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SCREENING FOR HEAT TOLERANCE IN TOMATO (*SOLANUM LYCOPERSICUM* L.) GENOTYPES USING HOT WATER

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Abstract

Abiotic stress conditions, such as heat stress limit tomato productivity in Ghana. Several studies recommend using stress-tolerant genotypes as a long-term and cost-effective approach for managing the impact of abiotic stress on crop productivity. However, there is limited availability of heat-tolerant tomato genotypes in Ghana; It is therefore imperative to identify locally available tomato genotypes with heat-tolerant ability to improve their productivity under stressful conditions. An experiment was conducted using hot water treatment (55 ℃ for 2 hours) to assess the heat tolerance potential of 71 tomato genotypes. Completely Randomised Design (CRD) with three replications was used. Tomato genotypes were tied separately in a cheesecloth and placed in a water bath at 55 ℃ for 2 hours. Seeds were removed from the cheesecloth and sown directly in a seed tray. Data were collected on germination and seedling parameters. The results showed that genotypes GPT14, U13, GPT28, and T03 were tolerant to the stress induced by the hot water treatment at the germination stage. A gene responsible for the expression of heat Shock Protein (HSP) has been found to confer heat tolerance in tomato; hence, HSP expression in the promising tomato genotypes was used to explain the observed heat tolerance in the seed germination experiment. Genotype GPT28 amplified the HSP gene Hsa 32. Therefore, this study revealed a few tomato genotypes as heat stress tolerant at the germination stage, with one genotype (GPT28) amplifying the heat stress tolerance gene Hsa 32. While this finding suggests the potential presence of heat tolerance traits in the Ghanaian tomato germplasm, further genetic and physiological studies are recommended to validate and characterize key stress tolerance traits across a wider set of tomato genotypes.

Keywords
Hot water; Seed germination; Heat shock proteins; Heat tolerance; Heat tolerant gene

Introduction

Tomato (*Solanum lycopersicum* L.) forms an essential component of food consumed in Ghana. It serves as a primary ingredient in many Ghanaian dishes (Tambo and Gbemu, 2010). Tomato is a valuable dietary source of folates and a wide spectrum of phytonutrients, particularly carotenoids. Among these, lycopene is the predominant carotenoid, accompanied by beta-carotene, gamma-carotene, phytoene, phytofluene, neurosporene, and small amounts of other carotenoids, all contributing to antioxidant activity and health benefits (Chaudhary et al., 2018). There has been a significant impact of heat stress on tomato production, causing alteration in the crop's biochemical, morphological, and physiological functions, leading to a decrease in growth and development (Kissoudis et al., 2015; Wahid et al., 2007). Hatfield and Prueger (2015) reported that tomato crops are sensitive to temperatures beyond the optimal growth temperature ranging between 15 and 32°C. Typically, when tomato plants are exposed to heat stress, the concentration of reactive oxygen species (ROS) increases above the normal, which potentially leads to a disruption of the balance of the radical scavenging system and compromising its overall cellular function or homeostasis (Sharkey, 2005). The harmful effects of heat stress on seed germination and subsequent seedling development in rice, okra, tomato, and hot pepper have successfully been managed by identifying heat-tolerant genotypes (Ibrahim and El-Muqadam, 2019). Seed treatment using hot water has successfully been used to induce heat stress in rice and is widely used to characterize heat tolerance during germination (Permana et al., 2017). Chang et al. (2007) reported that, for plants to balance the homeostasis of cellular proteins under heat stress, the plant cells up-regulate several heat-shock genes. These heat shock genes encode heat shock proteins that aid plants to survive under high temperatures. Small heat shock proteins (HSPs) are the most prevalent type of heat shock proteins in plants. Under heat stress, their expression can increase up to 200-fold (Wang et al., 2004). These proteins have been shown to positively influence thermo-tolerance by maintaining the threshold levels of ROS-scavenging enzymes in the cytosol (Driedonks et al., 2015). Liu et al. (2006) investigated a novel heat shock protein gene, *Hsa32*, which encodes a heat stress-associated 32 kDa protein. Their study revealed that, this gene is present in tomato plants and contributes to thermo-tolerance. Hence, this study also aimed to investigate the presence of this novel gene *Hsa32* and its potential role in conferring heat tolerance in selected tomato genotypes in Ghana. Specifically, this study sought to determine the ideal experimental conditions (hot water temperature and duration of seed exposure combination causing minimum seed germination) for screening the genotypes, screen and identify genotypes tolerant to the heat

stress and confirm the expression of HSP gene in promising heat-tolerant genotypes.

