Accepted: 29 March 2018

DOI: 10.1111/jac.12282

HEAT STRESS

WILEY Journal of Agronomy and Crop Science

Evaluating heat tolerance of a complete set of wheat-*Aegilops geniculata* chromosome addition lines

A. Green[1] | B. Friebe[2] | P. V. V. Prasad[3] | A. Fritz[3]

[1]North Dakota State University, Fargo, North Dakota

[2]Kansas State University, Manhattan, Kansas

[3]Agronomy, Kansas State University, Manhattan, Kansas

Correspondence
A. Green, North Dakota State University, Fargo, ND.
Email: andrew.j.green@ndsu.edu

Abstract

Heat stress limits wheat (*Triticum aestivum* L.) yield potential in many areas of the world, and wild relatives represent an important novel source of genetic tolerance. In a previous study of various *Aegilops* species, an accession of *Aegilops geniculata*, TA2899, was reported to be heat tolerant. Prior to that, a complete set of wheat-*Ae. geniculata* chromosome addition lines were developed using the same accession. The objective of this study was to screen the full set of addition lines to identify the chromosome(s) which carried the heat tolerance. The addition lines, Chinese Spring, as well as heat tolerant, and susceptible controls were screened twice for post-anthesis heat tolerance in growth chambers. Genotypes varied for temperature treatment ($p < .05$), but no differences were found between Chinese Spring and the addition lines. Additionally, no genotypes were superior to positive controls for grain fill duration. The proposed reason is that the TA2899 which was previously identified as heat tolerant should be reclassified as *Aegilops peregrina*. This is supported by spike morphology and marker correlations using genotyping-by-sequencing. Despite negative results, the methodology is valid and the results remain important to report, if for no other reason than to prevent another researcher from investigating this question.

KEYWORDS
Aegilops geniculata, heat stress, wheat

1 | INTRODUCTION

Many growth stages during the life of the wheat plant are susceptible to temperature extremes, but temperature extremes surrounding anthesis and the grain fill period are known to have a profound impact (Barkley et al., 2013; Farooq, Bramley, Palta, & Siddique, 2011; Pradhan, Prasad, Fritz, Kirkham, & Gill, 2012a; Prasad & Djanaguiraman, 2014).

Heat stress decreases grain yield by several factors. A primary response of heat stress is early leaf senescence (Al-Khatib & Paulsen, 1990; Blum, 1988; Yang, Sears, Gill, & Paulsen, 2002). Heat stress also inhibits leaf photosynthesis primarily as a result of thylakoid membrane damage (Al-Khatib & Paulsen, 1984; Ristic, Bukovnik, & Prasad, 2007) and the electron transport mechanisms in Photosystem II (Prasad, Pisipati, Mutava, & Tuinstra, 2008). The effect of heat stress is the acceleration of development and growth at all stages (Farooq et al., 2011; Shpiler & Blum, 1986). The yield component most affected by post-anthesis heat stress is kernel size (Yang et al., 2002). Post-anthesis heat stress decreases kernel size because of decreasing grain fill duration, even though heat increases the grain filling rate (Prasad, Boote, Allen, Sheehy, & Thomas, 2006).

Genetic improvement and cultivar selection are key mechanisms for coping with heat stress. *Aegilops geniculata* (Roth, syn *Aegilops ovata*) shows great promise for use in wheat improvement, from disease resistance genes (Gill et al., 1985; Kuraparthy et al., 2007; Liu et al., 2011), to abiotic stresses such as heat and drought tolerance (Pradhan, Prasad, Fritz, Kirkham, & Gill, 2012b; Pradhan et al., 2012a; Zaharieva, Gaulin, Havaux, Acevedo, & Monneveux, 2001). Studying reproductive heat stress from *Aegilops*, Pradhan et al. (2012a) identified two moderately tolerant accessions of

Ae. geniculata, including TA2899. Previous and unrelated work by Friebe, Tuleen, and Gill (1999) yielded a full set of chromosome addition lines using this accession in a Chinese Spring background. A Chinese Spring/TA2899 F_1 plant was backcrossed with Chinese Spring, and one plant was identified with $2n = 8x = 56$ chromosomes. Following the procedure outlined in Friebe et al. (1999), chromosome addition lines were developed. The disomic addition lines are hexaploid Chinese Spring each also containing one *Ae. geniculata* chromosome pair each, for a total of 44 chromosomes. The objective of this study was to identify the chromosome(s) which contributed to heat tolerance in TA2899 by comparing the high temperature versus optimal temperature response of the full set of 14 wheat-*Ae. geniculata* chromosome addition lines with Chinese Spring. Known heat tolerant and susceptible wheat cultivars were included as controls. The heat tolerance level of Chinese Spring was unknown prior to the study.

