***Pak. J. Bot.*, 49(4): 1279-1284, 2017.**

DIGITAL IMAGE ANALYSIS OF SEED SHAPE INFLUENCED BY HEAT STRESS IN DIVERSE BREAD WHEAT GERMPLASM

MUHAMMAD JAMIL[1*], AAMIR ALI[1], ABDUL GHAFOOR[2], KHALID FAROOQ AKBAR[3], ABDUL AZIZ NAPAR[4], NAIMA HUMA NAVEED[1], NASIM AHMAD YASIN[4], ALVINA GUL[5] AND A. MUJEEB-KAZI[6]

[1]Department of Botany, University of Sargodha, Sargohda, Pakistan
[2]Crop Science Institute (CSI), National Agriculture Research Center (NARC), Islamabad, Pakistan
[3]Department of Botany, University of Lahore (UOL), Sargodha Campus, Sargodha, Pakistan
[4]Horticulture wing, University of the Punjab, Pakistan
[5]Atta-ur-Rehman School of Applied Biosciences (ASAB),
National University of Science and Technology (NUST), Islamabad, Pakistan
[6]Texas A&M University, USA
[*]Corresponding author's email: jshahid80@yahoo.com; cell # (0092-333-6291799)

Abstract

Shape changes in wheat grain under heat stress become necessary to determine when shrinkage causes quality loss. High throughput seed morpho-metery is required to quantify the shape changes precisely. In this study, shrinkage of the seed under heat stress has been quantified thus can be used as the indicator of heat stress. Randomized complete block design was followed in which 49 genotypes were examined in 16 replicates, under two treatments (normal and late sown). SmartGrain software was used to quantify the digital images of seeds picked up from two treatments for each genotype in 16 replicates. Seed shape descriptors (length, width, length width ratio, area, perimeter, circularity) were precisely and robustly determined by the use of this software. ANOVA results showed significant effect of heat stress on seed shape. Critical difference, chi-square value mentioned the disparity in seed shape of normal and stressed wheat crop. Maximum heat susceptibility index for seed area was noted (2.38) and the minimum remained (0.02). Principal component analysis (PCA) revealed the relative importance of seed shape attributes and genotype by explaining about 85.02% (F1 = 59.87 % and F 2 = 25.16 %) variability.

Key words: Wheat, Smart grain, Seed shape, Digital imaging, Heat susceptibility index.

Introduction

To explore computer software-aided seed morphological details is an image analysis technique which can be applied to attain variety of purposes (Varma *et al.,* 2013). Digital image analysis (DIA) is the procedure in which digital image (camera captured) is transformed into digital values (Williams *et al.,* 2013). Previously kernel length (KL) and kernel width (KW) was determined by Vernier calipers (Ramya *et al.,* 2010). Kernel dimensions (KD) along with kernel length (KL), kernel width (KW) and kernel diameter ratio (KDR) were determined with the help of a simple ruler by Cui *et al.* (2011). Without digital assistance, measurement of seed shape attributes becomes less precise and laborious.

Tri dimensional details of wheat seed images can't be obtained without high through put digital imaging (DI). Reduction of size and shape in any dimension can be assessed through DIA. Through this technique changes in seed shape parameters due to heat stress can digitally be measured. Precise determination of grain size and shape was performed by Rasheed *et al.* (2014) by using software package Image J. Braadbaart & Bergen (2005); Breseghello and Sorrells (2007) also used Image J to determine seed shape and size. DI technique can highlight even the minor changes in the seed shape.

Heat stress affects the size and shape of wheat grain. Seed shape descriptors has been quantified and the variation obtained as a result of heat stress was analyzed in the present study. Through a multivariate data analysis, highly tolerant and highly susceptible lines were detected in this study on the basis of heat susceptibility indices of shape attributes. Novelty of this work resides in the time saving and new approach to assess the effect of heat stress on the degree of shrinkage of wheat grain size through such robust digital morphometric technique. Moreover this method to record the extent of seed shrinkage can be applied on the grains of other different species.

