Received: 14 March 2022 | Accepted: 19 September 2022 | Published online: 16 December 2022

DOI: 10.1002/csc2.20853

Crop Science

ORIGINAL ARTICLE

Crop Breeding & Genetics

Comparative analysis of heat-stress-induced abscisic acid and heat shock protein responses among pea varieties

Shaoming Huang[1] | **Haixia Zhang**[1,2] | **Randy W. Purves**[1,3] | **Rosalind Bueckert**[1] | **Bunyamin Tar'an**[1] | **Thomas D. Warkentin**[1]

[1]Dep. of Plant Sciences, Univ. of Saskatchewan, 51 Campus Dr., Saskatoon, SK S7N 5A8, Canada

[2]Dep. of Chemistry, Univ. of Winnipeg, 599 Portage Ave., Winnipeg, MB R3B 2G3, Canada

[3]Centre for Veterinary Drug Residues, Canadian Food Inspection Agency, 116 Veterinary Rd., Saskatoon, SK S7N 2R3, Canada

Correspondence
Thomas D. Warkentin, Dep. of Plant Sciences, Univ. of Saskatchewan, 51 Campus Dr., Saskatoon, SK, Canada, S7N 5A8.
Email: tom.warkentin@usask.ca

Assigned to Associate Editor Cory Hirsch.

Abstract

Heat shock proteins (HSPs) and abscisic acid (ABA) play important roles in plant heat responses but have not been extensively studied in pea (*Pisum sativum* L.), whose heat susceptibility is well known. In this study, four pea varieties varying in heat tolerance based on field trials were evaluated. Plants were heat stressed for 3, 6, 12, or 24 h at 38 ˚C before pollination. Anther and stipule RNA from the same flowering node were sampled for transcriptional profiling of *PsHSP18.1* and *PsHSP71.2*. Additional stipules were sampled for the quantification of ABA concentration and its five key catabolites from the four major ABA catabolic pathways by ultra-high performance liquid chromatography–selected reaction monitoring mass spectrometry (UHPLC-SRM/MS). The transcription of both HSP genes was upregulated because of heat stress (HS). In stipules, the upregulation was greatest at 3 h HS, whereas in anthers, the induced transcription was similar among different hours of HS. Likewise, more ABA accumulated in the ABA metabolism pool because of HS, and the ABA response started rapidly after 3 h of treatment. Heat-tolerant varieties had a higher ABA synthesis and turnover rate at 3 h HS than their respective heat-susceptible counterparts. This study provides new insights into different heat tolerance among Canadian pea varieties regarding HSP and ABA hormone regulation.

1 | INTRODUCTION

Pea (*Pisum sativum* L.) belongs to the cool-season legume family, among which, pea is considered as the most heat susceptible. In Canada, which accounts for one-third of global pea production, lower grain yield was seen in summers when the daily maximum temperature exceeded 28 ˚C during flowering or the seasonal temperature was over 17.5 ˚C (Bueckert et al., 2015). In comparison, heat stress (HS) in growth chambers was not fully expressed until daily maximum temperatures reached 36 ˚C (Jiang et al., 2019). In 2021, an unusually long episode of heat waves was seen in July and August across western Canada. For instance, in Saskatoon (52˚10′15″ N, 106˚42′00″ W, meteorological station: CA4057152), a record high at 40 ˚C was observed on 2 July, and there were 22 d in July and August where the daily maximum temperature exceeded 30 ˚C, on average, 6 h of the day. As a result, the production of field pea and other crops were

---

Abbreviations: ABA, abscisic acid; ABA-GE, abscisic acid–glucose ester; Ct, cycle threshold; DPA, dihydrophaseic acid; HS, heat stress; HSP, heat shock protein; neoPA, neophaseic acid; PA, phaseic acid; PCR, polymerase chain reaction; qRT-PCR, quantitative real-time PCR; UHPLC-SRM/MS, ultra-high performance liquid chromatography–selected reaction monitoring mass spectrometry.

detrimentally affected. In plant cellular defense against HS, the induction of heat shock proteins (HSPs) is one of the major responses. Heat shock proteins act as molecular chaperones to facilitate folding of other functional proteins at HS, thus alleviating their functional damage. Heat shock proteins, in general, are classified into HSP100, HSP90, HSP70, HSP60, and small HSP (sHSP) based on protein molecular weight. In pea, most HSPs are identified in the subfamilies of sHSP and HSP70 (DeRocher & Vierling, 1995; DeRocher et al., 1991; Lenne et al., 1995). Among these reported pea HSP genes, *PsHSP17.9*, *PsHSP18.1*, *PsHSP71.2*, and *PsHSP70b* transcripts seemed transcriptionally induced at HS (DeRocher & Vierling, 1995; DeRocher et al., 1991). Srikanthbabu et al. (2002) further provided evidence that the HSP induction at moderately high temperature improved survival rate of pea seedlings at subsequent lethal HS.

The phytohormone abscisic acid (ABA) also plays an important role in mediating plant adaptation to stress. The relationship between ABA-dependent heat response and HSP heat induction in leaf tissue is yet to be clarified, though some HSP70s seemed both heat and ABA inducible, for example, HSP71.1 in rice (*Oryza sativa* L.) (Ye et al., 2012; Zou et al., 2009). On the contrary, Bray (1991) found that HS-induced HSP accumulations at similar patterns between tomato (*Solanum lycopersicum* L.) ABA-deficient mutant 'flacca' and wild-type tomato 'Ailsa Craig'. Temperature stress can activate numerous genes involved in ABA biosynthesis, catabolism, and transport; ABA biosynthesis genes in *Arabidopsis thaliana* (L.) Heynh. (e.g., *ABA1*, *ABA2*, *ABA4*, *AAO3*, and *NCED3*) were differentially expressed (up- or downregulated) in an organ-specific manner among stem leaves on the flowering nodes, inflorescence meristem, and developing silique. As a result, a significant accumulation of ABA was detected in 37 ˚C-treated leaves compared with leaves at 22 ˚C (Baron et al., 2012). The induction of ABA under high temperature was observed after 8 h at 45 ˚C in canola (*Brassica napus* L.) seedlings (Kurepin et al., 2008). Transcriptomic comparison of rice seedlings revealed multiple genes in ABA synthesis and signal transduction were differentially regulated between elevated temperature and control temperature, and the pattern could vary depending on HS intensity (Sharma et al., 2021). Also, ABA profiling of cereal crops in various abiotic stresses, including heat, cold, and drought, suggested that increased ABA accumulation within reproductive structures was negatively correlated with abiotic stress tolerance (Bheemanahalli et al., 2020; Ji et al., 2011; Oliver et al., 2007; Tang et al., 2008). Little is known as to whether ABA metabolism or signaling responds differently between legume crops contrasting in their heat tolerance. In general, plants have four ABA catabolic pathways, among which, conjugation and hydroxylation of the 8′-carbon atom are the major pathways. The balance between ABA biosynthesis, catabolism, and transport determines ABA homeostasis in plant tissue. An ultra-high performance liquid chromatography–selected reaction monitoring mass spectrometry (UHPLC–SRM/MS) approach was developed for the quantification of ABA and its major metabolites. In this study, the authors are not only curious whether ABA per se accumulates in HS but, more importantly, how the ABA homeostasis would change with HS.