Materials and Methods

Plant materials used

Seventy-one tomato genotypes were used for this study, fifty-one came from WACCI, twelve from CSIR-CRI and eight from Agro-Input shops in Ghana. Seeds were stored in a cold room for a short period before work began (Table 1).

Table 1. Seventy-one tomato genotypes used for the experiment and their sources.

Code	Genotype name	Source
G1	102	WACCI
G2	Local	Agro-Input shop
G3	CRI 3	CSIR-CRI
G4	Seed Manica	Agro-Input shop
G5	Rio Grande	Agro-Input shop
G6	T22	WACCI
G7	G001	WACCI
G8	CRI 4	CSIR-CRI
G9	B	WACCI
G10	ST	WACCI
G11	G003	WACCI
G12	U006	WACCI
G13	T13	WACCI
G14	CRI 1	CSIR-CRI
G15	C004	WACCI
G16	T08	WACCI
G17	TO9	WACCI
G18	T001	WACCI
G19	CRI 8	CSIR-CRI
G20	U001	WACCI
G21	Pectomech	Agro-Input shop
G22	B001	WACCI
G23	F1	WACCI
G24	T01	WACCI
G25	CRI 1	CSIR-CRI
G26	T21	WACCI
G27	GPT 14	WACCI
G28	CRI 7	CSIR-CRI
G29	GPT 06	WACCI
G30	Roma	Agro-Input shop
G31	CRI 10	CSIR-CRI
G32	U11	WACCI
G33	T16	WACCI
G34	U10	WACCI
G35	U13	WACCI
G36	GPT 28	WACCI
G37	T19	WACCI
G38	J	WACCI
G39	127	WACCI
G40	Tropimech	Agro-Input shop
G41	107 S	WACCI
G42	U005	WACCI
G43	Peto 86	Agro-Input shop
G44	G002	WACCI
G45	CRI 11	CSIR-CRI
G46	123	WACCI
G47	Lebombo	Agro-Input shop
G48	T03	WACCI
G49	95	WACCI
G50	UC 82B	WACCI
G51	CRI 2	CSIR-CRI
G52	CRI 12	CSIR-CRI
G53	G5	WACCI
G54	E	WACCI
G55	T19	WACCI
G56	T20	WACCI
G57	GPT 27	WACCI
G58	U004	WACCI
G59	GPT 11	WACCI
G60	CRI 13	CSIR-CRI
G61	U008	WACCI
G62	H	WACCI
G63	U002	WACCI
G64	U003	WACCI
G65	B2	WACCI
G66	I	WACCI
G67	U12	WACCI
G68	GPT 30	WACCI
G69	U007	WACCI
G70	G005	WACCI
G71	CRI 15	CSIR-CRI

Location of study

The study was conducted in the Greenhouse of the Department of Crop Science, University of Ghana, Legon, from February 2020 to September 2020. All the laboratory works were conducted in the Biotechnology Laboratory of the Crop Science Department.

Experiment 1

Three tomato genotypes (G001, U002, and Pectomech) were used for this experiment. Four temperatures (40, 45°C, 50°C, 55°C) and three-time regimes (0.5hr, 1hr, and 2hr) were combined to give 12 treatments: 40°C * 0.5Hr, 40°C * 1Hr, 40°C * 2Hr, 45°C * 0.5Hr, 45°C * 1Hr, 45°C * 2Hr, 50°C * 0.5Hr, 50°C * 1Hr, 50°C * 2Hr, 55°C * 0.5Hr, 55°C * 1Hr, and 55°C * 2Hr. Distilled water was used as a control treatment. The seeds were tied in cheesecloth and placed in a water bath at the various temperatures and time regimes. The seeds were then placed on filter paper in a Petri dish, moistened with 10 ml of distilled water, and then sealed with parafilm. The Petri

dishes were placed in a germinator (Seedburo) at room temperature (25°C). The experiment was observed for 2 weeks (14 days) and the number of seeds that germinated within this period were counted and recorded. The treatments were used as a single factor and randomly assigned to the seeds of three tomato genotypes. The experiment was arranged in a completely randomised design (CRD) with three replications.