Materials and Methods

To determine the effect of heat stress on the shape and size of the wheat seeds, seeds of 49 genotypes were collected. Germplasm was provided by wheat wide crosses (WWC) program at National Agriculture Research Center (NARC), Islamabad. Originally the germplasm was developed by Maize and wheat improvement center (CIMMYT), Mexico under the project – Cereal Systems Initiative for South Asia (CSISA). Seeds of 49 genotypes were examined separately.

Design of experiment and statistical analysis: Randomized complete block design was followed in the study. About 16 replicates were taken as blocks and each block contained completely randomized set of 49 genotypes under two treatments, one timely sown (second week of November) and the other late sown (first week of January). The experiment was planted at the field of NARC, Islamabad. Analysis of variance (ANOVA) was performed by taking genotypes and treatments as sources of variation. Chi-square and Principal Component Analysis (PCA) was also performed to evaluate the inherent variability of the germplasm.

Image quantification: Digital image of the wheat seed was quantified with respect to specific dimensions by the technique known as Digital image analysis (DIA). A software "Smart Grain" was developed by Tanabata *et al.* (2012) to analyze the digital images. This software determines seven parameters: Area (A), Perimeter (P), Length (L), Width (W), Length to width ratio (L/W), Circularity (Cs) and Distance of Length-width intersection point to Centre of gravity (Ds).

Two photographs (one from Normal sown, second from Late sown) of 16 unharmed seeds for each genotype with digital camera (Samsung NX1000 20.3 MP) in horizontal orientation, crease side down on black background grid were taken. The distance between seeds and the camera lens was kept constant (20cm) for all the observations. Background black grid was divided in 16 uniform squares to keep the seed distance constant, so that 16 seeds at the corners of 9 squares were placed to take the image (Fig. 1). Photographs were converted in to JPEG format and further processed with SmartGrain software package (version 1.1).

Heat susceptibility index: Heat susceptibility index (HSI) for each parameter was calculated as reported by Tiwari *et al.* (2013). In the present study HSI of all the shape parameters were determined to assess the actual change in shape and size under heat stress. So the formula for seed area for each genotype is:

$$\mathbf{HSIA = [1\text{-}A_{(heat\ stress)} / A_{(control)}] / D} \text{ and } \mathbf{D = [1\text{-}A_{(mean\ in\ stress)} / A_{(mean\ in\ control)}]}$$

where: HSIA = Heat susceptibility index for Area; A (heat stress) = Area of the seed (mm^2) of late sown genotypes; A (control) = Area of the seed (mm^2) of timely sown genotypes; A (mean in stress) = Average area of the seed (mm^2) of all 49 late sown genotypes; A (mean in control) = Average area of the seed (mm^2) of all 49 timely sown genotypes.

In the same way as written above, HSI for all other parameters was calculated for each genotype.

Statistical analysis: Descriptive statistics was performed by Microsoft Excel 2010. Analysis of variance was done by assuming genotypes, treatment (sowing time) and replicates as sources of variation using software STATISTICA 7. After observing the significant difference of shape parameters in timely and late sown, heat susceptibility index was the unit reduction of specific parameter (i.e. area) due to heat stress. HSI for all the seven shape attributes was calculated and this multivariate data set was subjected to principal component analysis (PCA) with the software XLSTATS 2010.

Results

In the present study about 31.72% reduction in the wheat seed area (Table 1) has been observed due to heat stress. The major reduction in the area was due to the width that was 21.41% reduced. This high reduction in width causes, the length width ratio to be increased about 10.69% due to heat stress.

Although the seed length also reduced 13.84 % (Table 1), even then it shows that the shrinkage of the wheat seed occurred width wise majorly. Minimum seed length was observed 6.14mm in normal sown and 4.71mm in late sown crop. That was in accordance with Rasheed *et al.* (2014) and Masood *et al.* (2016). Chi-square value is the indicator that to what extant the studied attributes have been influenced by the heat stress. Difference between the normal and stressed value of any attribute is zero, the probability of the acceptance of this hypothesis has been denoted by $p \geq \chi^2$ (Table 1).