Core Ideas

- Heat-induced expression of *PsHSP18.1* and *PsHSP71.2* was a conserved heat response among four pea varieties.
- ABA response differential at 3 h heat stress was associated with heat tolerance in pea, similar to previous field trials.
- The involvement of HSP and ABA in heat response in pea was independent.

We hypothesized that (a) both pea HSP genes (*PsHSP18.1* and *PsHSP71.2*) and stipule ABA are involved with pea response to HS and (b) response variation would be observed among different pea varieties and the differentials would link with varieties' heat tolerance characterized at the field trial level.

2 | MATERIALS AND METHODS

2.1 | Plant material

Two pairs of heat-tolerant and heat-susceptible pea varieties—cultivars CDC Meadow vs. Nitouche and recombinant inbred lines PR11-2 vs. PR11-90—had been identified from different genetic backgrounds in previous studies in Canada. CDC Meadow (Warkentin et al., 2007) demonstrated superior heat tolerance out of the 24 genotypes studied by Tafesse et al. (2019) and Jiang et al. (2019), as it had consistently superior performance in canopy temperature depression, high in vitro pollen germination under HS, and high grain yield in field trials. Nitouche, originating from DLF Trifolium in Denmark, is considered as heat sensitive because of its high ovule abortion rate and relatively poor yield performance under high temperature (Jiang et al., 2019). PR11-2 and PR11-90 are recombinant inbred lines at F_8 from the population PR11, which was derived from the cross 'CDC Centennial'//'CDC Sage' made in 2008 at the Crop Development Centre, University of Saskatchewan (Huang et al., 2017). Both varieties have white flowers and green cotyledons, but PR11-2 has higher pod number per plant, longer flowering duration, and greater grain yield than

TABLE 1 Characteristics of flowering- and yield-related traits of ‘CDC Meadow’, ‘Nitouche’, PR11-2, and PR11-90 in 2017–2019 (rep = 3 in each year trial) at Saskatoon, Canada

Variety	Seeding date	Days to flowering	Flowering duration	Reproductive node # mainstem	Pod # mainstem	Plot yield
		d	d			kg ha^{-1}
‘CDC Meadow’	normal	54.3	16.3	5.6	9.0	2,784
	late	49.4	14.6	5.1	8.9	2,762
‘Nitouche’	normal	58.0	13.7	4.8	7.2	2,534
	late	50.7	12.3	4.4	6.7	1,816
PR11-2	normal	56.9	15.6	5.4	7.7	2,828
	late	51.9	12.7	4.8	7.4	2,665
PR11-90	normal	48.0	17.7	4.5	7.1	2,289
	late	47.7	13.0	3.3	5.2	1,145

Note. The late-seeded trial was planted 2–3 wk later than the normal seeded trial to expose the plots to greater heat stress. Both normal and late seeding trials had an average 9 d > 28 ˚C, 5 d > 30 ˚C during flowering; however, late seeding trials had its daily max temperature average (27.2 ˚C) 1 ˚C higher than the max average (26.3 ˚C) of normal seeding.

PR11-90 based on field trials under normal seeding and late seeding conditions, and thus, PR11-2 is considered as more heat tolerant than PR11-90 (Table 1). The late seeding date typically exposes the pea crop flowering period to HS analogous to that of the warmer regions of the North American prairies. The late seeding method was previously reported by Huang et al. (2017) and Tafesse et al. (2019) to expose the pea flowering period in mid-July and early August to relatively high temperatures.

2.2 | Experimental design and heat treatment

Seed samples of heat-tolerant varieties CDC Meadow and PR11-2 and heat-susceptible varieties Nitouche and PR11-90 were obtained from CDC, University of Saskatchewan. Three seeds of each variety were planted in a 3.8-L pot containing Sunshine mix #4 (Sun Gro) at control temperature condition (22/18 ˚C, 16/8 h day/night) which simulates a nonstress pea field environment in western Canada. The three plants in one pot were bulked later as one biological replicate. A randomized complete block design with three biological replications of each variety was used. Starting from 1 wk after seedlings were germinated, the plants were watered twice or three times per week based on the soil medium moisture, plant’s growth stage, as well as water use of each variety, so as to avoid drought stress. Pots were fertilized weekly with 100 ml of quick release fertilizer (20 N:20 P_2O_5:20 K_2O) prepared at a concentration of 3 g L^{-1}. At the stage when plants developed flower buds but prior to anther dehiscence, plants assigned to heat treatment groups were transferred from the control temperature chamber to a chamber at 38/18 ˚C, 16/8 h day/night for 3, 6, 12, or 24 h respectively. The setting of HS at 38 ˚C was consistent with Huang et al. (2021) and DeRocher et al. (1991) and was similar to the HS temperature peak used by Jiang et al. (2019). Then all the anthers and stipules on the first flowering node from the three plants within one pot were freshly frozen in liquid nitrogen and stored at –80 ˚C in a freezer. The whole experiment yielded a respective library of 60 samples of each plant organ type from four varieties, five heat treatments, and three biological replicates.

2.3 | RNA extraction and qRT-PCR

For each organ sample, the extraction of total RNA was conducted using QIAGEN RNeasy Plant Mini Kit. The quantity of extracted RNA was then determined by optical density at 260 nm and the OD260/OD280 absorption ratio using NanoDrop 8000 UV spectrophotometer. The remaining RNA of each tissue sample was then stocked at –80 ˚C. One microgram of total RNA was reversely transcribed to cDNA using SensiFAST cDNA synthesis kit. Four reference genes (*PP2A*, *GH720808*, *β-tubulin*, and *GH720838*) were recommended for abiotic stress experiments in pea (Die et al., 2010). *GH720808*, encoding histone H3, and *GH720838*, encoding transcription factor IIA, were tested in this experiment, and *GH720838* was selected as the reference gene to normalize the relative expression quantities of the target genes because it had the consistent expression among different time points and genotypes (i.e., among anther samples) cycle threshold (Ct) value was 19.4 ± 0.63, CV = 3.3; among stipule samples, Ct value was 20.7 ± 0.66, CV = 3.2. Specific primers of *PsHSP18.1* and *PsHSP71.2* were designed via IDT Primer quest tool (Integrated DNA Technologies Inc) with the following criteria: Tm of 62 ± 1 ˚C, polymerase chain reaction (PCR) amplicon lengths of 90–120 bp, primer length of 20–22 bp, and GC contents of 45–55%. Primer efficiency (%) of each gene was equaled to $(10^{-1/\text{slope}} - 1) \times 100$, and all primers had their efficiency rates between 90–110% and qualified for assay use (Supplemental Figure S1). Then SensiFAST SYBR No-ROX kit was used for the target gene expression using optical 384-well plate on BIO-RAD CFX384 quantitative real-time PCR (qRT-PCR) machine in accordance with the manufacture's protocols.

