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Impact of hydropriming on germination and seedling establishment of sunflower seeds at elevated temperature

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Abstract: High temperature is a limiting factor in the seed germination of most crops. This study evaluated the effects of hydropriming at 6, 12 and 18 h on germination performance and seedling establishment of sunflower seeds under high air temperature. Results showed that germination of unprimed seeds was suppressed at an average elevated temperature of 44.3 °C (range of 39.3 °C to 53.3 °C) for eighteen days indicated by an increased lag time to onset of germination and decreased germination percentage. Conversely, priming seeds for 12 h to 18 h increased the germination percentage, time to 50% seedling emergence (T_{50}), germination index and vigour index. Seedlings emerging from primed seeds exhibited uniform 16-day old seedlings (18 days after sowing), leading to greater seedling dry weight and shoot length as compared to unprimed seeds. Conversely, the total chlorophyll content remain unchanged for all seeds. The significant increase in the shoot parameters suggested a positive association with priming and stress tolerance. The priming duration of 12 h to 18 h showed improvement at elevated air temperature through the reduction of ungerminated seeds and increase in seedling growth characteristics.

Keywords: *Helianthus annuus* L.; stress factor; heat; high-temperature condition; climate change

Changes in temperature occur faster than any other environmental stress factors brought about by climate change. It is expected that there will be an increase of 2.3 ± 0.3 °C thresholds in world temperature in the next few years, as reported by Brown and Caldeira (2017). This poses a threat to many crops as the high temperature is known to negatively affect the survival and germination of seeds.

Sunflower (*Helianthus annuus* L.) is an economically important crop cultivated for its edible oil and of significant value to the ornamental industry. Its susceptibility to heat stress severely affected its growing cycle resulting in low yield (Debaeke et al. 2016). This is attributed to poor germination and subsequent seedling establishment caused by supra-optimal temperature during sunflower production. The increase in temperature is inevitable because of the ongoing environmental crisis. Moreover, adverse effects are more evident among low-vigour seeds resulting in a greater decrease in germination percentage and lower yield.

Seed priming is a promising technology to improve seed performance and offset the negative effects of abiotic stresses (Tabassum et al. 2017). There

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are several methods of priming that uses various chemicals and techniques in many seeds. Among these, hydropriming is considered the pragmatic approach as it is a cost-effective, safe and eco-friendly method of priming. The maximum efficiency of priming can be achieved with the appropriate duration. Priming duration is a crucial factor as it may affect the initiation of biochemical events necessary for early germination. In onion seeds, the most effective way for improving germination percentage is by hydropriming for a longer duration of 96 h (Caseiro et al. 2004). Conversely, the highest germination percentage and seedling dry weight for pinto beans were reported to be at a shorter priming duration of 7 h to 14 h (Ghassemi-Golezani et al. 2010). This indicates that a standard priming duration cannot be established as seeds have different physiological responses. Hence, it is vital to determine the specific optimum duration for priming each crop and cultivar of sunflower seeds to induce heat tolerance.

The beneficial effects brought about by priming is often tested when seeds are exposed to stress. The growth performance of primed seeds under unfavourable conditions improved as manifested by an increase in germination percentage, rate and uniform emergence of seedlings (Lemmens et al. 2019). The results obtained in these experiments are often carried out in controlled chambers, having one variable at a time and a constant temperature during the entire observation period of the crop. Thus, responses are not comparable to experiments where seeds are subjected to the natural environment.

The ability of seeds to germinate rapidly and the uniformity of seedling establishment are the parameters being assessed since these are the crucial stages in crop production (Eskandari 2013). The growth attributes of the seedlings are likewise of equal importance. Taking each aspect into account, this research was carried out to evaluate the potential of hydropriming low-vigour sunflower seeds at different durations and determine its germinative responses and seedling performance under high-temperature conditions.

MATERIAL AND METHODS

Seed materials and priming. Naturally-aged F_1 hybrid sunflower seeds (*Helianthus annuus* L. cv. Aguara 6) with an initial moisture content of 5.65 ± 0.5% were provided by a seed company in Thailand. Preliminary tests were done to verify viability and vigour of the seeds (germination percentage for standard test and accelerated ageing test are 95% and 83%, respectively).

