stages and the interaction with genotypes also resulted in significant differences.

When ANOVA was done to partition the variation due to year and its interaction effect along with two sowing dates and 36 genotypes (Table 3), the variation due to year and its interaction with genotypes was non significant (P>0.05). This implies that weather parameters during two seasons of observation did differ to a greater extent and reduced heterogeneity in SC and other traits. The data on SC was pooled for two season and genotype wise mean SC was estimated for further analysis. Prior to pooling the data was tested for homogeneity of variances.

Mean stomatal conductance of genotypes were averaged over two years for three growth stages along with genotypic ranks are given in Tables 4 and 5 for timely sown and late sown conditions respectively. Genotype, KAUZ/AA//KAUZ ranked 1st at late boot and late milk stages under timely sowing and late boot and early milk under late sowing. Lowest SC was recorded for BAV 92/SERI-1 at late boot and late milk and NW 2036 at early milk under timely sown conditions. Similarly, under late sown condition DBW 14 recorded lowest SC at late boot and early milk and WH 147 at late boot stage (Tables 4 and 5). Staggered sowing on two different dates, 15th November and 6th January resulted in imposing different atmospheric temperatures on crop during the time of measuring SC. Recording SC at different growth stages gave better understanding of plant behaviour and genotypic differences due to varying atmospheric temperature and phenology. Rebetzke et al. (2003) advised the preliminary studies to measure leaf conductance at different growth stages of the crop cycle to ascertain best expressed differences between genotypes. In general, stomatal conductance is more in plants during their peak vegetative stage as observed in late boot stage and decreases along with maturity due to senescence of leaves. In our studies also, genotypes had higher SC in late boot stage in both the sowing condition and later decreased over early milk and late milk stage as leaves proceed towards senescence. Nevertheless, there were genotypic differential responses. Under timely sown condition, the contrast between boot and early milk stage was positive and significant for most of the genotypes; however, six and two genotypes have negative, significant and negative non significant contrast respectively. The contrast between early milk and late milk stages was negative for 16 genotypes in which three among them were non significantly contrast. It was observed that none of the genotypes expressed stable stomatal conductance across the growth stages and there was variation in terms of growth stages. This variation is very clear to understand the differences in genotypic response for stomatal conductance to increasing temperature. As the daily maximum temperature increases from late boot to early milk and late milk due to seasonal changes (Table 1), the genotypes recorded higher SC to maintain transpiration cooling. The genotypic differential response is mainly due to their variation in genetic makeup.

Under late sown condition, the contrast between late boot and early milk stages was negative for nine genotypes where, two were non-significant. The positive contrast was high for most of the genotypes, but unexpectedly genotype GW273 exhibited significant negative contrast. Since the genotype is adopted for timely sowing under irrigated condition (Singh et al., 2011), elevated SC during later stages is observed responding to increased temperature due to late sowing during early milk stage. The contrast between early milk and late milk stage was positive and highly significant for all the genotypes. Though four genotypes maintained stable and low SC from late boot to early milk stage, but this stability did not proceed to next stage. SC recording from timely and

Table 1. Temperature (^{o}C) and Relative humidity (percentage) during recording of stomatal conductance