Analysis of variance (two-way) showed that on the basis of DS, circularity and length width ratio the genotypes differ significantly from one another irrespective to the heat stress. On the other hand area, perimeter, length, width does not differ across the genotypes. But these traits have been significantly affected by the heat stress. Interaction of the genotypes and treatment expressed that the width, length and circularity are meaningful traits. Heat stress does not affect the seed circularity, perimeter and length. Seed area and width are the character with prime importance when change in seed shape changes under heat stress is to be discussed. The non-significance in all the studied character across 16 replicates expresses the precision of the experiment (Table 2).

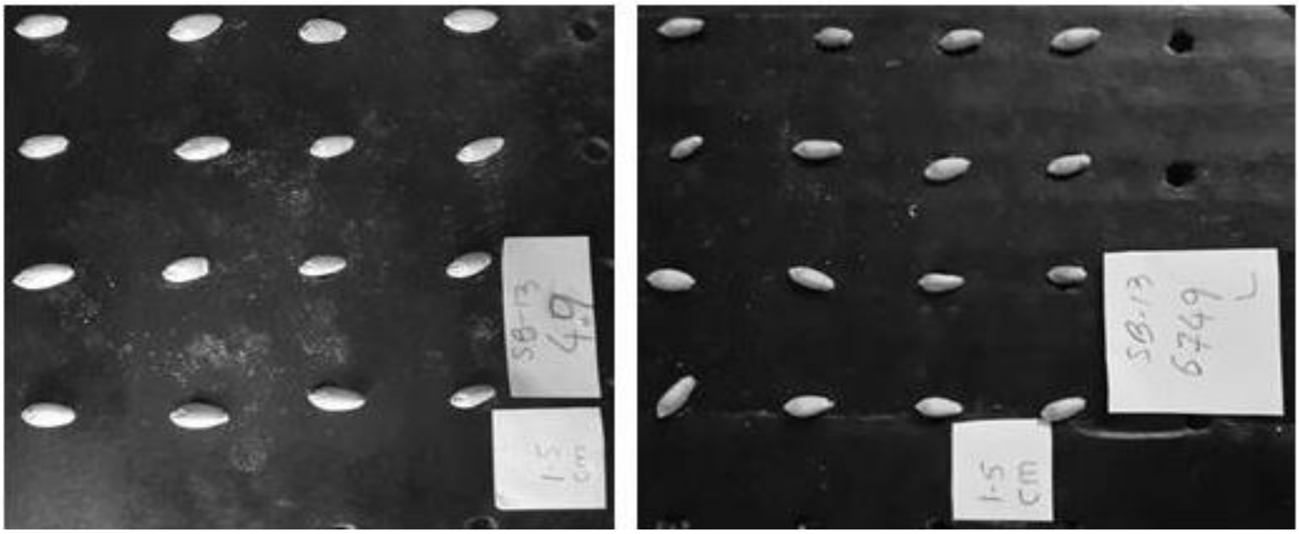

Fig. 1. Wheat seed image of one genotype in Normal (left) and late sown (right) conditions.

Table 1. Descriptive statistics showing difference between the seed shape characters of 49 genotypes under normal and late sown condition.

Description	Sowing	A	P	L	W	LWR	CS	DS
Mean	N	18.86	18.51	6.99	3.54	1.99	0.68	0.65
	L	12.88	15.56	6.02	2.78	2.20	0.66	0.64
% Reduction		31.72	15.90	13.84	21.41	-10.69	3.54	2.22
Critical difference (CD)		2.549	1.312	0.503	0.296	0.180	0.045	0.224
SD	N	5.42	2.10	0.80	0.45	0.13	0.03	0.12
	L	2.92	1.63	0.60	0.37	0.21	0.05	0.13
SE	N	0.135	0.07	0.0268	0.0158	9.63	2.39	0.012
	L	0.132	0.068	0.0262	0.0154	9.4	2.33	0.011
Minimum	N	12.84	15.97	6.14	2.83	1.76	0.60	0.40
	L	6.33	11.84	4.71	1.81	1.92	0.53	0.39
Maximum	N	48.34	28.88	10.82	5.78	2.39	0.75	1.00
	L	20.77	19.31	7.44	3.64	2.70	0.75	1.09
CV %		23.91	11.25	11.27	13.69	12.41	9.63	50.66
χ^2		42.5	0.04	1.51	5.86	133	10.3	3.61
$p \geq \chi^2$		0.000	0.8485	0.219	0.0155	0.000	0.0014	0.0573