2.4 | qRT-PCR data analysis

For each time point, transcription fold change of HSP gene relative to reference gene is demonstrated as $2^{(-\Delta Ct)}$, where $\Delta Ct = (\text{Ct of HSP gene}) - (\text{Ct of reference gene})$ (Schmittgen & Livak, 2008). Mean transcriptional fold change of *PsHSP18.1* gene and *PsHSP71.2* among different time points were compared using SAS Proc Mixed model (v9.4, SAS Institute Inc.). Following that, fold change values were further normalized via $2^{-\Delta\Delta Ct}$ method in order to compare the two genes' transcription changes among different HS periods between stipules and anthers among four genotypes.

2.5 | Extraction of ABA and its major catabolites and quantification via UHPLC-SRM/MS

In parallel, additional stipule samples were freeze dried and ground into dry, fine powder. Sixty stipule samples were prepared by weighing ∼50 mg into a 2-ml microtube for the quantification of ABA and its catabolites using UHPLC-SRM/MS. For a negative control nonheat-stressed stipule sample of the pea 'wilt' mutant, line JI 1069 was used. JI 1069 was documented as an ABA-deficient variety at drought stress (Wang et al., 1984).

One milliliter of solvent consisting of 80:19:1 methanol/water/formic acid containing deuterated internal standards (d_6–ABA, d_3–PA, d_3–DPA, d_4–7-OH-ABA, and d_5–ABA-GE; Toronto Research Chemicals) was added to each tube. After vortexing the tube for 5–10 s, the samples were placed onto a Thermo mixer for 30 min at 23.3 Hz (room temperature). Samples were then centrifuged for 5 min at 5,630 × g. An 800-µl aliquot of the supernatant was transferred into a new 2-ml microtube. A second extraction was carried out by adding 500 µl of the extraction solvent with no internal standard. After vortexing, mixing, and centrifuging as described above, 500 µl from the second extraction was transferred and combined with the first extraction. Three hundred ninety microliters of the supernatant was transferred to a new micro tube and dried using a speed vac (Labconco Corp.). The dried sample was then reconstituted in 130 µl of 79:20:1 water/methanol/formic acid, vortexed, and then mixed on the Thermo mixer at room temperature for 30 min at 23.3 Hz. A volume of 110 µl was transferred to a HPLC vial containing a 150-µl insert prior to UHPLC-SRM/MS.

Abscisic acid and its five metabolites (phaseic acid [PA] and dihydrophaseic acid [DPA] via C′8 hydroxylation, neophaseic acid [neoPA] via C′9 hydroxylation, 7′-OH ABA via C′7 hydroxylation, and abscisic acid–glucose ester [ABA-GE] via β-D-glucopyranosyl conjugation) were selected for quantification. Five deuterated standards were selected as internal standards, and an eight-point calibration curve was prepared for quantification (Table 2).

The UHPLC-SRM/MS was performed on a TSQ Altis triple quadrupole mass spectrometer equipped with a Vanquish Flex UHPLC (Thermo Fisher Scientific) in the negative ion mode. A volume of 10 µl was injected from the vial, and analytes were separated using an Agilent Zorbax Eclipse Plus C18 column (2.1 by 50 mm, 1.8 µm) and 5-mm guard column. The mobile phase consisted of 1% formic acid in water (solvent A) or in 90% acetonitrile (solvent B). The flow rate was set to 0.4 ml min^{-1} and a 17-min solvent gradient was used. The gradient started with 10% solvent B and gradually increased to 20% at 5 min and to 30% at 7 min; it then was increased to 42% at 11 min, ramped to 90% at 12 min, and then maintained for 2 min. It was returned to 10% at 14.5 min and maintained for 2.5 min to equilibrate the column. The liquid chromatography column temperature was controlled at 25 ˚C and the autosampler at 6 ˚C. The mass spectrometer parameters were as follows: electrospray voltage 2500 V, vaporizer temperature 350 ˚C, sheath gas pressure 60, auxiliary gas pressure 10, sweep gas 1, and capillary temperature 325 ˚C. The SRM parameters, such as the selection of precursor and fragment ions, collision energies and radio frequency lens for the compounds are shown in Table 2.

2.6 | Data quality check and outlier data removal

For the dataset of each measured parameter from individual heat treatments, outliers were filtered among varieties according to Tukey's criteria. An outlier was defined as any data outside the range [Q1 – 1.5(Q3 – Q1), Q3 + 1.5(Q3 – Q1)], where Q1 and Q3 are the lower and upper quartiles, respectively.

2.7 | ABA statistical analysis

For the concentration of ABA and its five catabolites, individual analysis of variance was conducted via Proc Mixed program in SAS v9.4 (SAS Institute Inc.). Multiple variety means were compared based on least significant difference at significance level .05.

TABLE 2 Selected reaction monitoring parameters for abscisic acid (ABA) and its selected catabolites on a Thermo Fisher Altis triple quadrupole mass spectrometer

Compound	Retention time	Precursor → fragment	Collision energy	Radio frequency lens
	min	m/z	V	V
ABA	7.3	263 → 153	10.2	48
PA	4.8	279 → 139	12	54
DPA	2.4	281 → 171	18	73
7′-OH–ABA	5.8	279 → 151	15.2	47
ABA–GE	4.6	425 → 263	10.2	77
neo–PA	6.4	279 → 205	12	56
d_6–ABA	7.3	269 → 159	10.2	48
d_3–PA	4.7	282 → 142	10.2	53
d_5–ABA–GE	4.5	430 → 268	10.2	65
d_4–7′-OH–ABA	5.8	283 → 221	10.2	48
d_3–DPA	2.4	284 → 240	13	69

Note. ABA-GE, abscisic acid–glucose ester; DPA, dihydrophaseic acid; neoPA, neophaseic acid; PA, phaseic acid.

TABLE 3 Relative *PsHSP18.1* and *PsHSP71.2* gene transcription to the reference gene *GH720838* on nonheat-stressed anther and stipule samples

	Stipules		Anthers	
Variety	*Pshsp18.1*	*Pshsp71.2*	*Pshsp18.1*	*Pshsp71.2*
PR11-2	0.14	0.08	0.33	0.12
PR11-90	0.07	0.05	0.24	0.05
‘CDC Meadow’	0.17	0.05	0.24	0.06
‘Nitouche’	0.17	0.11	0.35	0.10
Mean[a]	0.1 ± 0.05	0.1 ± 0.03	0.3 ± 0.06	0.1 ± 0.03

Note. Expression fold change of heat shock proteins relative to reference gene is demonstrated as $2^{(-\Delta Ct)}$, where ΔCt = (Ct of HSP gene − Ct of reference gene). Ct, cycle threshold.

[a] Each mean fold change shown in the last row is the average across the four varieties.

3 | RESULTS

3.1 | Transcriptional profiling of *PsHSP18.1* and *PsHSP71.2* between control temperature and heat stress

In the control temperature environment, the transcriptional levels of both *PsHSP18.1* and *PsHSP71*.2 were minimal compared with the transcription of the reference gene *GH720838* on both anther and stipule samples (Table 3). Whereas their transcription levels were significantly induced because of HS (Figure 1). Our results supported previous classification of both genes as heat inducible. The transcriptional level of *PsHSP18.1* was greater than that of *PsHSP71*.2 in both stipules and anthers at control temperature.