Sowing season	Growth stgaes	Date	Tmax°C	Tmin^{o}C	Tmean^{o}C	RH(M)%	RH(E)%	RHmean%
Timely sown 2012-13	Late Boot	09-02-2013	20.2	6.8	13.5	87	42	64.5
		10-02-2013	21.8	4.7	13.25	83	41	62.0
		11-02-2013	22.9	5.5	14.2	94	44	69.0
	Early milk	09-03-2013	30.5	10.7	20.6	95	26	60.5
		10-03-2013	32.6	15.1	23.85	64	30	47.0
		11-03-2013	30.8	13.4	22.1	96	40	68.0
	Late milk	19-03-2013	29.7	16.4	23.05	90	43	66.5
		20-03-2013	31	14.6	22.8	96	18	57.0
		21-03-2013	34.6	15.3	24.95	90	28	59.0
Late sown 2012-13	Late Boot	13-03-2013	30.5	12.4	21.5	93	36	65.0
		14-03-2013	32.5	16.3	24.4	88	43	66.0
		15-03-2013	28.0	10.3	19.2	90	25	58.0
	Early milk	29-03-2013	32.4	16.6	24.5	82	75	78.5
		30-03-2013	28.3	13.6	20.95	94	47	70.5
		31-03-2013	29.6	15.2	22.4	94	42	68.0
	Late milk	08-04-2013	37.2	17.6	27.4	60	34	47.0
		09-04-2013	37.0	19.4	28.2	71	29	50.0
		10-04-2013	38.0	22.0	30.0	63	41	52.0
Timely sown 2013-14	Late Boot	10-02-2014	19.0	2.3	10.7	97	51	74.0
		11-02-2014	19.0	3.0	11.0	97	46	71.5
		12-02-2014	21.0	3.0	12.0	97	56	76.5
	Early milk	07-03-2014	24.5	7.7	16.1	98	50	74.0
		08-03-2014	24.6	10.6	17.6	95	54	74.5
		09-03-2014	25.7	10.7	18.2	98	50	74.0
	Late milk	19-03-2014	29.0	14.7	21.9	92	37	64.5
		20-03-2014	27.5	11.8	19.7	85	41	63.0
		21-03-2014	28.0	11.4	19.7	95	35	65.0
Late sown 2013-14	Late Boot	13-03-2014	26.2	11.4	18.8	89	48	68.5
		14-03-2014	25.0	10.0	17.5	89	48	68.5
		15-03-2014	26.0	9.4	17.7	95	45	70.0
	Early milk	30-03-2014	31.5	15.8	23.7	78	28	53.0
		31-03-2014	30.5	14.5	22.5	72	29	50.5
		01-04-2014	31.0	14.5	22.8	77	31	54.0
	Late milk	08-04-2014	31.6	18.4	25.0	78	34	56.0
		09-04-2014	31.7	16.7	24.2	78	42	60.0
		10-04-2014	33.0	16.0	24.5	61	39	50.0

Tmax = Maximum temperature, Tmin = Minimum temperature, Tmean = Mean temperature, RH(M) = Relative humidity at morning, RH(E) = Relative humidity at evening

Table 2. Mean sum of squares for stomatal conductance for three growth stages under timely sown (TS) and late sown (LS) conditions during 2013 and 2014

Source	d f	TS 2013	TS 2014	LS 2013	LS 2014
Genotype	35	77173.22**	64435.67**	79605.17**	58301.78**
Stage	2	471390.90**	2327571.00**	1106505.00**	1877300.00**
Genotype x Stage	70	30216.22**	17081.85**	30930.24**	14587.29**
Replication (Stage)	3	2469.25ns	6382.39ns	1919.47ns	4752.76ns
Error	105	360.481	319.43	347.19	325.32
CV		2.97	5.17	2.93	5.48

Table 3. Mean sum of squares for stomatal conductance at late boot (SC-LB), early milk (SC-EM), late milk (SC-LM), canopy temperature at late boot (CT-LB), early milk (CT-EM), late milk (CT-LM), grain yield (GY), Kernel weight per spike (KWPS) and kernel number per spike (KNPS) across the genotypes, years and sowing conditions

Source	df	SC-LB	SC-EM	SC-LM	CT-LB	CT-EM	CT-LM	GY	KWPS	KNPS
Year	1	77.66ns	309.72ns	931.67ns	3.83ns	29.85ns	58.61ns	5415.22*	1059.75*	0.241ns
Sowing	1	5485950.9**	2980764.3**	12591077.2 **	2527.7**	1146.0**	2855.2**	9795539.6**	18894.4**	21.24**
Year x sowing date	1	155491.3**	22330.1**	6734.1**	419.8**	0.1ns	141.3*	3113.2**	26.9**	0.019**
Year x sowing date x replication	4	438.5ns	1114.1*	16936.8**	1.1ns	1.9*	1.1ns	2198.2**	1.2ns	0.0001ns
Genotype	35	152996.3**	55550.9**	47278.4**	1.8*	2.5**	2.0**	20619.3**	157.1**	0.083**
Genotype x sowing date	35	118401.8**	40717.2**	49702.3**	2.1**	2.0**	2.4**	22204.7**	134.2**	0.086**
Genotype x year	35	175.3ns	30.2	12.5	1.0*	1.3*	1.1*	1915.0**	2.3*	0.001ns
Genotype x year x sowing date	35	241.2ns	27.8	13.1	1.5*	1.3*	1.8*	2423.6**	1.6*	0.001ns
Error	140	191.2	270.6	552.5	0.3	0.4	0.6	1000.5	5.1	0.002
CV		2.2	3.4	7.0	2.4	2.3	2.5	11.4	5.6	3.47