Experiment 2

Seventy-one tomato genotypes were used for this experiment, as indicated in Table 1. Based on experiment 1, the treatment of 55 °C for 2 hours resulted in the lowest germination percentage and this was used to impose the heat stress on the 71 tomato genotypes. 45 seeds per genotype were tied in a cheesecloth and placed in a water bath at 55°C for 2 hours. After the hot water treatment, seeds were removed from the cheesecloth and sown in a seed tray filled with potting media (Coco Peat). The seeds were watered twice daily using tap water. The study was monitored daily for seedling emergence, growth, and development for 6 weeks. The experiment was arranged in a completely randomised design with three replicates in a screen house. Each replicate had 15 seeds per genotype, planted in a row. There was a total of 3,195 seeds sown in 71 seed trays.

Data Collection

Germination count was taken from day one to fourteen-day for experiment 1 and day one to eighteen day for experiment 2. The number of seeds that germinated over this period were used to calculate for the percentage germination, mean germination time, mean germination rate, coefficient of variation of the germination time, uncertainty of the germination process, and synchrony using Microsoft Excel (Ranal et al., 2009). Seedling growth parameters were also taken at 4, and 6 weeks after sowing (WAS). Data were collected from six (6) record plants per replicate. The growth parameters taken included:

i. **Plant height**: This was measured using a meter rule.

ii. **Girth**: This was measured using a pair of vernier callipers.

iii. **Number of leaves**: This was determined by counting the leaflets.

iv. **Leaf area**: This was measured using a leaf area meter.

v. **Root volume**: This was estimated using the volume displacement method. Roots of the sampled plants were collected at the end of the experiment. The potting media attached to the root system were carefully removed by shaking before immersion into a displacement can containing a known volume of water. The displaced water was collected in a measuring cylinder and recorded.

vi. **Chlorophyll content**: This was measured using a chlorophyll meter.

vii. **Root and shoot dry weight**: Roots and shoots were placed in envelopes and dried in an oven at 72°C for three days. Thereafter, dry weights were determined using an electronic weighing scale.

viii. **Plant sturdiness**: The sturdiness quotient, an indicator of seedling survivability in the field, was calculated by dividing plant height by girth. A lower quotient indicates a sturdier plant with a higher chance of survival, whereas values greater than 6 are considered undesirable.

Data Analysis

Data collected were analysed using ANOVA in Genstat (12th Edition). Treatment means were compared using the Least Significant Difference (LSD) procedure at a 5% probability level. The germination percentage and the plant growth parameters were used to assess tolerance level of each genotype to the imposed stress. Thus, genotypes that recorded higher percentage germination and vigorous seedling growth were classified as stress tolerant.

Experiment 3

Five genotypes (GPT28, GPT14, T03, U13, and Pectomech) were used for this experiment. Molecular analysis was conducted to investigate the presence of the gene coding HSP in promising heat-tolerant genotypes (GPT28, GPT14, T03 and U13). Pectomech, which was susceptible to the heat stress was used as control treatment. Seeds were tied in a cheese cloth and placed in a water bath at 55°C for 2 hrs to induce heat stress. Seeds were defatted in petroleum ether for 30 mins and then ground with 100 ul of DPEC water (v/v) using mortar and pistil. Fresh leaves were obtained from the selected seedlings and ground with DPEC water (v/v) in Eppendorf tubes. Plant RNA Extraction Mini Kit (Zimo Research) was used to extract the RNA following the manufacturer's protocol. RNA was converted to cDNA using a cDNA conversion Kit (Zimo Research) in accordance with the manufacturer's protocol.

RT-PCR Amplification of Heat-tolerant gene using Lepsl-1/Lepsl-2 primer

The reaction mix was composed of 6.5 µl of PCR-water, 12.5 µl of Quick-Load 2X Master Mix with Standard Buffer (New England Biolabs, Inc.), 1.0 µl each of Lepsl-1 (5'-ATGGCAGCATACACGTGGAAAAGTTTCAAC-3') and Lepsl-2 (5'-ACAGATGAGTGATTCAC-CCAGG-3') primers (Liu et al., 2006) and 4.0 µl of cDNA. PCR cycling conditions were as follows; initial denaturation at 95°C for 4 minutes, further denaturation at 95°C for 30 seconds, and annealing via touchdown at 63°C for 1 minute with temperature decreasing at 0.5°C per cycle for 40 cycles. Initial extension or elongation was set to occur at 72°C for 3 minutes and a final extension at 72°C for 10 minutes. A final hold temperature was set to 4°C. Amplicons were further analysed on a 1.2% agarose gel stained with 3.0 µl Ethidium bromide, run at 80 V for 1 hr 30

mins and viewed in a Trans-illuminator (Syngenes, Inc.) for further assessment.