Area size (A) [mm^2], Perimeter length (P) [mm], Length (L) [mm], Width (W) [mm], Length to width ratio (LWR), Circularity (CS) Distance (DS) [mm]

Table 2. Mean squares with p-value of F-ratio from Two-way ANOVA for seed shape attributes.

SOV	DF	Area (A)		Perimeter (P)		Length (L)	
		MS	p≥F	MS	p≥F	MS	p≥F
GEN.	48	247.09	0.52	349.27	0.16	52.45	0.13
Treat	1	6634.92	**0.00**	508.26	0.19	32.43	0.38
Reps.	15	253.04	0.94	182.95	0.99	27.48	0.99
GEN.*Treat	48	323.22	0.10	417.83	0.03	59.68	**0.03**
GEN.*Reps.	720	24.53	1.00	18.15	1.00	2.58	1.00
Reps.*Treat	15	18.79	1.00	17.20	1.00	2.68	1.00
		Width (W)		**L W Ratio (LWR)**		**Circularity (CS)**	
		MS	p≥F	MS	p≥F	MS	p≥F
GEN.	48	11.81	0.19	8.28	**0.00**	0.68	**0.01**
Treat	1	66.30	**0.01**	62.81	**0.00**	0.68	0.22
Reps.	15	6.06	1.00	1.92	1.00	0.19	1.00
GEN.*Treat	48	14.62	**0.02**	7.43	**0.00**	0.77	**0.00**
GEN.*Reps.	720	0.67	1.00	0.22	1.00	0.02	1.00
Reps.*Treat	15	0.45	1.00	0.51	1.00	0.01	1.00
		Distance (DS)					
		MS	p≥F				
GEN.	48	0.775	**0.014**				
Treat	1	0.908	0.182				
Reps.	15	0.385	0.975				
GEN.*Treat	48	0.650	0.101				
GEN.*Reps.	720	0.108	1.000				
Reps.*Treat	15	0.119	0.999				

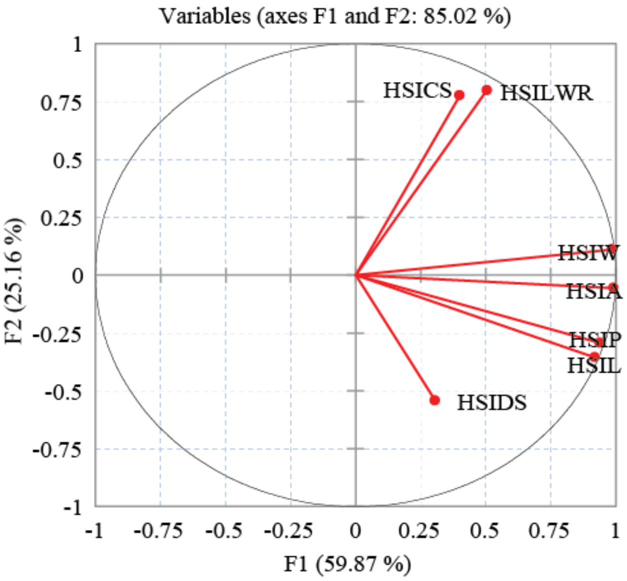

Fig. 2. Correlation circle of Heat susceptibility index (HSI) of seed shape variables loaded on Principal components. F1 (eigenvalue = 4.191) and F2 (eigenvalue = 1.761)