late sown crops clearly shows the response of genotypes to increase in atmospheric temperature; however the relation between air temperature and SC is not linear (Aphalo 1991). Therefore, during late sowing condition, SC was low. It has been reported that heat stress is associated with premature leaf senescence in wheat (Harding et al. 1990; Reynolds et al. 1994). Chakrabarti et al. (2013) observed decline in SC when wheat and chickpea were raised inside temperature gradient tunnel. In late sowing condition, low SC was recorded while the temperature was more than 30^{o}C during all the three stages of recording. SC recorded high in an optimum temperature of about 23 to 25^{o}C and declines when temperature is 28 to 35^{o}C (Blum 1986). Genotypes showing high SC are due to their inherent capacity for adopting to heat stress through evoporational cooling (Radin et al. 1994). Plants with higher SC promote evaporative cooling and thereby reduce thermal stress (Reynolds 2001). In late sown condition the temperature is high in boot stage itself and further increases in later stages, the genotypes have to maintain an optimum range of stomatal conductance to maintain transpirational cooling without much transpirational losses. Therefore SC was low among all the genotypes in all the growth stages under late sown condition when compared to timely sown condition. The closure of stomata may increase leaf temperature depending mainly on the radiation load on the canopy but will result in a better water economy or increased transpiration efficiency (Condon et al. 2004). Heat stress tolerance adaption mechanisms like leaf rolling, leaf drooping, which was observed in late sown crop also effected SC. Nevertheless under heat stress condition, early senescence is observed resulting in drop off in SC. Genotypes which can tolerate heat exhibit late senescence and therefore maintain optimal stomatal conductance, so that photosynthetic activity is continued for grain filling. Under heat stress condition, the genotypes such as KAUZ/AA//KAUZ, FLW 18, BERKUT, RAC 875, HD 2864 and RAJ 3765 maintained high and optimum SC over the growth stages. High stomatal conductance permits leaf cooling through evapotranspiration; this along with higher leaf chlorophyll content and stay green are associated with heat tolerance (Reynolds et al. 1994). SC is a direct function of evap-transpiration rate and cool canopy which is determined by a number of physiological and metabolic processes such as photosynthetic rate, vascular capacity, etc., High SC under heat stress may be indicative of a high demand for photo-assimilation caused by many, rapidly filling

Table 4. Mean stomatal conductance (mmol^{-2}m^{-2}s^{-1}) under timely sown averaged over two crop years at late boot (LB), early milk (EM) and late milk (LM) stages and also contrast between late boot and early milk and early milk and late milk

GENOTYPES	LB	EM	LM	RANK-LB	RANK-EM	RANK-LM	LB-EM	EM-LM
KAUZ/AA//KAUZ	1108.1	613.9	831.4	1	16	1	494.2**	–217.5**
SOKOLL-1	1076.9	685.3	768.7	2	7	3	391.6**	–83.4**
HALNA	1051.1	471.1	770.3	3	30	2	579.9**	–299.2**
GW 366	1030.6	517.7	726.1	4	26	7	512.8**	–208.3**
NI 5439	1026.9	412.7	727.3	5	35	6	614.2**	–314.6**
WL 711	982.2	596.4	731.0	6	17	5	385.8**	–134.6**
HD 2967	955.7	847.6	684.9	7	2	9	108.2**	162.6**
NW 2036	951.6	400	735.6	8	36	4	551.6**	–335.6**
MP 4010	940.7	688.8	705.8	9	6	8	251.9**	–17.0ns
HI 1563	870.8	619.2	625.0	10	15	12	251.6**	–5.8ns
EXCALIBUR	853.5	501.7	662.1	11	28	10	351.8**	–160.4**
BERKUT	853.2	455.9	626.8	12	32	11	397.3**	–170.8**
GW 273	821.5	527	611.9	13	25	14	294.4**	–84.8**
HW 2004	819.7	539.1	580.4	14	23	17	280.6**	–41.0*
PASTOR	796.7	676.2	617.5	15	9	13	120.5**	58.7**
GW 322	795	583	598.7	16	19	15	211.9**	–15.7ns
DBW 17	777.8	679.4	594.6	17	8	16	98.4**	84.8**
HD 2987	741.7	897.5	568.7	18	1	18	–155.9**	328.8**
WR 544	740.4	577.1	551.4	19	20	19	163.2**	25.8*
HD 2932	713.1	623.3	501.8	20	14	24	89.8**	121.5**
FLW 18	700.2	722.3	526.3	21	5	22	–22.0ns	195.9**
WH 730	699.8	437.3	548.6	22	33	20	262.5**	–111.3**
HI 1544	680.6	768.5	537.3	23	3	21	–87.9**	231.2**
RAJ 3765	671.2	586	506.1	24	18	23	85.2**	79.9**
HD 2781	650	557	492.3	25	21	25	93.0**	64.7**
WH 147	600.9	527.8	456.8	26	24	28	73.1**	71.0**
BABAX	588.2	476.8	443.9	27	29	29	111.4**	32.9*
PBW 343	584.9	426.5	475.1	28	34	26	158.3**	-48.6*
TEPOCA/RABE	583.3	553.9	463.5	29	22	27	29.4ns	90.5**
CHIRYA 7	581	514.3	418.9	30	27	31	66.7**	95.4**
PBW 550	565.2	637.2	443.2	31	12	30	–71.9**	194.0**
HI 617	526.4	638.2	407.4	32	11	32	–111.8**	230.8**
HD 2864	505.8	664.8	393.4	33	10	33	–159.1**	271.4**
RAC 875	493.6	728.2	391.7	34	4	34	–234.6**	336.6**
DBW 14	443.4	457.8	346.5	35	31	35	–14.4ns	111.3**
BAV 92/SERI-1	349.4	629.6	297.7	36	13	36	–280.2**	331.9**
MEAN	753.6	590	565.8					
SE(d)	5.8	10.8	19.7					
LSD at 5%	11.6	21.5	39.4					