Priming was done by putting 50 g of seeds on a sieve tray and placing it on a container with 75 mL water for either 6, 12, or 18 h at 20 °C. Thereafter, the imbibed seeds were dried at 40 ± 2 °C for about 24 h or until moisture content was close to the initial level. All primed seeds were allowed to acclimatise inside a desiccator at room temperature for two days before running the germination tests. Unprimed seeds were used as the control.

Standard germination test (SGT) and index (SGI) under laboratory conditions. Primed seeds (6, 12 and 18 h) and control (0 h) were germinated at 25 °C using between paper method (ISTA 2007). Each treatment had four replicates with 100 seeds per replicate. The normal seedlings were counted on the 4th (SGT4) and 10th day (SGT10) based on standard criteria. The germination percentage and ungerminated seeds (SUGS) were calculated based on ISTA (2007).

The normal seedlings that emerged daily for a period of ten days were also counted, and the standard germination index was calculated as:

$$SGI = \Sigma (n/d)$$

where: n – number of seedlings emerging on day d; d – days from sowing (Gupta 1993).

Seed germination and seedling cultivation under greenhouse conditions. Black plastic bags (15 × 6 cm) were filled with the soil-like substrate (Mai long mai ru, Thailand). A total of 400 seeds (4 replicates × 100 seeds) per treatment were individually sown in each plastic bag at a depth of 2 cm. The seeds were grown in a greenhouse with natural dawn and dusk transition of photoperiod and were watered thrice a week with tap water until the eighteenth day.

Greenhouse temperature and relative humidity. The air temperature and relative humidity were monitored using MicroLite III temperature logger (LITE5032L-RH, Fourtec, Israel). The data obtained were dependent on the daily local weather. The average maximum air temperature in the greenhouse within the experimental period of eighteen days is 44.3 °C, with the highest on the 3rd day (53.3 °C) (Figure 1A). The range of the maximum temperature is 45.6 °C to 53.3 °C for day 1 to 4, 39.9 to 47.7 °C for day 5 to 10 and 39.3 to 44.7 °C for day 11 to 18. The temperature gradually increased by daytime and dropped by night time. The average minimum temperature is 26.9 °C.

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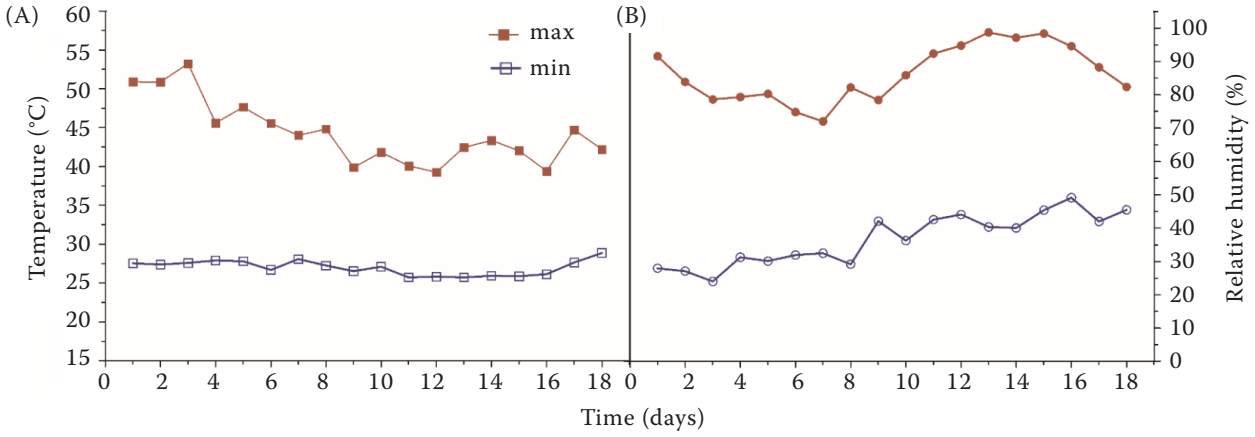

Figure 1. Daily maximum and minimum (A) air temperature and (B) relative humidity of the greenhouse starting from the day the seeds were sown up to the 18th day

On the other hand, the relative humidity (RH) is relatively low during the day and high by night time. The average maximum and minimum RH is 86.14% and 36.7%, respectively (Figure 1B).