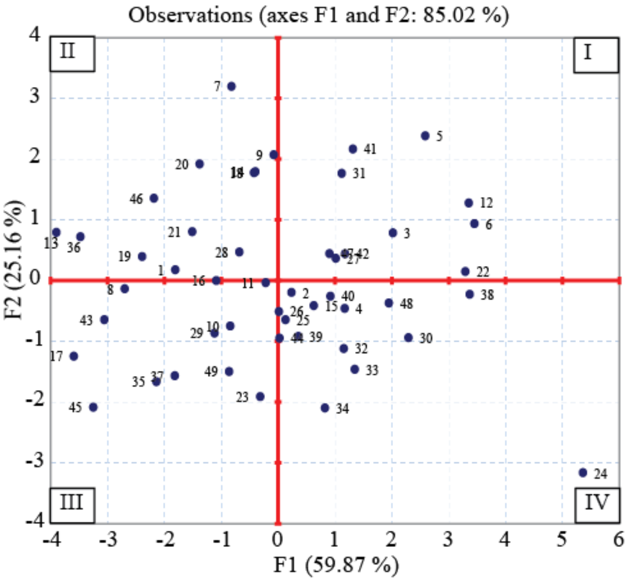

Fig. 3. Distribution of 49 genotypes on the basis of factor score provided to each genotype by principal component analysis.

Multivariate data regarding heat susceptibility index was subjected to Principal Component Analysis (PCA). So the lines with the best PCA score have been declared highly susceptible. First two factors covered 85.02% variation (Table 3). The strength of the relationship between variables and factors has been expressed in correlation circle (Fig. 2). PCA indicates that the most important variables for seed size are area and length. In Fig. 3, genotypes in cluster I and IV are severely affected. Genotypes in cluster II and III are tolerant due to the inverse relation with HSI vectors of all the variables. So the tolerant genotypes must have the lowest PCA score. (Table 3) (Figs. 2 & 3).

Top five genotypes with high heat susceptibility index have been selected as heat susceptible on the basis of grain shrinkage. On the other hand five genotypes with lowest heat susceptibility index have been declared as heat tolerant (Table 4). Heat susceptibility index of all the seed shape attributes was calculated and then the whole variability in this multivariate data set was analyzed with PCA. (Table 4).

Discussion

Besides grain yield reduction due to heat stress, grain size is of prime importance. Dziki & Laskowski (2004) reported that grain size have significant effect on grinding property. Grains of higher size are easy to grind and thus the flour yield can be maximized by increasing the grain size. That is dependent on plant genotype and grain filling duration. Grain filling duration is reduced if plant faces terminal heat stress and the gain shrinks ultimately. Seed area, Length, width, length width ratio and circularity are the key components of the seed size. If these attributes are properly addressed to check the grain size reduction under heat stress then grain size might be the good and precise indicator to assess the heat tolerance of the genotype.

The reduction in width and increase in length width ratio, these are the characters determining precisely the seed shape changes under heat stress. Cervantes *et al.* (2016) reviewed the work done by Balkaya & Odabas (2002), because the L / W is the Eccentricity Index, thus can be the initial parameter for seed shape determination. Seed area and length width ratio were noted as highly influenced characters under heat stress, while seed length and the perimeter are the least effective features.

Grain size and shape remain the most important during the course of evolution of bread wheat domestication, and is necessary for germination and growth of seedling (Eckardt, 2010). Changes in temperature during growth of crop significantly affect plant development and seriously influence the crop production (Willis *et al.*, 2008). Longer exposure to heat stress has detrimental effects on viability of seed (Delouche & Baskin, 1973). Enhanced reactive oxygen species (ROS) production as a result of high temperature can cause cell death (Apel & Hirt, 2004) hence induce seed shape changes. Response of the wheat crop to thermal stress as well as biotic stress (Jamil *et al*., 2016) compels to understand the need of genetic variations (Chopra *et al.*, 2017). Grain shape and size are independently inherited traits (Gegas *et al.*, 2010). Quantification of seed shrinkage and the details of seed dimensions influenced due to heat stress have been identified in the present research work. Besides this, seed size analysis can reveal varietal identification, characterization and germination percentage (Varma *et al.*, 2013). Inter specific and intra specific variability can be analyzed through seed shape changes (Cervantes *et al.*, 2016), an informative set of seed shape descriptors has been highlighted in a robust morphometric technique used in the present study. In this way, heat tolerant cultivars would be identified to address the effect of biotic stresses on food crops (Hasanuzzaman *et al*., 2013).