** = contrast significant at 0.01%, * = contrast significant at 0.05%, ns = non significant

Table 5. Rank wise performance of mean stomatal conductance (mmol^{-2} m^{-2}s^{-1}) averaged over -two crop years under late sown at late boot (LB), early milk (EM) and late milk (LM) stages and also contrast between late boot and early milk and early milk and late milk stages

GENOTYPES	LB	EM	LM	RANK-LB	RANK-EM	RANK-LM	LB-EM	EM-LM
KAUZ/AA//KAUZ	975.02	744.01	283.81	1	1	3	231.0**	460.2**
FLW 18	892.61	522.57	314.83	2	4	1	370.0**	207.7**
HD 2932	799.42	436.12	84.94	3	8	27	363.3**	351.2**
BERKUT	777.04	562.07	197.88	4	2	11	215.0**	364.2**
MP 4010	697.14	293.84	78.09	5	33	30	403.3**	215.7**
SOKOLL	660.39	356.13	231.68	6	20	7	304.2**	124.4**
RAC 875	601.43	520.81	239.43	7	5	6	80.6**	281.4**
HD 2864	598.01	511.03	183.88	8	6	13	87.0**	327.1**
CHIRYA 7	569.92	396.34	170.87	9	12	15	173.6**	225.5**
HI 1544	526.36	433.01	278.54	10	9	4	93.4**	154.5**
HD 2781	508.34	411.23	182.19	11	11	14	97.1**	229.0**
HI 617	483.15	349.34	143.06	12	22	17	133.8**	206.3**
EXCALIBUR	478.65	360.44	64.41	13	18	33	118.2**	296.0**
RAJ 3765	474.36	354.35	311.61	14	21	2	120.0**	42.7*
GW 366	470.45	365.89	92.93	15	17	19	104.6**	273.0**
WH 730	442.10	368.13	202.91	16	16	10	74.0**	165.2**
BAV 92/SERI	430.71	413.80	124.07	17	10	18	17.0ns	289.7**
TEPOCA/RABE	419.71	357.37	210.76	18	19	9	62.3**	146.6**
HALNA	418.55	262.80	85.12	19	35	26	155.8**	177.7**
BABAX	417.55	334.45	86.15	20	24	25	83.1**	248.3**
PBW 343	415.30	456.60	154.73	21	7	16	–41.3**	301.9**
NI 5439	391.25	332.12	63.88	22	25	34	59.1**	268.2**
HD 2987	382.75	395.25	59.62	23	13	35	–12.5ns	335.6**
GW 322	381.61	321.43	90.34	24	27	22	60.2**	231.1**
DBW 17	372.14	306.54	229.53	25	30	8	65.6**	77.0**
HW 2004	370.88	315.61	190.32	26	28	12	55.2**	125.3**
WH 147	369.56	295.33	36.25	27	32	36	74.2**	259.0**
GW 273	346.75	549.29	89.85	28	3	23	–202.5**	459.4**
NW 2036	339.45	305.88	247.10	29	31	5	33.5*	58.8**
PASTOR	339.30	325.93	79.96	30	26	29	13.4ns	246.0**
PBW 550	336.51	383.42	91.56	31	14	21	–46.9*	292.0**
WR 544	332.72	276.84	87.10	32	34	24	55.9**	189.7**
HD 2967	313.41	346.88	82.72	33	23	28	–33.5*	264.1**
HI 1563	301.60	378.23	92.02	34	15	20	–76.6**	286.2**
WL 711	289.50	308.87	74.50	35	29	32	–19.4ns	234.4**
DBW 14	270.38	262.50	77.42	36	36	31	7.9ns	185.1**
MEAN	477.61	386.51	147.61					
SE(d)	12.67	12.16	12.16					
LSD at 5%	25.27	24.27	24.27					