Results and Discussion

Results

Temperature and time combination causing minimum seed germination

The inhibitory activity of hot water at different temperatures combined with varying seed exposure times on seed germination was examined. The results in Table 2 showed a combination of hot water at 55°C with an exposure time of 2 hrs causing a low germination percentage, with a mean germination rate significantly different from the other treatment, and low germination synchrony. The seeds treated with hot water at 40°C combined with a 0.5 hrs exposure time had a germination percentage (100%) similar to the control treatment. The selected combination (55°C for 2 hrs) was used to screen the 71 tomato genotypes for heat tolerance.

Screening of 71 tomato genotypes for tolerance to heat stress (hot water treatment)

The study showed that the seed germination parameters were significantly ($p \leq 0.05$) influenced by the tomato genotypes (Table 3). Out of the 71 genotypes examined, twenty-seven genotypes germinated, four (G48, G35, G36, and G27) were rated Highly tolerant (HT), one (G7) was Moderately tolerant (MT), and the rest (93%) were either Moderately susceptible (MS) or Highly susceptible (HS) to heat stress. Genotype G48, G35, G36, and G27 recorded high germinability of 86.67%, 86.67%, 84.44%, and 75.56% that corresponded to a mean germination time of 7 days, 12 days, 7 days, and 9 days, respectively. There was no significant difference among the highly tolerant genotypes (G48, G35, G36, G27) with regards to the mean germination rate; G27 recorded the lowest synchrony in germination. Majority of the genotypes (62%) failed to germinate (0%) after the heat treatment. Based on the germination percentage, the genotypes were rated as HT, MT, MS, or HS, as shown in Table 3.

Implications of seeds treated with hot water on vegetative growth at four weeks after sowing (WAS)

Six (6) out of the 27 genotypes that germinated died four weeks after sowing (WAS). The differences in plant height, plant girth, and the number of leaves among the genotypes were significant ($p \leq 0.05$) at 4 WAS (Table 4). The average plant height ranged from 4 -54cm, plant girth from 0.03 – 1.87 mm, and the number of leaves from 1 – 4. Genotype G36 gave the tallest seedlings (54cm), while G8 gave the shortest seedlings (4cm). The thinnest and thickest girth were obtained in G60 (0.03mm) and G27 (1.87mm), respectively. Four genotypes (G50, G55, G44, and G35) produced more leaves (4 leaves each) while three (G8, G12, and G57) produced the least (1 leaf each).

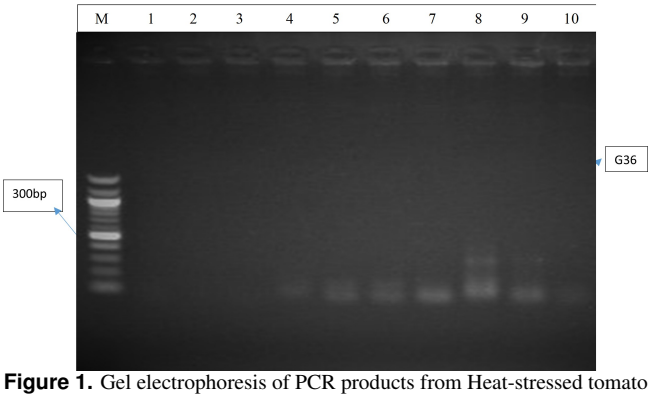

Figure 1. Gel electrophoresis of PCR products from Heat-stressed tomato leaf and seed samples using *Lepsl*-1/ *Lepsl*-2 specific primer. Lanes: 1-4 (Heat-stressed leaf samples), Lanes: 6-9 (Heat-stressed seed samples), Lane 5: Negative control (Heat susceptible genotype) and Lane 10: Negative control (Sterile nuclease-free water). M= 100bp DNA ladder (100 – 10000)

Influence of seeds treated with hot water on vegetative growth at six weeks after sowing (WAS)

There was no significant difference among the genotypes for plant height and girth. However, there was significant difference among the genotypes for the number of leaves.