Table 3. Principal component analysis (PCA) of Heat susceptibility index (HSI) of seed shape determinants.

	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	4.191	1.761	0.801	0.231	0.008	0.005	0.002
Variability (%)	59.868	25.157	11.445	3.307	0.121	0.073	0.030
Cumulative %	59.868	85.025	96.470	99.777	99.897	99.970	100.000
Contribution of the variables (%):							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
HSIA	23.487	0.177	0.566	0.968	64.296	4.660	5.845
HSIP	20.982	4.874	3.977	0.326	4.078	17.244	48.517
HSIL	20.179	7.174	1.321	6.166	28.301	0.802	36.058
HSIW	23.236	0.694	0.935	1.227	0.001	70.893	3.014
HSILWR	6.072	36.224	0.317	45.152	2.398	5.859	3.978
HSICS	3.818	34.204	18.129	39.855	0.917	0.507	2.570
HSIDS	2.225	16.653	74.754	6.306	0.009	0.034	0.018

Heat Susceptibility Index of Area size (HSIA), Perimeter length (HSIP), Length (HSIL), Width (HSIW), Length to width ratio (HSILWR), Circularity (HSICS), Distance (HSIDS)

Table 4. Pedigree details of the most heat tolerant and susceptible genotypes sorted out through Principal Component Analysis (PCA).

	ID #	F1 score	HSIA	Parentage
Heat tolerant	13	-3.897	0.28	ATTILA/3*BCN//BAV92/3/TILHI/4/SUP152/5/SUP152
	17	-3.590	0.18	KACHU/KINDE
	36	-3.475	0.05	FRET2/KUKUNA//FRET2/3/FRNCLN
	45	-3.250	0.02	MUNAL*2//WAXWING*2/TUKURU
	43	-3.055	0.06	KACHU/PVN//KACHU
Heat susceptible	24	5.359	2.38	WAXBILL
	6	3.451	1.80	AGT YOUNG/VORB
	38	3.368	1.76	BAJ #1/FRNCLN
	12	3.354	1.78	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/FRANCOLIN #1/5/MUNAL
	22	3.293	1.89	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/HAR311

Conclusion

By evaluating the seed shape changes under heat stress, someone can guess the reduction in quality of the seed after gauging the degree of shrinkage being introduced in the work done. Grain yield reduction is one of the indicators to heat stress but some other agronomic affairs also involved in such yield reduction; but seed shape changes are under the direct influence of heat stress so can be actually measured as emphasized here. High throughput seed morpho-metery becomes compulsory if the effect of heat stress is to be watched on seed shape that has already been focused in the present effort.

References

Apel, K. and H. Hirt. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 55: 373-399.

Balkaya, A. and M.S. Odabas. 2002. Determination of the seed characteristics in some significant snap bean varieties grown in Samsun, Turkey. *Pak. J. Biol. Sci*., 5: 382-387.

Braadbaart, F. and P.F. van Bergen. 2005. Digital imaging analysis of size and shape of wheat and pea upon heating under anoxic conditions as a function of the temperature. *Veg. Hist. Archaeobot*., 14(1): 67-75.

Breseghello, F. and M.E. Sorrells. 2007. QTL analysis of kernel size and shape in two hexaploid wheat mapping populations. *Field Crop Res*., 101(2): 172-179.