When subjected to HS, the transcription of both genes was significantly increased in all anther and stipule samples of all four varieties at every time point measured. In stipules, both genes had greater expression at 3 h than at 6, 12, and 24 h (Figure 1a,b). At each time point under HS, the induction fold change of both genes was statistically similar among the four varieties. In anther samples of each variety, the induction fold change of *PsHSP71.2* was similar among the four time points measured (Figure 1c). PR11-90 and Nitouche had greater induction than PR11-2 and CDC Meadow at some time points (e.g., 3 h at 38 ˚C). For the heat response of *PsHSP18.1* in anthers, the transcriptional induction was at similar fold change across the four time points in both PR11-2 or Nitouche (Figure 1d). Whereas in CDC Meadow, *PsHSP18.1* had the lower fold change at 6 h but similar fold changes at 3 , 12, and 24 h. In the anthers of PR11-90, *PsHSP18.1* had less induction at 24 h than the induction levels at 3 and 12 h. For individual time points under HS, the transcriptional fold change of *PsHSP18.1* had no significant variation at 24 h among four varieties. At 3 h, the transcription of *PsHSP18.1* was more induced in PR11-90 than PR11-2. At 6 h, PR11-90 had greater

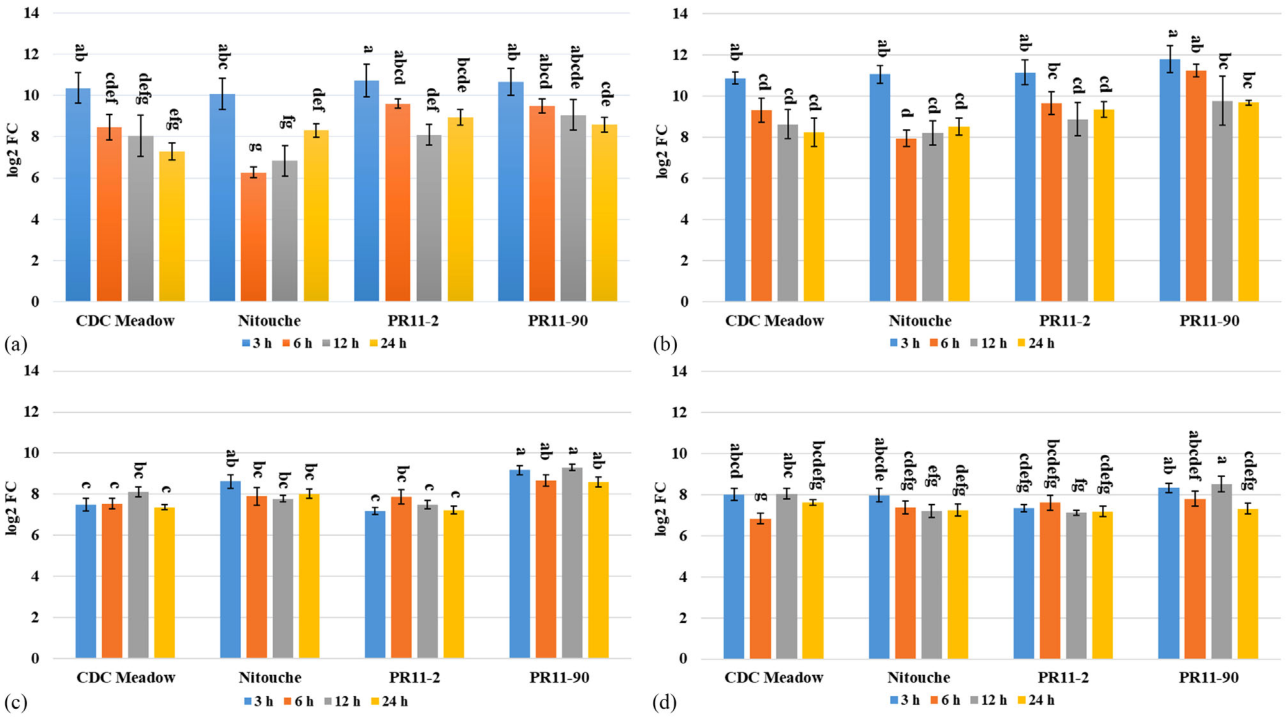

FIGURE 1 Transcriptional response of *PsHSP71.2* and *PsHSP18.1* at 3, 6, 12, or 24 h 38 ˚C heat stress in stipules (a) *PsHSP71.2* and (b) *PsHSP18.1*) and whole anthers (c) *PsHSP71.2* and (d) *PsHSP18.1*) at the same flowering node among four pea varieties. Each bar represents average gene transcriptional fold change in $\log_2$ ($2^{-\Delta\Delta Ct}$) across three biological replications and two technical replications and error bar represents standard error. Within a panel, mean values followed by the same letter did not differ significantly at $P < .05$

expression of *PsHSP18.1* than PR11-2 and CDC Meadow. At 12 h, CDC Meadow and PR11-90 had greater induction than Nitouche and PR11-2.

3.2 | Genetic variation in stipule ABA homeostasis under control temperature condition

Total concentration, that is the sum of ABA and its catabolites, was documented as an amount accounting for the ABA pool. The total ABA concentration of the negative control, JI 1069, was profiled to be 1.1 nmol g^{-1} dry wt. in our assay, which was much lower than the total ABA concentration of the four varieties of interest at control temperature (mean = 11.4 nmol g^{-1} dry wt.; Table 4). A means comparison of individual ABA catabolites and the total concentration among the four varieties was conducted on nonheat-stressed stipule samples. Even at the control temperature condition, significant variety differences were detected in DPA, neoPA, and the total ABA pool concentration (Table 4). However, these variations among the four varieties did not depend on their heat tolerance classification. One heat-susceptible cultivar, Nitouche, had the highest concentration of all ABA catabolites and the total ABA pool, but the other susceptible line, PR11-90, had the second lowest DPA and total concentrations. The heat-tolerant cultivar CDC Meadow had the lowest concentrations of DPA and the total ABA pool among the four varieties, but line PR11-2 had relatively high concentrations.

3.3 | ABA homeostasis response toward heat stress

To understand the whole ABA pool response to HS, ANOVA on the effects of varieties and different hours of HS on the total amount of ABA metabolites showed that varieties and high-temperature treatment both had significant effects (Table 5). More ABA in the pool accumulated at all heat treatments compared with the control temperature, with double peaks at 3 and 24 h (Table 5). Although the whole ABA pool concentration at 3 and 24 h 38 ˚C were similar, the composition of the ABA pool was different. At 3 h 38 ˚C, DPA accounted for 85% of the total; at 24 h 38 ˚C, DPA only accounted for ∼50% of the total, whereas a big amount of ABA was synthesized, implying a new round of ABA signaling is about to begin.