Germination percentage and indices at elevated temperature under greenhouse conditions. The number of normal seedlings were recorded after four days (GT4) and ten days (GT10). Seedlings were considered normal when the first pair of true leaves spread out. The germination percentage, ungerminated seeds percentage (UGS) and germination index (GI) at elevated temperature were calculated accordingly.

The normal seedlings were counted daily for ten days, and time to 50% seedling emergence (T_{50}) was calculated based on the equation from Farooq et al. (2005):

$$T_{50} = t_i + \frac{(N/2 - n_i)(t_c - t_i)}{n_c - n_i}$$

where: N – total number of seedlings emerged; n_c – cumulative number of seedlings at times t_i and t_c, respectively, when $n_i < N/2 < n_c$.

The vigour index (VI) was calculated following Vashisth and Nagarajan (2010):

VI = germination % (GT10) × seedling dry weight (SEDW).

Seedling growth attributes. Eighteen days after sowing, twenty bags per replicate were randomly selected, and the seedlings were harvested. The soil particles were carefully washed off from the roots, and thereafter, the seedling length (SEL), root length (RL), and shoot length (SHL) were measured. The sampled roots and shoots were subsequently dried at 60 °C for 48 h to determine each dry weight (DW): seedling (SEDW), shoot (SHDW) and root (RDW) (Imran et al. 2018).

Another set of ten seedlings per replicate were harvested for the measurement of the leaf (LFW) and stem fresh weight (SFW). Total chlorophyll (TC) from the leaf samples was quantified based on the method of Witham et al. (1971).

Statistical analysis. A complete randomised block design with four replicates for each treatment was used in the experiment. Percentage values were arcsine-transformed before further analysis. Significant differences were tested by analysis of variance (ANOVA) and means separated using Fisher's least significant difference (*LSD*) at $P \leq 0.05$ and statistical association evaluated by Pearson correlation test using SPSS software (Version 17.0, SPSS Inc. Chicago, USA).

RESULTS

Effect of priming on germination at standard conditions (ISTA 2007). All seeds exhibited a high standard germination percentage of 91% to 94% after ten days showing no statistically significant difference among the treatments (data not shown).

The seeds primed for longer durations exhibited a higher standard germination index (Figure 2). A strong positive correlation was established between priming and SGI (r = 0.865), as shown in Table 1.

Effect of priming on germination percentage and vigour at elevated temperature. The germination percentage of the seeds under high temperature displayed statistically significant differences on the

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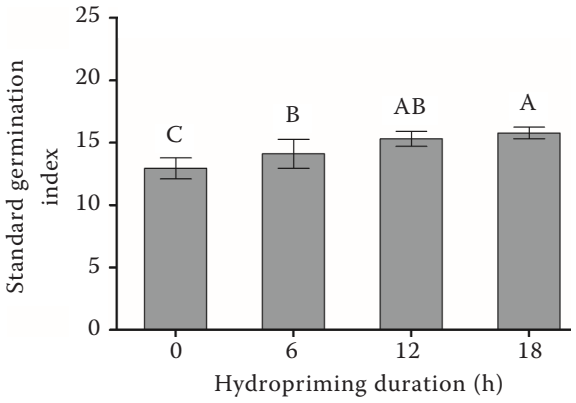

Figure 2. Effect of hydropriming duration on germination index of sunflower seeds at standard conditions. Bars with different letters show significant differences at $P \leq 0.05$ according to Fisher's least significant difference test. Error bars represent standard deviation

4th (45.6 °C) and 10th (41.84 °C) day after planting (Figure 3A). Seeds primed for 12 h and 18 h improved germination on both counts as compared to the control. After the final count, 18 h-primed seeds showed the highest germination with an average increase of 29% from the control and 13% to 19% from seeds primed for shorter durations. On the other hand, the percentage of ungerminated seeds (Figure 3B) significantly decreased with a longer priming duration of 12 h and 18 h. The decrease in 18 h-primed seeds was 60% compared to the control.