** = contrast significant at 0.01%, * = contrast significant at 0.05%, ns = non significant kernels (i.e. sink strength) in physiologically well-adapted genotypes. Alternatively high SC reflects an intrinsically higher metabolic capacity; cooler canopy is indicative of a good vascular system capable of meeting evaporative demand.

Grain yield per plant, kernel number per spike (KNPS) and kernel weight per spike (KWPS) was significantly affected due to staggered sowing across the genotypes (Table 2). The genotypes were grouped into three categories based on the heat susceptibility index for grain yield (HSI): heat tolerant (HT HSI $\leq$ 0.75), moderately heat tolerant (MT HSI > 0.75- < 1.10) and heat susceptible (HS HSI > 1.10). Among 36 genotypes, 8 were heat tolerant, 14 medium heat tolerant (MT) and the remaining 14 were heat susceptible (HS), respectively. Genotypic response to heat stress was presented as relative percent change in SC under late sown condition over timely sown condition (Table 6). Heat tolerant genotypes which maintained relatively higher SC under stress condition have lower percent change over normal condition. Heat susceptible genotypes recorded low SC under stress and therefore relative percent change over normal condition was high. Response curve for relative percent change in SC drawn over percent change in KWPS due to heat stress shows significant relationship (Fig. 1).

Table 6. Relative percent change in stomatal conductance in late sown condition over timely sown condition at three growth stages and heat susceptibility index in genotypes

Genotypes	Late boot	Early milk	Late milk	HSI	Category
CHIRYA 7	1.91	22.94	59.21	0.27	HT
BAV 92/SERI	–23.27	34.28	58.32	0.41	HT
KAUZ/AA//KAUZ	12.01	-21.20	65.86	0.43	HT
WH 730	36.83	15.81	63.01	0.61	HT
FLW 18	–27.47	27.65	40.18	0.62	HT
RAC 875	–21.84	28.48	38.87	0.68	HT
HI 617	8.21	45.26	64.88	0.71	HT
RAJ 3765	29.33	39.53	38.43	0.75	HT
HI 1544	22.67	43.66	48.16	0.80	MHT
HD 2932	–12.11	30.03	83.07	0.85	MHT
DBW 14	39.03	42.66	77.66	0.89	MHT
WR 544	55.06	52.03	84.20	0.94	MHT
BERKUT	8.93	-23.28	68.43	0.96	MHT
NI 5439	61.90	19.52	91.22	0.99	MHT
DBW 17	52.15	54.88	61.40	1.01	MHT
WH 147	38.50	44.05	92.07	1.02	MHT
GW 273	54.35	29.33	87.20	1.02	MHT
TEPOCA/RABE	52.00	44.87	84.91	1.03	MHT
PBW 550	40.47	39.83	79.34	1.06	MHT
PBW 343	–18.24	23.13	53.26	1.07	MHT
HW 2004	54.75	41.46	67.21	1.07	MHT
SOKOLL	38.68	48.03	69.86	1.07	MHT
GW 322	28.05	35.49	54.53	1.10	HS
HD 2864	28.99	-7.05	67.43	1.11	HS
MP 4010	25.89	57.34	88.94	1.11	HS
WL 711	70.53	48.21	89.81	1.17	HS
GW 366	57.79	-4.22	85.32	1.18	HS
HD 2987	48.39	55.96	89.52	1.21	HS
HI 1563	65.37	38.92	85.28	1.25	HS
HD 2781	21.79	26.16	62.99	1.25	HS
EXCALIBUR	43.92	28.16	90.27	1.26	HS
PASTOR	57.41	51.80	87.05	1.27	HS
BABAX	29.02	29.86	80.59	1.30	HS
NW 2036	64.33	23.53	66.41	1.37	HS
HD 2967	67.21	59.07	87.92	1.42	HS
HALNA	60.18	44.22	88.95	1.54	HS
Mean	32.57	32.51	72.27		