Regarding ABA and its various metabolites, ANOVA results showed that ABA, DPA, PA, ABA-GE, and neoPA had significant responses to temperature treatment, whereas 7′-OH ABA had no response (Table 5). When pea stipules were exposed to 3 h HS, ABA concentration dropped significantly compared with control temperature, and it was

TABLE 4 Mean ± standard deviations ($n = 3$) of abscisic acid (ABA), dihydrophaseic acid (DPA), neophaseic acid (neoPA), and total ABA pool concentration on nonheat-stressed stipule samples among four pea varieties

Variety	Heat tolerance	ABA	DPA	neoPA	Total
		nmol g^{-1} dry wt.			
'CDC Meadow'	Tolerant	1.20 ± 0.45a[a]	5.99 ± 1.08b	0.06 ± 0.01b	8.3 ± 1.39c
PR11-2	Tolerant	1.41 ± 0.32a	10.47 ± 2.37a	0.05 ± 0.01b	13.1 ± 1.95ab
'Nitouche'	Susceptible	1.49 ± 0.40a	11.02 ± 1.05a	0.09 ± 0.01a	13.4 ± 0.79a
PR11-90	Susceptible	1.05 ± 0.40a	8.66 ± 0.37b	0.04 ± 0.02a	10.7 ± 0.60bc

Note. For each column, if the values share a common letter, it means that they are not significantly different according to LSD (.05). PA, 7′-OH ABA and ABA-GE were not shown in the table because of nonsignificant variation among varieties. The full are provided in Supplemental Table S1.

converted to DPA via 8′-hydroxylation pathway; as a result, DPA concentration increased accordingly. At 6 and 12 h, ABA concentration increased to its similar level of control temperature. For neoPA derived from ABA via 9′- hydroxylation, its concentration did not vary between control temperature and HS at 3, 6, and 24 h, but its concentration decreased at 12 h 38 ˚C.

3.4 | ABA response variation between heat tolerant and heat susceptible pairs of varieties

The concentration changes of ABA, its catabolites, and total ABA pool between control temperature and different heat treatments was characterized for each of the four varieties. For each variety, the ABA concentration was the lowest after 3 h at 38 ˚C (Figure 2). The ABA pool concentration was the highest at 3 and 24 h at 38 ˚C, which was consistent with the result in Table 5. A contrasting pattern of stipule total ABA pool concentration between the two pairs of heat tolerant and heat-sensitive varieties was observed. In the pair of CDC Meadow and Nitouche, CDC Meadow, the more heat-tolerant cultivar, had a lower level than Nitouche (susceptible counterpart); whereas in the pair of PR11-2 and PR11-90, heat tolerant PR11-2 had a higher level than PR11-90. But from the perspective of ABA response to heat treatment, at 3 h HS, the total ABA pool concentration of CDC Meadow increased by 53% compared with control temperature, and the corresponding increase percentage was 12, 36, and 33% for Nitouche, PR11-2, and PR11-90, respectively. The increased percentage of ABA converting to DPA was 74, 18, 48, and 35% for CDC Meadow, Nitouche, PR11-2, and PR11-90, respectively. Both PR11-2 and CDC Meadow had a faster ABA turnover rate, with more ABA synthesized in the pool after 3 h at 38 ˚C than their sensitive counterparts. The similar response pattern in the two groups suggests that heat tolerance might correlate with a greater ABA induction and a faster turnover rate at the early stage of HS.

4 | DISCUSSION

4.1 | Induced transcription of *PsHSP18.1* and *PsHSP71.2* at heat stress

Based on their expression patterns, HSPs within each family can be classified into three subcategories, which are heat induced, expressed constitutively but not heat induced, and expressed constitutively with additional heat induction. The two HSP genes, *PsHSP18.1* and *PsHSP71.2*, whose expression was identified as heat inducible on a vegetable pea variety (DeRocher and Vierling, 1995; DeRocher et al., 1991), were validated in this study on field pea varieties with various thermal tolerance. Both genes were minimally expressed in the stipules and anthers at nonstressed control temperature condition (Table 3), but their expression was dramatically induced when subjected to high temperature treatment (Figure 1).

In plant cellular defense against heat, the induction of HSP is one of the major responses. The HSPs act as molecular chaperones, which are proteins that facilitate folding of other functional proteins especially at the secondary and tertiary structure during nonstress periods and prevent them from denaturation and aggregation during exposure to HS. Overexpression of HSP genes in transgenic plants helped plants exhibited improved tolerance to high temperature (Fragkostefanakis et al., 2015; Mishra et al., 2018). For stipule samples in this study, 3 h high-temperature HS treatment was the most heat-responsive time point, as the fold change of both genes reached the maximum on every variety (Figure 1a,b). This rapid response corresponded with the characterization of *PsHSP18.1* and *PsHSP71.2* in the vegetable pea cultivar Little Marvel (DeRocher & Vierling, 1995; DeRocher et al., 1991) as well as the findings reported in other plant species (Wahid et al., 2007). For anthers of individual variety, the transcriptional level of both genes was steady during HS (Figure 1c,d). Greater expression was seen in stipule than anther, suggesting a better thermal tolerance in vegetative organs than in

TABLE 5 Effects of variety and temperature stress levels on pea stipules

	df	ABA	DPA	PA	7′-OH ABA	ABA–GE	neoPA	Total
		nmol g^{-1} dry wt.						
Stress level								
Control	–	1.29 ± 0.50b[a]	9.03 ± 2.38bc	0.73 ± 0.14d	0.24 ± 0.12a	0.03 ± 0.01b	0.06 ± 0.02a	11.37 ± 2.42b
3 h 38 ˚C	–	0.43 ± 0.13c	12.64 ± 3.05a	1.42 ± 0.25c	0.35 ± 0.17a	0.05 ± 0.02b	0.07 ± 0.02a	14.95 ± 3.05a
6 h 38 ˚C	–	1.09 ± 0.61b	8.93 ± 2.83bc	1.80 ± 0.94b	0.25 ± 0.11a	0.04 ± 0.01b	0.07 ± 0.04a	12.19 ± 3.92b
12 h 38 ˚C	–	1.43 ± 0.73b	9.71 ± 1.92b	1.03 ± 0.42d	0.28 ± 0.10a	0.08 ± 0.04b	0.04 ± 0.01b	12.56 ± 2.60b
24 h 38 ˚C	–	3.80 ± 1.98a	7.91 ± 2.07c	3.32 ± 0.88a	0.32 ± 0.10a	0.31 ± 0.25a	0.06 ± 0.02a	15.71 ± 3.95a
		ANOVA						
Effects								
Variety (V)	3	ns[†]	***	***	***	ns	ns	***
Stress level (S)	4	***	***	***	ns	***	**	***
V × S	12	ns	ns	***	ns	*	*	ns

Note. ABA-GE, abscisic acid–glucose ester; DBA, dihydrophaseic acid; neoPA, neophaseic acid; PA, phaseic acid. For each column, if the values share a common letter, it means that they are not significantly different according to least significant difference (.05).

[a] Values are the mean concentrations across four varieties with standard deviations.

*Significant at .05 probability level.