2 | MATERIALS AND METHODS

The fourteen chromosome addition lines (noted by their Wheat Genetics Resource Center (WGRC) collection number and shown in Table 1), Chinese Spring, two heat tolerant checks [*Ventnor* (Yang et al., 2002) and *Jefimija* (Ristic et al., 2007)], and two heat sensitive checks (*Jagger* and *U1275* (Talukder et al., 2015)) were germinated on germination paper which was wetted with a solution containing 5 g/L terraclor (Quintozene) wettable powder fungicide. All check genotypes were hexaploid (*Triticum aestivum*, L.) with a winter growth type. Two days after germination, the seminal roots of each seedling were removed and fixed in ice water overnight. Roots were then fixed in a solution of three parts ethanol (99% v/v) to one part glacial acetic acid. After 1 week, roots were acetocarmine (1% carmine, 45% acetic acid) stained and the root tip caps were extracted and squashed. Chromosome counts were completed to identify at least four plants of the only monosomic addition line (TA7666). Roots of disomic addition lines were kept in the acetic acid-ethanol solution for future analysis. The disomic addition lines are meiotically stable with an approximately 90% transmission rate (Bernd Friebe, personal communication).

Seedlings were transplanted into Sungro Professional Growing Mix (Sungro Horticulture, Agawam, MA) and vernalized at 4.4°C for 3 weeks. During both repeats, every genotype was transplanted to four pots, with two seedlings in each 2.45 L round pot (17.5 cm tall; with a top diameter of 15.24 cm-Nursery Supplies Inc, Orange, CA). The four pots were divided into two pairs—one pot in each pair was randomly assigned to the high-temperature treatment, and the other to the optimal temperature. Each pair was grown adjacently in the greenhouse until they were moved to a growth chamber for temperature treatment, 10 days after anthesis (noted by anther extrusion). Genotype pairs were randomized together in the greenhouse, and all pots were completely randomized in the growth chamber. Greenhouse conditions consisted of a 16-hr photoperiod with controlled 21°C daytime temperatures and 15.5°C night-time temperatures.

TABLE 1 Differences in least square means for grain fill duration with Dunnett's adjustment for multiple comparisons, Chinese Spring control

Heat treatment 35°/30°			Optimal treatment 25°/20°		
Genotype	Difference[a]	Adj. *p**	Genotype	Difference	Adj. *p*
7655	0.50	1.00	7655	4.25	.96
7656	−1.50	.89	7656	0.00	1.00
7657	1.50	.89	7657	1.25	1.00
7658	2.00	.79	7658	5.50	.87
7659	1.25	.94	7659	1.50	1.00
7660	−0.50	1.00	7660	−2.00	1.00
7661	2.00	.79	7661	5.25	.90
7662	1.75	.84	7662	2.75	1.00
7663	−0.75	.99	7663	−1.00	1
7664	−9.25	.23	7664	−6.00	.81
7665	−2.25	.74	7665	−4.25	.98
7666	3.00	.61	7666	4.00	.99
7667	0.00	1.00	7667	−0.75	1.00
7688	1.00	.97	7688	0.75	1.00
Jagger	1.25	.94	Jagger	7.00	.65
Jefimija	5.50	.37	Jefimija	9.25	.33
U1275	−1.00	.97	U1275	7.00	.65
Ventnor	2.00	.79	Ventnor	12.50	.09

[a]Taken as difference between lsmeans of each genotype minus Chinese Spring.
*Adjusted *p* value.

Light intensity in the greenhouse from artificial lights was around 400 μM m^{-2} s^{-1}, plus ambient light. Plants were well watered to avoid any low-moisture stress. At jointing (Feekes 6; Large, 1954), plants were tethered to bamboo stakes to avoid lodging. Pots were treated with "Marathon" systemic granular insecticide (1% imidacloprid; OHP Inc, Mainland, PA) at rate of 1.4 g per pot to prevent insect damage. All measurements were based on the phenology of the primary tiller of each plant, which was tagged at spike emergence.