HT = heat tolerant; MHT = medium heat tolerant; HS = heat susceptible

Association of Stomatal Conductance with canopy temperature (CT) and grain yield related traits

Canopy temperature is highly suitable for selecting physiologically superior genotypes in warm, low relative humidity environments, where high evaporative demand leads to leaf cooling of up to 10°C below ambient temperatures. This permits differences among genotypes to be detected relatively easily using infrared thermometry. However, such differences cannot be detected in high relative humidity environments because the effect of evaporative cooling of leaves is negligible. Nonetheless, leaves maintain their stomata open to permit the uptake of CO_2 and differences in the rate of CO_2 fixation may lead to differences in leaf

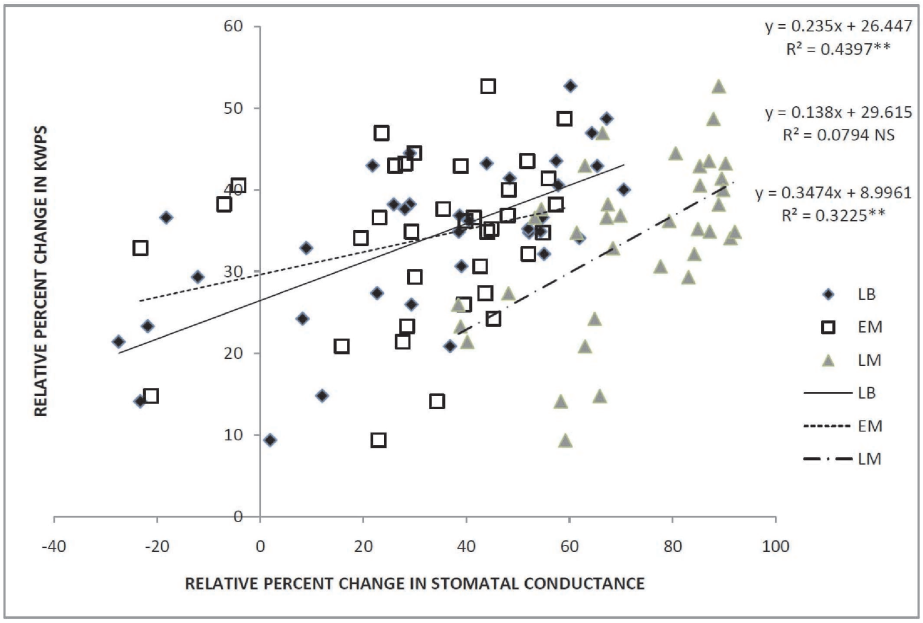

Fig. 1. Relationship between relative percent change in stomatal conductance and kernel weight per spike (KWPS) at late boot (LB), early milk (EM) and late milk (LM) stages

conductance that can be measured using a porometer (Reynolds et al. 2001). Unlike canopy temperature, stomatal conductance is measured on individual plant and therefore increases the precision and helps in selection of plants with high or low conductance thereby improving the genotypic effect for CT. Finally, genotypes with higher SC will have cooler canopies and are suitable for late and very late sowing under irrigation, terminal heat stress in Indian wheat cropping pattern. The handheld porometer provides rapid measurement of leaf stomatal conductance under irrigated conditions (Rebetzke et al. 2000). Pearson's correlation coefficients between SC under timely sown and CT significantly correlated during late boot (r = –0.64**), early milk (r = –0.63**) and late milk (r = –0.64**) stage. Correlations were significant for KWPS (r = 0.44**, 0.46**) and HSI (r = 0.37*, 0.40**) at late boot CT and late milk stage. Under late sowing condition correlations between SC at all growth stages were significantly correlated with CT, KNPS, KWPS and HSI (Table 7). Stomatal conductance and grain yield was significantly correlated (r = 0.93; p<0.01) in

Table 7. Correlation between stomatal conductance (SC) at late boot (LB), early milk (EM) and late milk (LM) with kernel number per spike (KNPS), kernel weight per spike (KWPS), heat susceptibility index (HSI), grain yield (GY) and canopy temperature at late boot (LB), early milk (EM) and late milk (LM) stages