The time to 50% seedling emergence (T_{50}) also decreased among all primed seeds (Figure 3C). The daily count of normal seedlings (Figure 3D) showed distinct differences among treatments starting by the 4th day wherein 12 h-primed seeds increased by two folds and the 18 h-primed seeds reached a count unattainable by the control even until the 10th day. Likewise, the germination index significantly increased among primed seeds in reference to the control with the peak in seeds primed for18 h (Figure 3E). The vigour index showed an increase when seeds were primed for 12 h and 18 h with a 37% to 43% difference from the control (Figure 3F).

All the above-mentioned parameters at elevated temperature showed a strong positive (r = 0.773 to 0.894) and negative (r = –0.799 to –0.805) correlation with priming, as reflected in Table 1.

Effect of priming on the seedling growth attributes. The seedlings that emerged from primed seeds significantly increased in growth attributes such as seedling DW, shoot length, shoot DW, leaf FW, and

Table 1. Pearson correlation coefficient (r) calculated between seed hydropriming and germination percentages, indices and seedling growth attributes

	PRMG	SGT4	SGI	GT4	GT10	T_{50}	GI	VI	UGS	SEDW	SHL	SHDW	LFW	SFW
PRMG	1													
SGT4	**0.893**	1												
SGI	**0.865**	**0.993**	1											
GT4	**0.773**	0.668**	0.636**	1										
GT10	**0.800**	0.687**	0.656**	**0.910**	1									
T_{50}	**–0.799**	**–0.800**	**–0.804**	**–0.745**	–0.630**	1								
GI	**0.894**	**0.789**	**0.757**	**0.922**	**0.948**	**–0.801**	1							
VI	**0.866**	0.687**	0.643**	**0.821**	**0.878**	–0.671**	**0.914**	1						
UGS	**–0.805**	–0.671**	–0.635**	**–0.927**	**–0.983**	0.661**	**–0.957**	**–0.874**	1					
SEDW	0.694**	0.547*	0.520*	0.614*	0.566*	–0.652**	0.684**	**0.838**	–0.599*	1				
SHL	0.608*	0.426	0.400	0.537*	0.503*	–0.486	0.606*	**0.765**	–0.535*	**0.945**	1			
SHDW	**0.741**	0.595*	0.574*	0.604*	0.563*	–0.684**	0.705**	**0.839**	–0.588*	**0.976**	**0.947**	1		
LFW	**0.823**	**0.810**	**0.790**	0.526*	0.594*	–0.670**	0.691**	0.639**	–0.634**	0.531*	0.422	0.587*	1	
SFW	**0.764**	0.723**	0.697**	0.597*	0.613*	–0.634**	0.684**	0.612*	–0.662**	0.478	0.367	0.531*	**0.938**	1

Bold – correlation is significant at the 0.001 level; **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level. PRMG – priming; SGT 4 – standard germination test 4th day; SGI – standard germination index; GT4 – germination test 4th day; GT10 – germination test 10th day; T_{50} – time to 50% seedling emergence; GI – germination index; VI – vigour index; UGS – ungerminated seeds; SEDW – seedling dry weight; SHL – shoot length; SHDW – shoot dry weight; LFW – leaf fresh weight; SFW – stem fresh weight