Chlorophyll content, leaf area, plant biomass, root volume and sturdiness among germinated tomato genotypes

Table 6 shows a comparison of chlorophyll content, leaf area, plant biomass, and root volume among the 21 surviving tomato genotypes. The results indicated that all the parameters studied were significantly ($P \leq 0.05$) influenced by the tomato genotypes. Chlorophyll synthesis was maximum in genotype G21 (16.80 $cmolcm^{-3}$) compared to the lowest 2.20 $cmolcm^{-3}$ recorded for G23. Genotype G26 recorded the highest leaf area of 47.88 cm^2. Additionally, shoot and root dry weights were very high in tomato genotype G36 (1.32 g and 0.92 g, respectively), while genotype G8 yielded 0.013g and 0.007g, the lowest shoot and root dry weights, respectively. Genotypes G7 and G5 had massive root volumes (4.67 cm^3 each), while G23 produced the smallest root volume (1.10cm^3). G7 recorded the lowest mean value, followed by the following genotypes 37, 15, 44, 55 and 57 for sturdiness

Discussions

Effect of hot water as heat stress-inducing agent on seed germination of tomato Genotypes

Seed treatment with hot water induces heat stress during seed germination (Permana et al., 2017). In this study, a progressive reduction in the germination percentage of tomato seeds was found when the seeds were exposed to hot water at temperatures above 50°C for more than 1 hr. The results suggest that long-term high temperatures in the tropics if left unmanaged, may severely reduce seed viability and germination in tomato. Secondly, the finding supports hot water as a stress agent that can be used to test heat stress tolerance in the tomato genotypes.

From this study, genotypes GPT14, U13, GPT28, T03 performed better in terms of germination percentage with geno-

Table 2. Mean germination measurement

TREATMENT	G (%)	MGT/(days)	MGR	CVt	U	Z
Temperature/°C * Time/Hr						
40°C * 0.5Hr	100.0 c	4	0.25 b	0.00	0.00 a	1.00 e
45°C * 0.5Hr	95.2 c	3	0.32 c	7.59	0.27 abc	0.87 de
50°C * 0.5Hr	98.4 c	3	0.33 c	5.44	0.27 abc	0.87 de
55°C * 0.5Hr	98.4 c	3	0.31 c	8.85	0.50 bcd	0.74 cd
40°C * 1Hr	95.2 c	4	0.25 b	4.17	0.28 abc	0.87 de
45°C * 1Hr	100.0 c	3	0.33 c	5.66	0.29 abcd	0.85 cde
50°C * 1Hr	93.7 c	3	0.32 c	8.22	0.24 ab	0.87 de
55°C * 1Hr	81.0 b	4	0.23 b	16.09	1.05 e	0.36 b
40°C * 2Hr	96.8 c	4	0.25 b	0.00	0.00 a	1.00 e
45°C * 2Hr	98.4 c	3	0.33 c	3.07	0.18 ab	0.91 de
50°C * 2Hr	93.7 c	3	0.31 c	11.88	0.63 d	0.69 c
55°C * 2Hr	33.3 a	3	0.18 a	8.71	0.59 cd	0.14 a

*Means followed by the same letters are not significantly different according to the LSD test at 5%. G%= germination percentage MGR = mean germination rate, MGT= mean germination time, CVt = coefficient of variation in germination time, U = uncertainty in germination, Z = synchrony in germination

types U13 and T03 recording the highest germination percentages; this indicates that these genotypes were highly tolerant to the heat stress induced by the hot water (55°C for 2hours) treatment. This agrees with what Song et al. (2005) reported, that the initial establishment of plant species in high-temperature areas is related to the germination response of the seeds to heat stress and early establishment usually determines the plant's survival to the maturity stage. Also, Fahad et al. (2017) reported that, seed germination potential was affected negatively by high temperature leading to reduced seed viability.