Traits	Timely sown			Late sown		
	SC-LB	SC-EM	SC-LM	SC-LB	SC-EM	SC-LM
KNPS	0.21	0.13	0.22	0.34*	0.36*	0.11
KWPS	0.44**	0.20	0.46**	0.47**	0.43**	0.35*
HSI	0.37*	–0.01	0.40*	–0.43**	–0.37*	–0.40*
GY	0.26*	0.17	0.28*	0.44**	0.49**	0.39**
CT-LB	–0.64**			–0.81		
CT-EM		–0.63**			–0.83**	
CT-LM			–0.64**			–0.82**

** = significant at 0.01%, * = significant at 0.05%

an historical series of bread wheat measured at Obregon, Mexico for three years (Rees et al. 1993). Similarly, Fischer et al. (1998) reported high correlation between SC and grain yield when crop temperature was between 22-35^{o}C (r = 0.94; p<0.01). CT and SC was significantly associated (R^2 = 0.40 to 0.64). Plants with higher SC promote evaporative cooling and thereby reduce thermal stress (Reynolds, 2001). Reynolds et al. (1994) obtained significant positive correlations between grain yield and SC measured at pre anthesis and post anthesis under heat stress. Amani et al. (1996) reported positive correlation between SC and canopy temperature depression; high and were significant correlation (r = 0.84) between grain yield and canopy temperature depression.

Phenological stages such as days to anthesis and days to maturity were shortened due to staggered sowing. Days to anthesis were 95.5±0.53 and 63±0.27 in timely and late sowing conditions respectively. Average days for maturity in timely sown was 132.1±0.95 and 86.36±0.4 in late sown condition. There was significant difference in atmospheric temperature and relative humidity during anthesis between timely sown and late sown condition (Student's t = –6.29, P< 0.001; t = 3.54, P < 0.001). To assess whether phenological stages had confounded effect on SC, data of early milk and late milk was analysed using as a covariate, days for anthesis and maturity. The covariate was not significant (P > 0.32-0.73) in any stage of development indicating that a value of SC was not systematically associated with anthesis. Reynolds et al. (1994) also reported decrease in SC along the growth stages when measured at booting, anthesis, post anthesis and grain filling in control and heat stress environments. In the present study a significant variation was found in the growth rate among the genotypes, but the effect of developmental stages on stomatal conductance was not significant at any of the phenological stages studied. This indicates that the genotypic differences in stomatal conductance for response to heat stress were not caused by differences in growth rate or phenology (Reynolds et al. 1994, Araus et al. 2002).

The study demonstrates stomatal conductance as an important physiological trait to select the best plants among best bulks of cooler canopy related to heat tolerance in crops. The handheld porometer provides rapid measurement of leaf stomatal conductance in irrigated condition. A higher value of SC under heat and irrigated condition is associated with cooler canopies and with higher yield. Therefore, genotypes with higher SC could serve as a source for future crop improvement programme for heat tolerance. Genotypes KAUZ/AA//KAUZ, SOKOOL, TEPOCA/RABE and RAC 875 had higher SC. Indian genotypes HD 2932, HD 2987, FLW-18, HW 2004 and RAJ 3765 were equally potential. These genotypes can be utilized for breeding to introgress heat tolerance related traits (high SC) and to improve yield under warm climate condition.

Authors' contribution

Conceptualization of research (KTR, GPS, KVP); Designing of the experiments (KTR, GPS, AA, KVP); Contribution of experimental materials (GPS, KPS); Execution of field/lab experiments and data collection (KTR, AA, NJ, BA); Analysis of data and interpretation (KTR, AA, NJ, GPA); Preparation of the manuscript (KTR, AA, PKS, GPS, KVP).

Declaration

The authors declare no conflict of interest.

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References

Amani I., Fischer R. A. and Reynolds M. P. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in hot climate. J. Agron. Crop Sci., **176**: 119-129.

Aphalo P. J. and Jarvis P. G. 1999. Do stomata respond to relative humidity? Plant Cell Env., **14**: 127-132.

Araus J. L., Slafer G. A., Reynolds M. P. and Royo C. 2002. Plant breeding and water relations in C_3 cereals: what should we breed for? Ann. Bot., **89**: 925-940.

Blum A. 1986. The effect of heat stresses on wheat leaf and ear photosynthesis. J. Exp. Bot., **37**: 111-118.