**Significant at .01 probability level.

***Significant at .001 probability level.

[†] ns, nonsignificant at the .05 probability level.

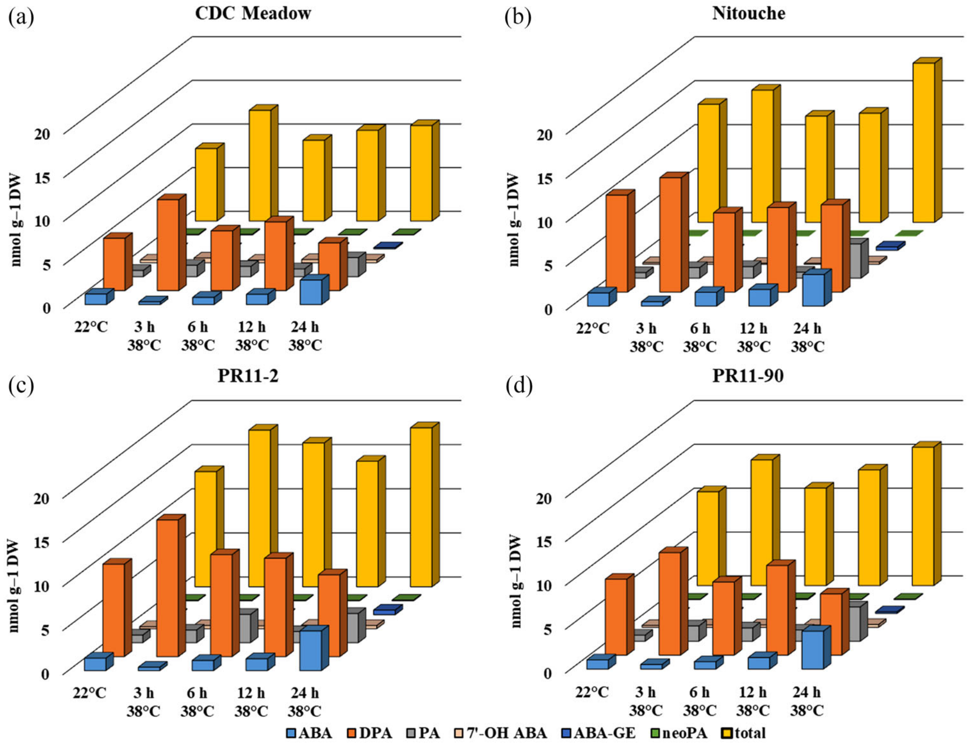

FIGURE 2 The concentration (nmol g^{-1} dry wt.) change of abscisic acid (ABA), phaseic acid (PA), dihydrophaseic acid (DPA), 7′-OH ABA, abscisic acid–glucose ester (ABA-GE), neophaseic acid (neoPA), and the total over different hours of heat stress at 38 ˚C on two heat-tolerant pea varieties, (a) 'CDC Meadow' and (c) PR11-2, and two heat-susceptible pea varieties, (b) 'Nitouche' and (d) PR11-90

reproductive organs. However, the induction threshold of both genes did not correlate with the varieties' heat-tolerance classification. For instance, in heat-stressed stipules, CDC Meadow and PR11-2 had similar accumulation levels of both transcripts as their susceptible counterpart, Nitouche and PR11-90, at individual time points (Figure 1a,b). This result supports the finding in Huang et al. (2021): that induced expression of genes associated with HSPs and their transcription factors was a common heat response among pea varieties with varying heat tolerance. Heat-induced transcription of HSP genes could be cultivar specific in rice (Chandel et al., 2013) and wheat (*Triticum aestivum* L.) (Mishra et al., 2017) when screening among multiple heat tolerant and susceptible varieties. Similar to this study, the differential induction of HSP genes did not depend on the classification of the variety's heat tolerance. They both found that heat-tolerant and heat-susceptible varieties could have strong induction in some of the HSP genes. One possible explanation on high HSP induction in a heat-susceptible variety is that a rescue mechanism is taking place, which is also likely the reason that both HSP genes had higher induction in anthers of PR11-90 than anthers of PR11-2 at many time points, *PsHSP71.2* in particular (Figure 1c,d).

4.2 | Pea ABA metabolic homeostasis at control temperature and heat stress

In the limited papers related to pea ABA, leaf ABA concentrations were reported in the range of 0.76–2.27 nmol g^{-1} dry wt. (Zhang & Davies, 1987; Zhang & Zhang, 1994), and the ABA concentration at control temperature condition in this experiment fell within the range (Table 4). The DPA derived from C-8′ hydroxylation was the most abundant among ABA catabolites, whereas neoPA via 9′ hydroxylation and 7′OH-ABA via 7′ hydroxylation were minimal. Though there was no resource in pea to compare, in the general review papers of plants, 8′ hydroxylation is believed to be the dominant catalytic hydroxylation pathway (Cutler & Krochko, 1999; Sah et al., 2016; Zeevaart & Creelman, 1988). At control temperature, free ABA levels in stipules were not statistically different among four pea varieties, which was in agreement with the finding of Ji et al. (2011) that there was no significant variation of endogenous ABA levels in unstressed leaves among four wheat varieties with varying drought tolerance. Whereas in our study, significant variety differences were detected in DPA, neoPA, and the total ABA pool concentrations (Table 4).

Phytohormone ABA is involved in the regulation of thermotolerance, and 7-d-old seedlings of *Arabidopsis aba1*, *aba2*, and *aba3* mutants with deficiency in different ABA synthesis genes displayed reduced basal and acquired heat tolerance compared with wild type (Larkindale et al., 2005). In our study, JI1069, an ABA-deficient line, demonstrated a wilting phenotype under control temperature particularly during the reproductive stage and, as a result fruit production, failed. Its heat susceptibility compared with other pea varieties mainly was due to low vigor at control temperature. When subjected to HS, ABA response could be rapid (i.e., within hours). Leaf ABA concentration started to increase within the first hour of HS in grapevine (*Vitis vinifera* L.) leaf (Abass & Rajashekar, 1993) and during the 4- to 8-h period of HS in canola leaf (Kurepin et al., 2008). Although we found the ABA pool concentration accumulated at 3 h of high temperature, the induction was not continuous (Table 5). Its concentration between 6 and 12 h HS dropped to the similar level of control temperature, but the concentration started to increase again during 12–24 h HS. The ABA pool accumulation after 24 h HS mainly was due to the significant increase in the concentration of active ABA. This induced active ABA was also found in *Arabidopsis* leaves when 24 h HS was applied (Baron et al., 2012). The accumulated ABA level did not bring about a further transcription induction of either *HSP71.2* or *HSP18.1* at 24 h HS compared with other durations of heat treatment (Figure 1), suggesting the expression of both genes was not ABA inducible. Bray (1991) drew a similar conclusion in tomato based on the finding that HS induced accumulation of HSPs were at similar patterns between tomato ABA-deficient mutant ‘flacca’ and wild-type ‘Ailsa Craig’. Contrastingly in rice, several studies showed that some HSPs could be induced by ABA (Ye et al., 2012; Zou et al., 2009), and based on this evidence, the authors proposed that ABA-mediated HSP induction could be one mechanism by which ABA confers heat tolerance. However, the involvement of ABA in heat response seemed complex, and ABA-related enhancement of heat tolerance in plant species was also achieved through the regulation of expressions of respiratory burst oxidase homologs genes and sucrose metabolism (Li et al., 2021).