High-temperature treatment in the growth chamber consisted of 35°day/30°night, 15-hr photoperiod and optimal was 25°day/20°night and a 15-hr photoperiod. In the growth chamber, physiological readings were initiated on the fourth day and taken every other day thereafter until tiller death, noted by complete flag leaf senescence or physiological maturity (yellow uppermost peduncle), whichever came first. After a 16-day temperature treatment, pots were returned to the greenhouse. Pairs within each genotype were compared across the two temperature treatments, and an average genotype response was used for analysis and comparison to Chinese Spring. The experiment was repeated once. One repeat was completed in May 2012 and the other in November 2012.

Plants were measured for chlorophyll index, as measured by SPAD (Konica-Minolta SPAD 502 Plus; Spectrum Technologies,

Aurora, IL), which measures leaf greenness and is correlated to chlorophyll content (Markwell, Osterman, & Mitchell, 1995). Photochemical efficiency of Photosystem II (PSII) was measured by Fv/Fm variable fluorescence with an OS-30P+ handheld fluorometer (Opti-sciences, Hudson, NH), which measures active PSII receptors and is correlated to photosynthetic leaf health and heat stress (Maxwell & Johnson, 2000; Ristic et al., 2007). Chlorophyll index readings were recorded as an average of three points on the flag leaf of the main tiller on the adaxial surface of the leaf. Fv/Fm readings were obtained with the handheld fluorometer on the adaxial surface of the same main tiller flag leaf as near to the culm as possible after a 30-min dark adaptation. Grain fill duration was derived as the total number of days from anthesis to tiller death. To compare the genotypic effect of heat tolerance, contrasts were calculated as the difference between least square (ls) means of the optimal minus the heat treatment. Spikelet number and seeds per spike were recorded at maturity. Seed weight per spike was obtained after 5 days of drying at 37°. Average individual seed weight was derived from seeds per spike and seed weight per spike.

SAS 9.3 (SAS Institute, 2013) was used for statistical analysis. The Glimmix procedure was used for an analysis of variance. Experiment ($n = 2$), entry ($n = 19$), temperature treatment ($n = 2$) and their two-way interactions were all analysed as fixed effects. Tukey's HSD was used for multiple comparisons. Dunnett's adjustment for multiple comparisons of means was also used with the genotype Chinese Spring as the control, as it is the base genome for the addition lines. A multiple regressions change point analysis of chlorophyll index and photochemical efficiency of PS II for genotypes by experiment was completed using Proc Reg in SAS (SAS, 2016) to detect the day during physiological measurements where the slope of the response curve changed to become negative (Schwarz, 2015).

As will be noted in "Results," these experiments called into question whether the accession tested by Pradhan et al. (2012a) was the same accession used by Friebe et al. (1999) to develop the addition lines. As a result, the seed requested from the WGRC for this study, seeds of the original spikes donated to the WGRC, as well as seed from each subsequent seed increase, were grown for analysis. DNA extraction was performed on bulked leaf tissue from two plants using the BioSprint 96 DNA Plant Kit (Qiagen) with the BioSprint 96 Workstation (Qiagen, Hilden, Germany). Genotyping-by-sequencing was used to identify single nucleotide polymorphisms (SNPs) in the extracted DNA following the methods of Poland et al. (2012). Markers with more than 70% missing data were discarded. The remaining SNPs were numerically coded as 1 for homozygotes of the most frequent allele, 0 for heterozygotes and –1 for homozygotes of the less frequent allele. Correlations between genotypes were compared for all available seed increases (Table 2).

3 | RESULTS

An analysis of variance of all genotypes for the three primary response variables of grain fill duration, seeds per spike and average seed weight completed. In an analysis of grain fill duration for only the addition lines and Chinese Spring, temperature was the only significant source of variability ($p < .05$; Table 3).