Vegetative growth response of seeds treated with hot water

At four weeks after sowing, significant differences were observed in the mean plant height among the tomato genotypes. Genotype GPT28 recorded the tallest height, while genotype CRI4 had the shortest. This suggests that, at this developmental stage, genotype GPT28 had a competitive advantage in intercepting sunlight, which is essential for efficient photosynthesis. Plant height is often associated with a genotype's competitive ability for light, especially in early growth stages, and it is a key trait linked to photosynthetic efficiency and biomass accumulation (Behera et al., 2020; Kumar et al., 2021). An increase in stem girth was observed from week to week across all genotypes, reflecting a general improvement in seedling vigour. Stem girth is often correlated with plant robustness and the capacity to transport water and nutrients efficiently (Kumar et al., 2021). The number of leaves per plant also increased progressively, indicating an enhanced photosynthetic capacity. A greater number of leaves facilitates increased surface area for light absorption and carbon dioxide uptake, both of which are critical for effective photosynthesis (Hu et al., 2020). Thus, genotypes producing more leaves may be better adapted to accumulate biomass and support growth. Significant differences were also noted among the genotypes for chlorophyll content, leaf area, shoot dry weight, root dry weight, and root volume. Chlorophyll plays a vital role in photosynthesis by enabling plants to absorb light energy. In this study, genotype Pectomech exhibited the highest chlorophyll content, implying a greater capacity to capture light energy for the synthesis of carbohydrates. Variation in chlorophyll content among genotypes has been previously reported, with implications for differences in photosynthetic efficiency and overall plant performance (Behera et al., 2020; Gandhi et al., 2022). Similarly, leaf area is a key determinant of a plant's ability to intercept solar radiation. Genotype GPT14, which recorded the highest leaf area, likely had an advantage in intercepting more light and absorbing more carbon dioxide, leading to higher photosynthate production. The amount of dry matter produced by a plant is closely linked to its leaf area, as larger leaves capture more light and contribute to greater assimilate production (Hu et al., 2020; Li et al., 2020). Together, these findings suggest that genotypic differences in morphological and physiological traits such as plant height, leaf number, chlorophyll content, and leaf area have direct implications for the growth potential and competitive ability of tomato seedlings.

Molecular confirmation of abiotic stress tolerance in promising tomato genotypes

Plants are capable of adapting to biotic and abiotic stresses through genetic modifications, including mutations and insertions or deletions (indels). These genetic modifications may result in transcriptional regulation or post-translational modifications of stress-related genes and proteins. Chang et al. (2007) reported that, for plants to balance the homeostasis of cellular proteins under heat stress, plant cell up-regulates several heat inducible genes, commonly referred as "heat shock genes" (HSGs). HSPs are broadly divided into two prominent families: the low and large molecular weight HSPs. They are further divided into five major classes: HSP100, HSP90,

Table 3. Mean germination measurements and tolerance of 71 tomato genotypes to heat stress imposed using hot water

Genotype	G (%)	Tolerance rating	MGR	MGT (days)	CVt	U	Z
G48	86.67 f	HT	0.14 fgh	7	24.99 cd	1.87 efg	0.27
G35	86.67 f	HT	0.08 bcdef	12	13.85 abcd	2.20 fg	0.14
G36	84.44 f	HT	0.16 gh	7	19.69 bcd	1.60 def	0.32
G27	75.56 f	HT	0.11 efgh	9	29.25 d	2.59 g	0.10
G7	37.78 e	MT	0.06 abcde	16	15.22 abcd	1.83 efg	0.12
G63	24.44 de	MS	0.04 abcd	11	6.46 ab	1.02 bcde	0.16
G13	23.07 cde	MS	0.07 bcde	14	16.64 abcd	0.83 abcd	0.44
G55	23.01 cde	MS	0.10 defg	11	29.77 d	1.25 cde	0.14
G50	20.00 bcd	MS	0.09 cdefg	11	19.88 bcd	1.31 cdef	0.03
G37	14.29 abcd	MS	0.17 h	7	15.15 abcd	0.67 abc	0.00
G44	14.29 abcd	MS	0.08 bcdef	13	6.06 ab	0.53 abc	0.50
G21	14.28 abcd	MS	0.07 bcde	15	16.06 abcd	1.00 bcde	0.00
G23	14.28 abcd	MS	0.05 abcde	9	4.56 ab	0.33 ab	0.00
G12	14.27 abcd	MS	0.04 abcd	11	4.27 ab	0.83 abcd	0.11
G15	13.89 abcd	MS	0.07 bcde	14	8.16 abc	0.67 abc	0.00
G26	13.33 abcd	MS	0.09 cdef	13	7.31 abc	0.86 abcd	0.00
G45	9.53 abcd	MS	0.05 abcde	10	2.22 ab	0.31 ab	0.11
G1	8.89 abcd	MS	0.03 abc	3	9.71 abc	0.50 abc	0.06
G14	7.70 abc	MS	0.05 abcde	9	0.00 a	0.00 a	0.33
G57	7.14 abc	MS	0.08 bcdef	6	15.71 abcd	0.33 ab	0.00
G60	6.67 ab	MS	0.08 bcdef	7	0.00 a	0.00 a	0.00
G20	5.56 ab	MS	0.02 ab	5	0.00 a	0.00 a	0.00
G68	4.77 ab	MS	0.11 efgh	4	0.00 a	0.00 a	0.00
G69	4.77 ab	MS	0.04 abcd	12	0.00 a	0.00 a	0.00
G18	3.03 a	MS	0.02 ab	6	0.00 a	0.00 a	0.00
G8	2.22 a	MS	0.02 ab	5	0.00 a	0.00 a	0.00
G58	2.22 a	MS	0.02 ab	6	0.00 a	0.00 a	0.00
G2	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G3	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G4	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G5	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G6	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G9	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G10	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G11	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G16	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G17	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G19	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G22	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G24	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G25	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G28	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G29	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G30	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G31	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G32	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G33	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G34	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G38	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00