Active ABA concentration at 3 h HS, on the contrary, was repressed among four pea varieties. The accumulated ABA pool concentration at this time was due to increased DPA via ABA C′8-hydroxylation pathway. Interestingly, heat-tolerant varieties CDC Meadow and PR11-2 had faster ABA turnover than their susceptible counterpart varieties, which appears to be linked with pea heat tolerance. Similarly, a faster ABA turnover was reported in a heat-tolerant variety of rice (Tang et al., 2008) and wheat (Bheemanahalli et al., 2020) compared with their susceptible checks. Our study provides a new direction in the understanding of hormonal regulation under HS in legumes, whose current emphasis is on other plant hormones (e.g., auxin or ethylene) (Ozga et al., 2017).

5 | CONCLUSION

The transcription of *PsHSP18.1* and *PsHSP71.2* identified in vegetable pea was validated as heat inducible in the field pea varieties. The transcription of both genes was most induced at 3 h at 38 ˚C in stipules. In anthers, the induction of both genes was similar at 3 and 24 h of HS. Compared with control temperature, the average ABA pool concentration among four varieties increased by >30% at 3 and 24 h of HS. Heat-tolerant varieties had greater ABA response than heat susceptible varieties in terms of 25% faster ABA turnover rate at early HS stage. This response differential between tolerant and susceptible varieties linked well with different heat tolerance of the four varieties at the field level. However, HSPs response appeared to be a conserved plant HS response as the expression induction did not differ significantly among varieties known to differ in heat tolerance under field conditions.

DATA AVAILABILITY STATEMENT

The data that supports this study will be shared upon reasonable request to the corresponding author.

ACKNOWLEDGMENTS

This research was funded by the Saskatchewan Ministry of Agriculture, Saskatchewan Pulse Growers, and the Western Grains Research Foundation. The authors would like to thank Dr. Sue Abrams at the Department of Chemistry, University of Saskatchewan, for advice in ABA metabolism and Dr. Arthur Davis at the Department of Biology, University of Saskatchewan, for advice in experiment design and data analysis. Thanks also to the staff of the Crop Development Center pulse crop breeding program for seed preparation and to the staff of the pulse crop breeding and molecular genetics lab, University of Saskatchewan, for technical support. The authors appreciated John Innes Center genetic resources for generously providing seeds of pea mutant JI 1069 used as negative control in ABA profiling assay.

AUTHOR CONTRIBUTIONS

Shaoming Huang: Conceptualization; Formal analysis; Investigation; Methodology; Writing-original draft. Haixia Zhang: Investigation; Methodology; Writing-review & editing. Randy W. Purves: Investigation; Methodology; Writing-review & editing. Rosalind Bueckert: Conceptualization; Writing-review & editing. Bunyamin Tar’an: Conceptualization; Formal analysis; Writing-review & editing. Thomas D. Warkentin: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest. None of these institutions had any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ORCID

Shaoming Huang https://orcid.org/0000-0002-9829-0303
Rosalind Bueckert https://orcid.org/0000-0002-3030-7762
Thomas D. Warkentin https://orcid.org/0000-0003-3059-0606

REFERENCES

Abass, M., & Rajashekar, C. (1993). Abscisic acid accumulation in leaves and cultured cells during heat acclimation in grapes. *HortScience*, *28*, 50–52. https://doi.org/10.21273/HORTSCI.28.1.50

Baron, K. N., Schroeder, D. F., & Stasolla, C. (2012). Transcriptional response of abscisic acid (ABA) metabolism and transport to cold and heat stress applied at the reproductive stage of development in *Arabidopsis thaliana*. *Plant Science*, *188*, 48–59. https://doi.org/10.1016/j.plantsci.2012.03.001

Bheemanahalli, R., Impa, S. M., Krassovskaya, I., Vennapusa, A. R., Gill, K. S., Obata, T., & Jagadish, S. K. (2020). Enhanced N-metabolites, ABA and IAA-conjugate in anthers instigate heat sensitivity in spring wheat. *Physiologia Plantarum*, *169*, 501–514. https://doi.org/10.1111/ppl.13109

Bray, E. A. (1991). Wild-type levels of abscisic acid are not required for heat shock protein accumulation in tomato. *Plant Physiology*, *97*, 817–820. https://doi.org/10.1104/pp.97.2.817

Bueckert, R. A., Wagenhoffer, S., Hnatowich, G., & Warkentin, T. D. (2015). Effect of heat and precipitation on pea yield and reproductive performance in the field. *Canadian Journal of Plant Science*, *95*, 629–639. https://doi.org/10.4141/cjps-2014-342

Chandel, G., Dubey, M., & Meena, R. (2013). Differential expression of heat shock proteins and heat stress transcription factor genes in rice exposed to different levels of heat stress. *Journal of Plant Biochemistry and Biotechnology*, *22*, 277–285. https://doi.org/10.1007/s13562-012-0156-8

Cutler, A. J., & Krochko, J. E. (1999). Formation and breakdown of ABA. *Trends in Plant Science*, *4*, 472–478. https://doi.org/10.1016/S1360-1385(99)01497-1

DeRocher, A. E., Helm, K. W., Lauzon, L. M., & Vierling, E. (1991). Expression of a conserved family of cytoplasmic low molecular weight heat shock proteins during heat stress and recovery. *Plant Physiology*, *96*, 1038–1047. https://doi.org/10.1104/pp.96.4.1038

DeRocher, A. E., & Vierling, E. (1995). Cytoplasmic HSP70 homologues of pea: Differential expression in vegetative and embryonic organs. *Plant Molecular Biology*, *27*, 441–456. https://doi.org/10.1007/BF00019312

Die, J. V., Román, B., Nadal, S., & González-Verdejo, C. I. (2010). Evaluation of candidate reference genes for expression studies in *Pisum sativum* under different experimental conditions. *Planta*, *232*, 145–153. https://doi.org/10.1007/s00425-010-1158-1

Fragkostefanakis, S., Röth, S., Schleiff, E., & Scharf, K. D. (2015). Prospects of engineering thermotolerance in crops through modulation of heat stress transcription factor and heat shock protein networks. *Plant, Cell & Environment*, *38*, 1881–1895. https://doi.org/10.1111/pce.12396

Huang, S., Gali, K. K., Lachagari, R. V., Chakravartty, N., Bueckert, R. A., Tar'an, B., & Warkentin, T. D. (2021). Identification of heat responsive genes in pea stipules and anthers through transcriptional profiling. *PLoS One*, *16*, e0251167. https://doi.org/10.1371/journal.pone.0251167

Huang, S., Gali, K. K., Tar'an, B., Warkentin, T. D., & Bueckert, R. A. (2017). Pea phenology: crop potential in a warming environment. *Crop Science*, *57*, 1540–1551. https://doi.org/10.2135/cropsci2016.12.0974