Grain fill duration (GFD) under heat stress is a key indicator of tolerance. The range of GFD for the chromosome addition lines in the heat treatment was from 12 to 24 days, with Chinese Spring averaging 21.25 days. Least square means (lsmeans) were compared in a pairwise Tukey–Kramer means separation, and no addition line was found to statistically differ from Chinese Spring. A Dunnett multiple comparison test with Chinese Spring as the control was also completed and confirmed that no chromosome addition lines varied from Chinese Spring (Table 1). For the response variable average seed weight, genotype, temperature and the interaction of temperature and genotype were found to significantly differ ($p < .05$).

The change point is the day during temperature treatment where a response curve for a plant health measurement significantly changes. Change points were estimated visually by plotting plant health measurements against days of treatment and then tested by linear regression, following Schwarz (2015). Significantly different slopes confirmed a change point day. In this case, the change point indicates an irreversible negative response to heat stress. One value per genotype was obtained by averaging the change point day of the chlorophyll index and photochemical efficiency of PS II for genotype, by experiment. The comparison between the wheat and addition lines suggests that there was no superior source of heat tolerance in the addition lines, as *Jefimija* consistently had a later change point date, and *Ventnor* in experiment 2 was superior to the addition lines for both physiological measures. No addition line had a consistently later change point day than Chinese Spring.

4 | DISCUSSION

To identify a chromosome significantly contributing to heat tolerance, two conditions must be met. First, the chromosome addition line must be significantly different from Chinese Spring. Otherwise, the alien chromatin is having no detectable effect as all lines contain the same hexaploid wheat background. A significant variance between Chinese Spring and an addition line could indicate a positive or negative effect on heat tolerance. Secondly, if an addition line is found to differ from Chinese Spring, then its heat tolerance can be assessed with response variables such as grain fill duration, or seed production. If the mean response for an addition line is superior to Chinese Spring, then a small difference between heat and optimal temperature treatments for a given genotype could indicate heat tolerance. Alternately stated, the genotype performed similarly regardless of heat stress.

The positive control cultivars *Ventnor* and *Jefimija* were previously reported as possessing heat tolerance (Narayanan, Prasad, & Welti, 2016b; Narayanan, Tamura, Roth, Prasad, & Welti, 2016a; Ristic et al., 2007; Talukder et al., 2015). Because these sources of tolerance are present in hexaploid wheat, any novel sources of tolerance from the tertiary gene pool would need to be clearly

TABLE 2 Whole genome correlations (*r*) determined by SNPs for TA 2899 seed increases, Chinese Spring, and unrelated *Ae. geniculata* control

Genotype	Description	TA 10437	TA 2899a	TA 2899b	TA 2899c	TA 2899d	TA 2899e	TA 2899f	TA 2899g	TA 2899h	TA 2899i	TA 2899j	TA 2899k	TA 2899l	TA 2899m	TA 2899n	TA 2899o
TA 10437	Unrelated *Ae. geniculata*	1.00															
TA 2899a	Original Donation	.92	1.00														
TA 2899b	Original Donation	.93	.98	1.00													
TA 2899c	Addition Line Donor	.91	.97	.98	1.00												
TA 2899d	Seed Increase 1-2	.69	.68	.68	.67	1.00											
TA 2899e	Seed Increase 2-1[a]	.68	.68	.68	.67	.98	1.00										
TA 2899f	Seed Increase 3-2	.69	.68	.68	.68	.98	.98	1.00									
TA 2899g	Seed Increase 4-1	.89	.95	.95	.94	.66	.66	.66	1.00								
TA 2899h	Seed Increase 6-3	.69	.68	.68	.67	.98	.98	.98	.65	1.00							
TA 2899i	Seed Increase 7-2	.93	.98	.99	.97	.68	.68	.68	.95	.68	1.00						
TA 2899j	Seed Increase	.92	.98	.98	.97	.67	.67	.68	.95	.67	.98	1.00					
TA 2899k	Seed Increase 11-2	.92	.98	.99	.97	.68	.67	.68	.95	.67	.98	.98	1.00				
TA 2899l	Seed Increase 13-2	.92	.98	.99	.97	.68	.68	.68	.95	.68	.99	.98	.98	1.00			
TA 2899m	Seed Increase 14-1	.93	.98	.99	.98	.68	.68	.69	.95	.68	.99	.98	.99	.99	1.00		
TA 2899n	Seed Increase 15-1	.93	.98	.99	.97	.68	.68	.69	.95	.68	.99	.98	.98	.99	.99	1.00	
TA 2899o	Seed Increase 15-2	.92	.98	.99	.97	.67	.67	.67	.95	.67	.98	.98	.98	.98	.99	.99	1.00
Chinese Spring	Chinese Spring wheat	.08	.06	.06	.06	.20	.20	.20	.06	.20	.06	.06	.06	.06	.06	.06	.06

[a]Seed source for study by Pradhan et al. (2012a,b).