Cervantes, E., J.J. Martín and E. Saadaoui. 2016. Updated Methods for Seed Shape Analysis. *Scientifica*, 2016: 1-10.

Chopra, R., G. Burow, J.J. Burke, N. Gladman and Z. Xin. 2017. Genome-wide association analysis of seedling traits in diverse Sorghum germplasm under thermal stress. *BMC BMC Plant Biol.*, 17(1): 12.

Cui, F.A., A. Ding, J.U.N. Li, C. Zhao, X. Li, D. Feng and H. Wang. 2011. Wheat kernel dimensions: how do they contribute to kernel weight at an individual QTL level? *J. Genet*., 90(3): 409-425.

Delouche, J.C. and C.C. Baskin. 1973. Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Sci Technol*., 1(2): 427-452.

Dziki, D. and J. Laskowski. 2004. Influence of kernel size on grinding process of wheat at respective grinding stages. *Pol. J. Food Nutr. Sci.*, 13(1): 29-34.

Eckardt, N.A. 2010. Evolution of domesticated bread wheat. *Plant Cell*., 22(4): 993-993.

Gegas, V.C., A. Nazari, S. Griffiths, J. Simmonds, L. Fish, S. Orford and J.W. Snape. 2010. A genetic framework for grain size and shape variation in wheat. *Plant Cell.,* 22(4): 1046-1056.

Hasanuzzaman, M., K. Nahar, M.M. Alam, R. Roychowdhury and M. Fujita. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.*, 14(5): 9643-9684.

Jamil, M., A. Ali, K.F. Akbar, A. Ghafoor, A.A. Napar, S. Asad and A. Mujeeb-Kazi. 2016. Relationship among water use efficiency, canopy temperature, chlorophyll content and spot blotch (*Cochliobolus sativus*) resistance in diverse wheat (*Triticum aestivum* L.) germplasm. *Pak. J. Bot.*, 48(3): 993-998.

Masood, R., N. Ali, M. Jamil, K. Bibi, J.C. Rudd and A. Mujeeb-Kazi. 2016. Novel genetic diversity of the alien D-Genome synthetic hexaploid wheat (2n=6x=42, AABBDD) germplasm for various phenology traits. *Pak. J. Bot.*, 48(5): 2017-2024.

Ramya, P., A. Chaubal, K. Kulkarni, L. Gupta, N. Kadoo, H.S. Dhaliwal and V. Gupt. 2010. QTL mapping of 1000-kernel weight, kernel length, and kernel width in bread wheat (*Triticum aestivum* L.). *J. Appl. Genet*., 51(4): 421-429.

Rasheed, A., X. Xia, F. Ogbonnaya, T. Mahmood, Z. Zhang, A. Mujeeb-Kazi and Z. He. 2014. Genome-wide association for grain morphology in synthetic hexaploid wheats using digital imaging analysis. *BMC Plant Biol*., 14(1): 1-21.

Tanabata, T., T. Shibaya, K. Hori, K. Ebana and M. Yano. 2012. SmartGrain: high-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiol*., 160(4): 1871-1880.

Tiwari, C., H. Wallwork, U. Kumar, R. Dhari, B. Arun, V.K. Mishra and A.K. Joshi. 2013. Molecular mapping of high temperature tolerance in bread wheat adapted to the Eastern Gangetic Plain region of India. *Field Crop Res*., 154: 201-210.

Varma, S.V., D.K. Kanaka and K. Keshavulu. 2013. Seed image analysis: its applications in seed science research. *Int. Res. J. Agric Sci*., 1(2): 30-36.

Williams, K, J. Munkvold and M. Sorrells. 2013. Comparison of digital image analysis using elliptic Fourier descriptors and major dimensions to phenotype seed shape in hexaploid wheat (*Triticum aestivum* L.). *Euphytica*, 190: 99-116.

Willis, C.G., B. Ruhfel, R.B. Primack, A.J. Miller-Rushing, and C.C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proc Natl Acad Sci.*, 105(44): 17029-17033.

(Received for publication 12 July 2016)