Ji, X., Dong, B., Shiran, B., Talbot, M. J., Edlington, J. E., Hughes, T., White, R. G., Gubler, F., & Dolferus, R. (2011). Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. *Plant Physiology*, *156*, 647–662. https://doi.org/10.1104/pp.111.176164

Jiang, Y., Lahlali, R., Karunakaran, C., Warkentin, T. D., Davis, A. R., & Bueckert, R. A. (2019). Pollen, ovules, and pollination in pea: Success, failure, and resilience in heat. *Plant, Cell & Environment*, *42*, 354–372. https://doi.org/10.1111/pce.13427

Kurepin, L. V., Qaderi, M. M., Back, T. G., Reid, D. M., & Pharis, R. P. (2008). A rapid effect of applied brassinolide on abscisic acid concentrations in *Brassica napus* leaf tissue subjected to short-term heat stress. *Plant Growth Regulation*, *55*, 165–167. https://doi.org/10.1007/s10725-008-9276-5

Larkindale, J., Hall, J. D., Knight, M. R., & Vierling, E. (2005). Heat stress phenotypes of *Arabidopsis* mutants implicate multiple signaling pathways in the acquisition of thermotolerance. *Plant Physiology*, *138*, 882–897. https://doi.org/10.1104/pp.105.062257

Lenne, C., Block, M., Garin, J., & Douce, R. (1995). Sequence and expression of the mRNA encoding HSP22, the mitochondrial small heat-shock protein in pea leaves. *Biochemical Journal*, *311*, 805–813. https://doi.org/10.1042/bj3110805

Li, N., Euring, D., Cha, J. Y., Lin, Z., Lu, M., Huang, L. -J., & Kim, W. Y. (2021). Plant hormone-mediated regulation of heat tolerance in response to global climate change. *Frontiers in Plant Science*, *11*, 2318. https://doi.org/10.3389/fpls.2020.627969

Mishra, D., Shekhar, S., Agrawal, L., Chakraborty, S., & Chakraborty, N. (2017). Cultivar-specific high temperature stress responses in bread wheat (*Triticum aestivum* L.) associated with physicochemical traits and defense pathways. *Food Chemistry*, *221*, 1077–1087. https://doi.org/10.1016/j.foodchem.2016.11.053

Mishra, D., Shekhar, S., Singh, D., Chakraborty, S., & Chakraborty, N. (2018). Heat shock proteins and abiotic stress tolerance in plants. In A. Asea, & P. Kaur (Eds.), *Regulation of heat shock protein responses* (pp. 41–69). Springer.

Oliver, S. N., Dennis, E. S., & Dolferus, R. (2007). ABA regulates apoplastic sugar transport and is a potential signal for cold-induced pollen sterility in rice. *Plant and Cell physiology*, *48*, 1319–1330. https://doi.org/10.1093/pcp/pcm100

Ozga, J. A., Kaur, H., Savada, R. P., & Reinecke, D. M. (2017). Hormonal regulation of reproductive growth under normal and heat-stress conditions in legume and other model crop species. *Journal of Experimental Botany*, *68*, 1885–1894. https://doi.org/10.1093/jxb/erw464

Sah, S. K., Reddy, K. R., & Li, J. (2016). Abscisic acid and abiotic stress tolerance in crop plants. *Frontiers in Plant Science*, *7*, 571. https://doi.org/10.3389/fpls.2016.00571

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, *3*, 1101. https://doi.org/10.1038/nprot.2008.73

Sharma, E., Borah, P., Kaur, A., Bhatnagar, A., Mohapatra, T., Kapoor, S., & Khurana, J. P. (2021). A comprehensive transcriptome analysis of contrasting rice cultivars highlights the role of auxin and ABA responsive genes in heat stress response. *Genomics*, *113*, 1247–1261. https://doi.org/10.1016/j.ygeno.2021.03.007

Srikanthbabu, V., Krishnaprasad, B. T., Gopalakrishna, R., Savitha, M., & Udayakumar, M. (2002). Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *Journal of Plant Physiology*, *159*, 535–545. https://doi.org/10.1078/0176-1617-00650

Tafesse, E. G., Warkentin, T. D., & Bueckert, R. A. (2019). Canopy architecture and leaf type as traits of heat resistance in pea. *Field Crops Research*, *241*, 107561. https://doi.org/10.1016/j.fcr.2019.107561

Tang, R. -S., Zheng, J. -C., Jin, Z. -Q., Zhang, D. -D., Huang, Y. -H., & Chen, L. -G. (2008). Possible correlation between high temperature-induced floret sterility and endogenous levels of IAA, GAs and ABA in rice (*Oryza sativa* L.). *Plant Growth Regulation*, *54*, 37–43. https://doi.org/10.1007/s10725-007-9225-8

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: an overview. *Environmental and Experimental Botany*, *61*, 199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Wang, T. L., Donkin, M. E., & Martin, E. S. (1984). The physiology of a wilty pea: Abscisic acid production under water stress. *Journal of Experimental Botany*, *35*, 1222–1232. https://doi.org/10.1093/jxb/35.8.1222

Warkentin, T. D., Vandenberg, A., Tar'an, B., Barlow, S., & Ife, S. (2007). CDC Meadow field pea. *Canadian Journal of Plant Science*, *87*, 909–910. https://doi.org/10.4141/CJPS07038

Ye, S., Yu, S., Shu, L., Wu, J., Wu, A., & Luo, L. (2012). Expression profile analysis of 9 heat shock protein genes throughout the life cycle and under abiotic stress in rice. *Chinese Science Bulletin*, *57*, 336–343. https://doi.org/10.1007/s11434-011-4863-7

Zeevaart, J. A., & Creelman, R. A. (1988). Metabolism and physiology of abscisic acid. *Annual Review of Plant Physiology and Plant Molecular Biology*, *39*, 439–473. https://doi.org/10.1146/annurev.pp.39.060188.002255

Zhang, J., & Davies, W. (1987). ABA in roots and leaves of flooded pea plants. *Journal of Experimental Botany*, *38*, 649–659. https://doi.org/10.1093/jxb/38.4.649

Zhang, J., & Zhang, X. (1994). Can early wilting of old leaves account for much of the ABA accumulation in flooded pea plants? *Journal of Experimental Botany*, *45*, 1335–1342. https://doi.org/10.1093/jxb/45.9.1335

Zou, J., Liu, A., Chen, X., Zhou, X., Gao, G., Wang, W., & Zhang, X. (2009). Expression analysis of nine rice heat shock protein genes under abiotic stresses and ABA treatment. *Journal of Plant Physiology*, *166*, 851–861. https://doi.org/10.1016/j.jplph.2008.11.007

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Huang, S., Zhang, H., Purves, R. W., Bueckert, R., Tar'an, B., & Warkentin, T. D. (2023). Comparative analysis of heat-stress-induced abscisic acid and heat shock protein responses among pea varieties. *Crop Science*, *63*, 139–150. https://doi.org/10.1002/csc2.20853