ϒTolerance rating based on seed germinability (%): >60% = highly tolerant (HT); 30–60% =moderately tolerant (MT); 1–30 =moderately susceptibly (MS); <1% = highly susceptible (HS).

Table 3 continue...

Genotype	G (%)	Tolerance rating	MGR	MGT (days)	CVt	U	Z
G39	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G40	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G41	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G42	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G43	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G46	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G47	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G49	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G51	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G52	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G53	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G54	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G56	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G59	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G61	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G62	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G64	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G65	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G66	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G67	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G70	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00
G71	0.00 a	HS	0.00 a	0	0.00 a	0.00 a	0.00

Table 4. Seedling height, girth, and number of leaves produced by germinated tomato genotypes at four WAS

Genotype	Plant Height (cm)	Plant Girth (mm)	Number of Leaves
G7	1.5 a^{X}	1.41 fg	3 bcde
G8	0.4 a	0.16 a	1 a
G12	1.2 abc	0.51 abcd	1 ab
G13	2.8 cde	0.86 bcde	2 abcde
G15	1.9 abcd	1.03 def	2 abcde
G21	1.9 abcd	1.07 ef	2 abcd
G23	2.5 bcd	0.90 cdef	2 abc
G26	2.9 def	1.66 g	3 bcde
G27	4.5 fg	1.87 g	3 bcde
G35	1.8 abcd	0.43 abc	4 e
G36	5.4 g	1.02 def	3 bcde
G37	2.7 cde	0.42 abc	3 cde
G44	2.2 bcd	0.35 ab	4 de
G45	1.0 ab	0.08 a	2 abc
G48	4.3 efg	0.53 abcd	3 bcde
G50	1.5 abcd	0.24 a	4 de
G55	3.0 def	0.53 abcd	4 de
G57	2.1 bcd	0.34 ab	1 ab
G60	1.4 abcd	0.03 a	2 abcd
G63	2.3 bcd	0.18 a	2 abcde
G68	2.5 bcd	0.43 abc	2 abcde

*Means followed by the same letters in a column are not significantly different based on the LSD test at 5%

Table 5. Seedling height, girth, and number of leaves produced by germinated tomato genotypes at six WAS

Genotype	Plant Height (cm)	Plant Girth (mm)	Number of Leaves
G7	80.3	2.37	4 bcd
G8	25.7	0.52	1 a
G12	67.0	1.39	2 ab
G13	112.0	2.07	4 bcd
G15	90.7	2.50	5 d
G21	112.0	1.98	4 bcd
G23	71.3	1.42	2 abc
G26	129.3	2.42	4 bcd
G27	111.0	2.56	4 bcd
G35	89.7	2.17	6 d
G36	122.7	2.79	4 bcd
G37	89.7	2.55	5 d
G44	90.7	2.41	6 d
G45	65.0	1.10	3 abcd
G48	114.7	2.65	5 cd
G50	94.7	1.76	5 d
G55	83.0	2.13	4 bcd
G57	67.7	1.72	2 abc
G60	74.7	1.67	4 bcd
G63	90.3	1.69	4 bcd
G68	91.3	1.77	3 abcd

[X]Means followed the same letters in a column are not significantly different based on the LSD test at 5%

Table 6. Mean seedling chlorophyll content, leaf area, shoot, and root dry weight, root volume and sturdiness produced by 21 tomato genotypes under heat stress