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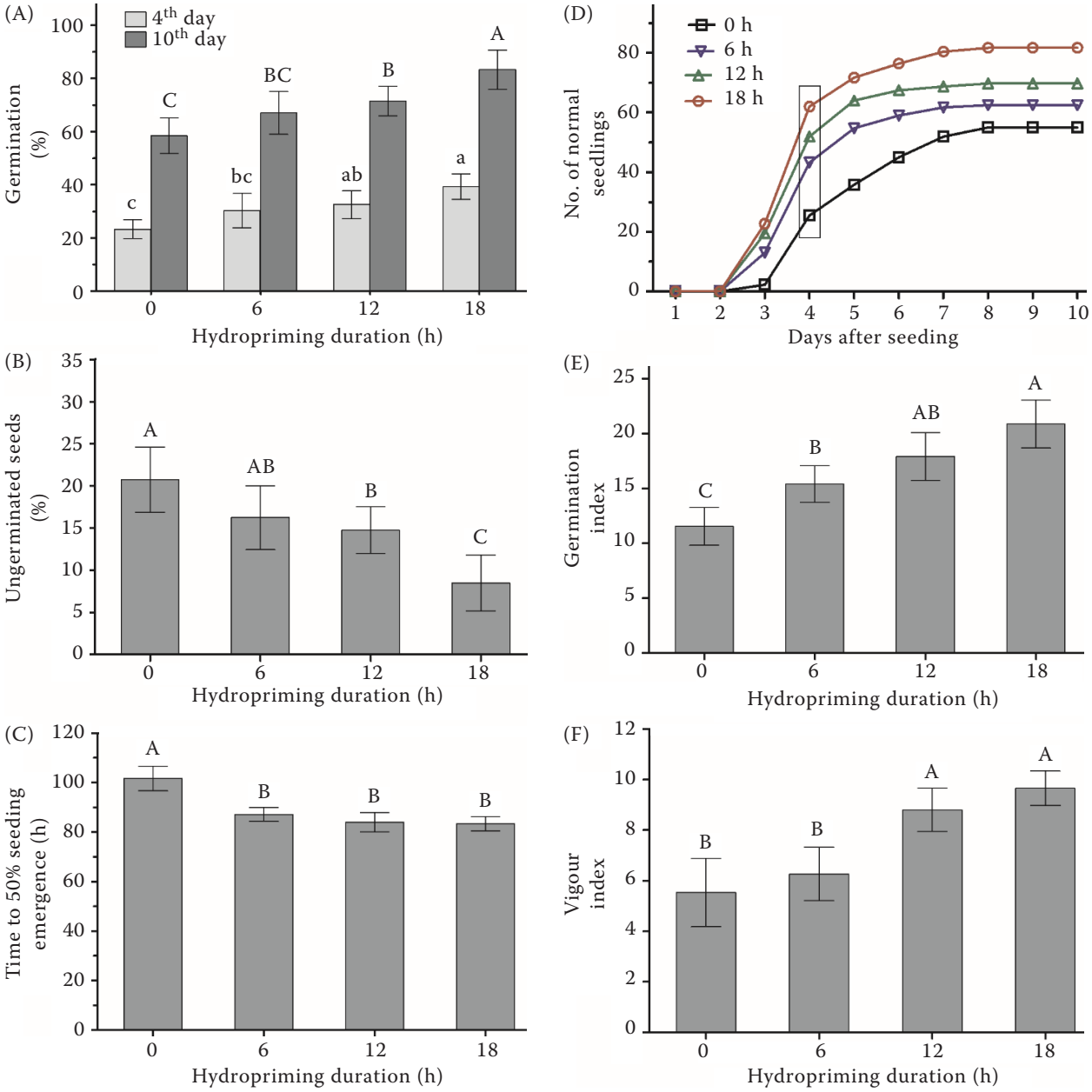

Figure 3. Effect of hydropriming duration on the (A) germination percentage; (B) ungerminated seeds; (C) time to 50% seedling emergence; (D) daily seedling emergence; (E) germination index and (F) vigour index of sunflower seeds at elevated temperature of 39.3 °C to 53.3 °C for eighteen days. Bars with different letters show significant differences at $P \leq 0.05$ according to Fisher's least significant difference test. Error bars represent standard deviation

stem FW (Figure 4, Table 2). The improvement of primed seeds over the control seeds was 13.53% to 31.10% for seedling DW, 6.90% to 17.71% for shoot length, 15.89% to 37.18% for shoot DW, 28.72% to 52.66% for leaf FW and 29.15% to 48.14% for stem FW. Moreover, Table 1 showed that these growth attributes have a strong (shoot DW, leaf FW and stem FW; r = 0.741 to 0.823) and moderate (seedling DW and shoot length; r = 0.608 to 0.694) positive correlation with priming.