TABLE 3 *F*-values from analysis of variance for grain fill duration, seeds per spike and average seed weight for addition lines and Chinese Spring only

	df	Grain fill duration	Seeds/Spike	Average seed weight
Experiment	1	3.09	5.98*	0.72
Genotype	18	1.86	25.72***	8.81***
Experiment * Genotype	18	0.5	4.04**	2.28
Temperature Treatment	1	42.50***	0.02	281.97***
Genotype * Temperature treatment	18	0.18	1.69	2.93*
Experiment * Temperature treatment	1	0.06	1.46	0.02

*$p < .05$; **$p < .01$; ***$p < .001$.

superior to warrant the work required for gene introgression into an adapted background. For the grain fill duration ANOVA of only addition lines, temperature was the only significant difference (Table 3), providing evidence of the lack of heat tolerance conferred by the *Ae. geniculata* chromatin.

Genotypes were found to significantly differ for seeds per spike and average seed weight. Genotypic differences between seed number were also expected because of the documented differences in spike type of the chromosome addition lines (Friebe et al., 1999).

No addition lines were found to have higher seed number or weight than Chinese Spring, despite variance differences (data not shown), indicating a negative effect of alien chromatin in some addition lines.

Photochemical efficiency of PS II and chlorophyll index are quantitative measures of plant health and are highly correlated to photosynthetic efficiency and heat stress responses (Ristic et al., 2007). Change point values for both measurements by experiment were analysed. A correlation between parameters in experiment one was $r = .69$, and $r = .82$ for experiment two. This supports the conclusion by Ristic et al. (2007) that the two measures are highly correlated measures of plant health.

There were no addition lines which had superior performance in heat stress. The lack of differences between Chinese Spring and the addition lines could be because any genetic variation for heat tolerance is quantitative and, therefore, not expressed in individual chromosomes added to the Chinese Spring background. If only one genome contains a tolerance gene, then genes which are present in TA2899 may also be having a lesser effect in the wheat genetic background because of dosage effects relating to only one homologue being present in each addition line.

Another explanation for heat tolerance not being expressed in the addition lines is that TA2899 was not heat tolerant, contradicting previous reports (Pradhan et al., 2012a). During the screening of the entire collection of *Ae. geniculata* for heat tolerance, the accession tested as TA2899 from the WGRC was first observed to have a different spike architecture. Personal communication on *Aegilops* morphology with local experts and van Slageren (1994) suggested that the accession might have been *Aegilops peregrina*, another allotetraploid with a $U^pU^pS^pS^p$ genome designation.

In the analysis of all available sources of TA2899, four entries (TA2899d, e, f and h) were significantly less correlated to the original TA2899 (a&b), and the seed source for the production of the addition lines (TA 2899c) in the work by Friebe et al. (1999) (Table 2). The four entries in question were highly related to each other, and interestingly, more highly correlated with Chinese Spring ($r = .2$) than the original sources of TA2899 ($r = .06$). This may also support the presence of an S genome, which is closely related to the B genome of wheat (Salse et al., 2008).

The marker data were consistent with the morphological data, which confirmed four seed increase sources (TA2899d, e, f &h) were different from *Ae. geniculata* based on heading date and spike morphology. Among them was the seed source for the current work on *Ae. geniculata* and the seed requested for the study by Pradhan et al. (2012a), which is TA2899d in Table 2. As further confirmation, genomic in situ hybridization was performed with total M and U genome DNA as probes primers to confirm that these plants were in fact not *Ae. geniculata*. The present speculation is that accession actually was *Ae. peregrina*. Two observations of the original source of TA2899 (2899a in Table 2) were also screened for heat tolerance using the same treatment as outlined above. It appeared to have very poor tolerance to heat stress, with senescence occurring <6 days after initiation of heat stress (data not shown). The original source of the accession was not screened in the study originally as it was very old seed available in limited quantities and had been subsequently regrown to produce fresh seed.