Chakrabarti B., Singh S. D., Kumar V., Harit R. C. and Misra S. 2013. Growth and yield response of wheat and chickpea crops under high temperature. Indian J. Plant Physiol., **18**: 7-14.

Clarke J. M. and Clarke F. R. 1996. Considerations in design and analysis of experiments to measure stomatal conductance of wheat. Crop Sci., 36: 1401-1405.

Condon A. G., Reynolds M. P., Rebetzke G. J., van Ginkel M., Richards R. A. and Farquhar G. D. 2007. Using stomatal aperture traits to select for high yield potential in bread wheat. In: Buck, H.T., Nisi, J.E., Salomo´n, N. (eds) Wheat production in stressed environments. Proc. 7th Inter. Wheat Conf., Mar del Plata, Argentina. Springer, Netherlands, 617-624.

Condon A. G., Richards R. A., Rebetzke G. J. and Farquhar G. D. 2004. Breeding for high water-use efficiency. J. Exptl. Botany, **55**: 2447-2460.

Fischer R. A. and Maurer, R. 1978. Drought resistance in spring wheat cultivars I. Grain yield responses. Aust. J. Agril. Res., **29**: 897-912.

Fischer R. A., Rees D., Sayre K. D., Lu Z. M., Condon A. G. and Larque Saavedra A. 1998. Wheat yield progress is associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci., **38**: 1467-1475.

Harding S. A., Guikema J. A. and Paulsen G. M. 1990. Photosynthetic decline from high temperature stress during maturation of wheat. II. Interaction with source and sink processes. Plant Physiol., **92**: 654-658.

Hasanuzzaman M., Nahar K., Alam M. M., Roychowdhury R. and Fujita M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Inter. J. Mol. Sci., **14**: 9643-9684.

Joshi A. K., Mishra B., Chatrath R., Ferrara G. O. and Singh R. P. 2007. Wheat improvement in India: Present status, emerging challenges and future prospects. Euphytica, **157**: 431-446.

Morison J. I. L. and Lawlor D. W. 1999. Interactions between increasing CO_2 concentration and temperature on plant growth. Plant Cell Env., **22**: 659-682.

Radin J. W., Lu Z., Percy R. G. and Zeiger E. 1994. Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proc. National Academy Sci. USA, **91**: 7217-7221.

Rebetzke G. J., Condon A. G., Richards R. A. and Farquhar G. D. 2003. Gene action for leaf conductance in three wheat crosses. Aust. J. Agril. Res., **54**: 381-387.

Rebetzke G. J., Condon A. G., Richards R. A. and Read J. J. 2001. Phenotypic variation and sampling for leaf conductance in wheat (*Triticum aestivum* L.) breeding populations. Euphytica, **121**: 335-341.

Rebetzke G. J., Read J. J., Barbour M. M., Condon A. G. and Rawson H. M. 2000. A hand-held porometer for rapid assessment of leaf conductance in wheat. Crop Sci., **40**: 277-280.

Rees D., Sayr K., Acevedo E., Nava-Sanchez T., Lu Z. M., Zeiger E. and Limon A. 1993. Canopy temperatures of wheat: relationship with yield and potential as a technique for early generation selection. Wheat Special Report 70. Mexico. D.F: CIMMYT.

Reynolds M. P., Balota M., Delgado M. I. B., Amani I. and Fischer R. A. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust. J. Plant Physiol., **2**: 717-730.

Reynolds M. P., Nagarajan S., Razzaque M. A. and Ageeb O. A. A. 2001. Heat tolerance. In *Application of Physiology in Wheat Breeding* 129-134 (Eds M. P Reynolds, J. I. Ortiz-Monasterio and A McNab). Mexico. CIMMYT.

SAS Institute Inc. 2009. SAS version 9.2. SAS Institute Inc., Cary, North Carolina, USA.

Singh G., Shoran J., Tyag, B. S. and Singh S. S. 2011. Wheat research and breeding strategies in India. In:The world wheat book, the history of wheat breeding Bonjeon A. P., Angus W. J., van Ginkel M. (eds.), Volume-2, Lavoisier publisher, France, 373-404.

Taiz L. and Zeiger E. 2010. Plant Physiology, Fifth Edition. Sinauer Associates. Sunderland, MA.

Wardlaw I. F. and Moncur L. 1995. The response of wheat to high temperature following anthesis. I The rate and duration of kernel filling. Aust. J. Plant Physiol., **22**: 391-397.