DISCUSSION

Seed germination and early seedling emergence are the critical aspects for the growth and development of any crops. Priming the seeds resulted to an early

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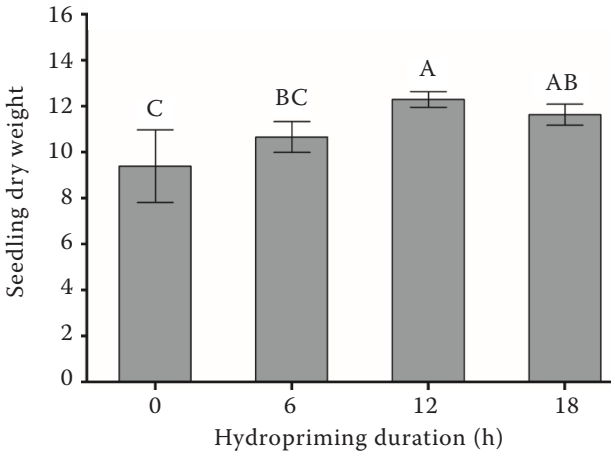

Figure 4. Effect of hydropriming duration on the seedling dry weight (g/100 seedlings) of sunflower seeds grown at elevated temperature of 39.3 °C to 53.3 °C for eighteen days. Bars with different letters show significant differences at $P \leq 0.05$ according to Fisher's least significant difference test. Error bars represent standard deviation

seedling emergence by the 4th day, but differences were not statistically significant by the 10th day as all conditions were optimum (data not shown). The significant increase in SGI (Figure 2) with longer priming durations indicated an improvement in seed vigour as it reflects both percentage and speed of germination (Kader 2005).

The results varied when seeds were planted in soil and exposed to natural conditions; the high air temperature profoundly influenced the decline in germination percentage of all seeds on both counts (Figure 3A). Such condition becomes unfavourable since the average maximum temperature of 44.3 °C (range: 39.3 °C to 53.3 °C) is way beyond the optimum germination temperature (25 °C to 30 °C) for sunflower seeds as reported by Corbineau et al. (1988). However, priming seeds for 12 h to 18 h significantly increased the germination percentage as compared to the control (Figure 3A). Primed seeds also showed earlier and more uniform germination and an improved seedling emergence as reflected in the greater T_{50}, GI and VI (Figures 3C–F). Furthermore, it took seven days for the control seeds to attain at least 50% normal seedlings, whereas primed seeds achieved this stage by the 4th day, increasing continuously until the 8th day (Figure 3D). This can be attributed to a head start among primed seeds as these seeds are partially hydrated, initiating early activities, and the stimulatory effect is conserved after re-drying (Chen and Arora 2011). Thus, the metabolic processes start sooner at a faster rate after re-imbibition.

The germination potential of seeds can be reduced at a higher temperature. Sunflower seeds have a maximum germination temperature that varies between 41.7 °C and 48.9 °C depending on genotypes (Khalifa et al. 2000). In the first three days (Figure 1A), the daytime air temperature of more than 50 °C have exceeded the threshold temperature of germination, which could result in thermoinhibition among seeds. The next days are still within the supra-optimal temperature range. This prolonged exposure to high temperature may have caused the seeds to enter into a state of thermodormancy (Hills and van Staden 2003), resulting in poor germination as reflected in Figures 3A–B. This arrest in germination can be influenced by the regulation between phytohormones abscisic acid (ABA) and gibberellic acid (GA) that are well known in controlling seed germination. The heat stress could stimulate the *de novo* synthesis of ABA and the repression of its catabolism, maintaining the

Table 2. Seedling growth attributes of sunflower seeds after priming at different time durations and exposed to elevated temperature of 39.3 °C to 53.3 °C for eighteen days