Genotype	Chlorophyll content (cmol/cm^3)	Leaf Area (cm^2)	Shoot Dry Weight (g)	Root Dry Weight (g)	Root Volume (cm^3)	Sturdiness (Height/Girth)
G7	12.77 de[X]	24.52 bcdef	0.44 bcdef	0.26 cdefg	4.67 c	3.39 a
G8	2.27 a	5.70 a	0.01 a	0.01 a	1.33 a	4.90 fghij
G12	8.50 abcd	11.79 ab	0.17 abc	0.12 abcde	2.50 ab	4.82 efghij
G13	8.87 abcd	32.31 cdefg	0.27 abcde	0.21 bcdefg	3.70 bc	5.39 hijk
G15	6.60 abcd	22.73 abcde	0.21 abcd	0.13 abcdef	4.67 c	3.66 bcd
G21	16.80 e	41.24 fg	0.27 abcde	0.18 abcdefg	2.60 ab	6.19 k
G23	2.20 a	19.15 abcd	0.14 ab	0.09 abc	1.10 a	5.11 ghijk
G26	10.60 cde	47.88 g	0.46 cdef	0.29 efg	2.10 a	5.36 hijk
G27	7.47 abcd	38.19 efg	1.19 hi	0.66 h	2.07 a	4.35 bcdefgh
G35	6.83 abcd	29.55 bcdef	0.97 gh	0.70 h	1.70 a	4.12 bcdefg
G36	10.00 bcde	30.78 cdefg	1.32 i	0.92 i	2.37 ab	4.39 bcdefgi
G37	5.10 abc	30.04 cdefg	0.50 def	0.31 fg	1.50 a	3.52 bc
G44	9.53 bcd	36.01 defg	0.54 ef	0.33 g	1.83 a	3.77 bcde
G45	3.07 ab	17.64 abc	0.17 abc	0.12 abcde	1.27 a	5.86 jk
G48	6.30 abcd	29.88 bcdefg	1.24 hi	0.59 h	2.40 ab	4.33 bcdefgh
G50	6.00 abcd	25.31 bcdef	0.45 cdef	0.23 bcdefg	2.37 ab	5.37 hijk
G55	4.10 abc	34.77 cdefg	0.57 ef	0.24 bcdefg	2.17 ab	3.91 bcdef
G57	8.73 abcd	18.90 abcd	0.22 abcd	0.10 abcd	1.33 a	3.96 bcdef
G60	4.93 abc	27.10 bcdef	0.15 abc	0.07 ab	1.50 a	4.63 defghi
G63	4.00 abc	30.40 cdefg	0.75 fg	0.28 defg	1.87 a	5.44 ijk
G68	8.40 abcd	21.08 abcde	0.17 abc	0.07 ab	1.53 a	5.17 ghijk

[X]Means followed by the same letters in a column are not significantly different based on the LSD test at 5%

HSP70, HSP60, and low molecular weight or small HSP (Wang et al., 2004). They identified that, small HSPs were the most prevailing heat shock proteins in plants for which under heat stress, their expression can rise to 200 folds Wang et al. (2004). Driedonks et al. (2015) added that, small heat shock proteins (sHsp) positively affect thermo-tolerance by maintaining the threshold levels of ROS scavenging enzymes that could initiate the signalling pathway of thermo-tolerance. Liu et al. (2006) reported on a novel HSP gene, *Hsa32*, which encodes an HS-associated 32 kDa protein. They found out

Table 7. Presence/Absence of heat-tolerant gene in tomato leaf and seed samples

Genotypes	Lab Code	Present (300bp)	Absent (300bp)
G27	1		-
G35	2		-
G36	3		-
G48	4		-
G21	5		-
G27	6		-
G35	7		-
G36	8	+	
G48	9		-

*(+) = Presence of Heat-tolerant gene
*(-) = Absence of Heat-tolerant gene

that the gene was present in tomato plants under heat stress and conferred thermo-tolerance. In view of the reports by Liu et al. (2006), we observed that genotypes GPT14, U13 and T03 were tolerant to the heat stress, however, they did not exhibit the expression of the HSP gene *Hsa32* in both leaves and seeds. This may be attributed to the fact that abiotic stress tolerance is controlled by multiple genes Avni et al. (2008), an indication that other HSP genes controlled the heat stress tolerance expressed by these genotypes. Furthermore, the presence of this novel gene in genotypes GPT28 (seeds) confirms that the gene *Hsa32*, control the heat tolerance of this genotype.

Conclusion

Out of the 71 genotypes studied, only four (GPT14, U13, GPT28, and T03) demonstrated the ability to tolerate heat stress induced by the hot water treatment. Among these heat-tolerant genotypes, GPT28 notably expressed the HSP gene *Hsa32*. This novel hot water protocol has proven effective for inducing heat stress in seeds. Further research is recommended to explore the presence of *Hsa32* gene in tomato and other crop genotypes cultivated in Ghana.

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