In conclusion, no source of heat tolerance was identified in the chromosome addition lines with TA2899. This was most likely due to identification of heat tolerance in a different genotype TA2899 by Pradhan et al. (2012a), which is not the source of *Ae. geniculata* used to produce the chromosome addition lines by Friebe et al. (1999). The tolerance source identified by Pradhan et al. (2012a) is currently being investigated to validate its potential use in wheat improvement.

Contribution no. 17-184-J from the Kansas Agricultural Experiment Station.

ACKNOWLEDGEMENTS

The authors would like to acknowledge collaborators Sarah Battenfield, Robert Gaynor and Jon Raupp for assistance and guidance in conducting this work.

ORCID

A. Green http://orcid.org/0000-0002-4359-4166

P. V. V. Prasad http://orcid.org/0000-0001-6632-3361

REFERENCES

Al-Khatib, K., & Paulsen, G. M. (1984). Mode of high temperature injury to wheat during grain development. *Physiologia Plantarum*, *61*, 363–368. https://doi.org/10.1111/j.1399-3054.1984.tb06341.x

Al-Khatib, K., & Paulsen, G. M. (1990). Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. *Crop Science*, *30*, 1127–1132. https://doi.org/10.2135/cropsci1990.0011183X003000050034x

Barkley, A., Tack, J., Nalley, L. L., Bergtold, J., Bowden, R., & Fritz, A. K. (2013). Weather, disease, and wheat breeding effects on Kansas wheat varietal yields, 1985 to 2011. *Agronomy Journal*, *106*, 227–235.

Blum, A. (1988). *Plant breeding for stress environments* (p. 201). Boca Raton, FL: CRC Press Inc.

Farooq, M., Bramley, H., Palta, J. A., & Siddique, K. H. (2011). Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Sciences*, *30*, 491–507. https://doi.org/10.1080/07352689.2011.615687

Friebe, B. R., Tuleen, N. A., & Gill, B. S. (1999). Development and identification of a complete set of *Triticum aestivum-Aegilops geniculata* chromosome addition lines. *Genome*, *42*, 374–380. https://doi.org/10.1139/g99-011

Gill, B. S., Sharma, H. C., Raupp, W. J., Browder, L. E., Hatchett, J. H., Harvey, T. L., … Waines, J. G. (1985). Evaluation of *Aegilops* species for resistance to wheat powdery mildew, wheat leaf rust, hessian fly, and greenbug. *Plant Disease*, *69*, 314–316.

Kuraparthy, V., Chhuneja, P., Dhaliwal, H. S., Kaur, S., Bowden, R. L., & Gill, B. S. (2007). Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. *Theoretical and Applied Genetics*, *114*, 1379–1389. https://doi.org/10.1007/s00122-007-0524-2

Large, E. C. (1954). Growth stages in cereals illustration of the Feekes scale. *Plant Pathology*, *3*, 128–129. https://doi.org/10.1111/j.1365-3059.1954.tb00716.x

Liu, W., Rouse, M., Friebe, B., Jin, Y., Gill, B. S., & Pumphrey, M. O. (2011). Discovery and molecular mapping of a new gene conferring resistance to stem rust, Sr53, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin. *Chromosome Research*, *19*, 669–682. https://doi.org/10.1007/s10577-011-9226-3

Markwell, J., Osterman, J., & Mitchell, J. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research*, *46*, 467–472. https://doi.org/10.1007/BF00032301

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—A practical guide. *Journal of Experimental Botany*, *51*, 659–668. https://doi.org/10.1093/jexbot/51.345.659

Narayanan, S., Prasad, P. V. V., & Welti, R. (2016b). Wheat leaf lipids during heat stress: II. Lipids experiencing coordinated metabolism are detected by analysis of lipid co-occurrence. *Plant, Cell and Environment*, *39*, 608–617. https://doi.org/10.1111/pce.12648

Narayanan, S., Tamura, P. J., Roth, M. R., Prasad, P. V. V., & Welti, R. (2016a). Wheat leaf lipids during heat stress: I. High day and night temperatures result in major lipid alterations. *Plant, Cell and Environment*, *39*, 787–803. https://doi.org/10.1111/pce.12649