Priming duration (h)	Shoot		Root		Leaf	Stem	Total chlorophyll (mg/100 mg FW)
	length (cm)	DW	length (cm)	DW	FW		
0	12.76 ± 1.75^{b}	6.67 ± 1.07^{c}	16.59 ± 1.43^{a}	2.72 ± 0.64^{a}	18.80 ± 1.52^{c}	29.53 ± 2.28^{b}	15.60 ± 1.19^{a}
6	13.64 ± 0.64^{ab}	7.73 ± 0.46^{b}	15.58 ± 1.88^{a}	2.93 ± 0.30^{a}	24.23 ± 1.85^{b}	38.08 ± 2.96^{a}	15.15 ± 1.32^{a}
12	15.02 ± 0.42^{a}	9.15 ± 0.30^{a}	16.25 ± 2.52^{a}	3.16 ± 0.15^{a}	26.48 ± 4.08^{ab}	38.15 ± 6.46^{a}	15.08 ± 0.97^{a}
18	14.50 ± 0.49^{a}	8.62 ± 0.49^{ab}	16.58 ± 1.12^{a}	3.01 ± 0.26^{a}	28.73 ± 2.50^{a}	43.68 ± 3.81^{a}	14.71 ± 0.83^{a}

Means in the same column followed by different letters are significantly different at $P \leq 0.05$ according to Fisher's least significant difference test. Values represent the average of four replicates and standard deviation per treatment. Sampling was done on the 18th day after planting. DW – dry weight (g/100 seedlings); FW – fresh weight (g/100 seedlings)

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ABA content above the threshold level (Toh et al. 2008). This condition suppresses the action of GA, preventing germination to take place. Furthermore, a complex interplay between other factors such as impairment of major enzymes, oxidative damage and biochemical changes may also have contributed (Fahad et al. 2017).

The data of the current study underlines that priming seeds improve germination percentage by significantly decreasing the number of ungerminated seeds and faster seedling emergence (Figures 3B–D). Seed priming treatments enhance the GA/ABA ratio promoting the germination process (El-Araby et al. 2006). The re-imbibition of the primed seeds at elevated temperature may have increased the ABA content, but the high amount of GA present after priming is sufficient to counteract the effect of ABA. Conversely, the 6 h priming duration is still in the early stage of metabolic activities; therefore, the higher temperature may have regulated the action of GA through the higher ABA content.

As the seedlings emerged, the absence of hydropriming and exposure to high temperature adversely affect the control seeds in which seedling growth attributes are at the bottommost (Figure 4, Table 2). Conversely, seedlings from seeds primed for 12 h and 18 h achieved a synchronised and more uniform emergence with an improved seedling dry weight as well as increased shoot length and fresh weight. In the study of Hussain et al. (2017), priming induces the *de novo* synthesis of hydrolases, breaking down food reserves and efficiently translocate to the growing embryo. This vigorous start has provided an advantage for the seedlings to complete developmental events even at stressful conditions. Another strategy assimilated by seed priming is in the form of a priming memory (Chen and Arora 2013), wherein the process of priming imposes stress on the seeds leaving imprints that enhances tolerance to future stresses.

It is also observed that at elevated temperature, priming benefits on seedling growth focus primarily on the shoots. The seedling length showed no significant difference (data not shown). However, when taken separately, shoot length significantly increased among seeds primed for 12 h and 18 h (Table 2). Likewise, the shoot DW, leaf FW and stem FW increased among primed seeds. Our results signify that the shoot growth is a good indicator of stress tolerance among primed seeds, whereas root growth is less sensitive to heat stress. This conforms with the review of Claeys et al. (2014) on plants under stress.

On the other hand, the total chlorophyll is not affected by the priming treatment (Table 2). Zhang et al. (2015) reported that priming could prevent severe chlorophyll loss under unfavourable conditions. Moreover, enhancement in chlorophyll accumulation after priming varies depending on the priming solution used for 12 h (Anwar et al. 2020). Therefore, priming efficiency on chlorophyll content may be dependent on seed priming solution and duration and the intensity of the environmental factors that the seedlings were exposed to.

In conclusion, priming seeds at 12 h to 18 h alleviate the adverse effects of high temperature by reducing the occurrence of ungerminated seeds, faster germination and enhancing the stress tolerance, as shown by the increase in seedling growth attributes. This pragmatic approach of hydropriming is beneficial, particularly for low-vigour seeds that are challenged by the increasing temperature due to climate change. For further studies, determining the longevity and storage conditions of the primed seeds would be an advantage.

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