Poland, J., Endelman, J., Dawson, J., Rutkoski, J., Wu, S., Manes, Y., … Jannink, J. L. (2012). Genomic selection in wheat breeding using genotyping-by-sequencing. *The Plant Genome Journal*, *3*, 103–113. https://doi.org/10.3835/plantgenome2012.06.0006

Pradhan, G. P., Prasad, P. V. V., Fritz, A. K., Kirkham, M. B., & Gill, B. S. (2012a). High temperature tolerance in species and its potential transfer to wheat. *Crop Science*, *52*, 292–304. https://doi.org/10.2135/cropsci2011.04.0186

Pradhan, G. P., Prasad, P. V. V., Fritz, A. K., Kirkham, M. B., & Gill, B. S. (2012b). Response of *Aegilops* species to drought stress during reproductive stages of development. *Functional Plant Biology*, *39*, 51–59. https://doi.org/10.1071/FP11171

Prasad, P. V. V., Boote, K. J., Allen, L. H., Sheehy, J. E., & Thomas, J. M. G. (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*, *95*, 398–411. https://doi.org/10.1016/j.fcr.2005.04.008

Prasad, P. V. V., & Djanaguiraman, M. (2014). Response of floret fertility and individual grain weight of wheat to high temperature stress: sensitive stages and thresholds for temperature and duration. *Functional Plant Biology*, *41*, 1260–1269.

Prasad, P. V. V., Pisipati, S. R., Mutava, R. N., & Tuinstra, M. R. (2008). Sensitivity of grain sorghum to high temperature stress during reproductive development. *Crop Science*, *48*, 1911–1917. https://doi.org/10.2135/cropsci2008.01.0036

Ristic, Z., Bukovnik, U., & Prasad, P. V. V. (2007). Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop Science*, *47*, 2067–2073. https://doi.org/10.2135/cropsci2006.10.0674

Salse, J., Chagué, V., Bolot, S., Magdelenat, G., Huneau, C., Pont, C., … Segurens, B. (2008). New insights into the origin of the B genome of hexaploid wheat: evolutionary relationships at the SPA genomic region with the S genome of the diploid relative *Aegilops speltoides*. *BMC Genomics*, *9*, 1–12.

SAS Institute (2013). *9.3 foundation for 64-bit Microsoft Windows*. Cary, NC: SAS Inst.

SAS Institute (2016). *9.4 foundation for 64-bit Microsoft Windows*. Cary, NC: SAS Inst.

Schwarz, C. J. (2015). Regression-hockey sticks, broken sticks, piecewise, change points. In: *Course Notes for Beginning and Intermediate Statistics*. Retrieved from http://www.state.sfu.ca/~cschwarz/CourseNotes. Retrieved 2 March 2016.

Shpiler, L., & Blum, A. (1986). Differential response of wheat cultivars to hot environments. *Euphytica*, *35*, 483–492. https://doi.org/10.1007/BF00021856

vanSlageren, M. W. (1994). *Wild wheats: A monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae)*. Wageningen Agricultural University Papers 94-7.

Talukder, S. K., Prasad, P. V. V., Todd, T., Babar, M. A., Poland, J., Bowden, R., & Fritz, A. K. (2015). Effect of cytoplasmic diversity on post anthesis heat tolerance in wheat. *Euphytica*, *204*, 383–394. https://doi.org/10.1007/s10681-014-1350-7

Yang, J., Sears, R. R., Gill, B., & Paulsen, G. (2002). Growth and senescence characteristics associated with tolerance of wheat-alien amphiploids to high temperature under controlled conditions. *Euphytica*, *126*, 185–193. https://doi.org/10.1023/A:1016365728633

Zaharieva, M., Gaulin, E., Havaux, M., Acevedo, E., & Monneveux, P. (2001). Drought and heat responses in the wild wheat relative *Aegilops geniculata* roth. *Crop Science*, *41*, 1321–1329. https://doi.org/10.2135/cropsci2001.4141321x

How to cite this article: Green A, Friebe B, Prasad PVV, Fritz A. Evaluating heat tolerance of a complete set of wheat-*Aegilops geniculata* chromosome addition lines. *J Agro Crop Sci*. 2018;204:588–593. https://doi.org/10.1111/jac.12282