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RESEARCH PAPER

Exploring the potential of heat priming in improving heat stress tolerance at anthesis stage in wheat (*Triticum aestivum*. L)

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Abstract: This study investigates the effect of seed priming with heat and further exposure of the plants to heat stress at anthesis on plant physiology and yield-related traits in RAJ3765 (RAJ) and HD2967 (HD), two diverse wheat varieties. Heat stress induced a significant reduction in chlorophyll content, chlorophyll content index (CCI), and membrane stability index (MSI), which were partially alleviated by heat priming. Heat-priming enhanced the root development, with significant increase in root length, surface area, volume, fresh weight, and dry weight as compared to non-primed ones. Further more, heat-primed plants exhibited a notable improvement in flag leaf length and flag leaf width. While both RAJ and HD varieties with heat priming tended to exhibit less reduction in spike length compared to non-primed ones, the effects on spikelets per spike and grains per spike varied. Overall, heat priming positively impacted flag leaf development and spikelet formation under heat stress, with variable effects on grain yield across varieties. These findings underscore the potential of heat priming as a strategy to enhance stress tolerance and improve yield stability in wheat cultivation under heat stress conditions.

Key words: Chlorophyll, Heat stress (HS), Heat priming (HP), HD2967, Raj3765

Introduction

Wheat, a staple food for rural populations, provides essential nutrients including proteins, carbohydrates, and vitamins (Umar *et al.,* 2019). Cultivated as a *rabi* crop, wheat offers greater planting flexibility compared to rice, being sown in winter and harvested in early summer. Climate factors such as precipitation and temperature play pivotal roles in wheat production, affecting soil moisture and water availability crucial for crop development (Nelson *et al*., 2009). Recent decades have seen unprecedented global temperature increases, notably impacting India's climate with temperatures exceeding multi-century records. Elevated temperatures induce heat stress in wheat, categorized as high ambient, mild, and severe stress, posing significant challenges to crop productivity. Delayed sowing exposes wheat to terminal heat stress during grain filling, leading to yield losses attributed to pollen infertility and reduced resource capture during critical growth stages. While resistant wheat varieties offer resilience against heat stress through mechanisms like antioxidant activation and compatible solute accumulation (Farooq *et al*., 2017), agronomic strategies such as seed priming provide effective mitigation. Seed priming, involving treatment with organic or inorganic compounds, enhances germination, activates metabolic processes, and induces systemic resistance against abiotic stresses like high temperature (Hilker, 2016). Notably, hydro priming improves seedling vigor and crop growth under drought stress conditions (Adinde *et al*., 2020). Environmental changes, such as the occurrence of drought stress, present substantial challenges to agricultural efficiency, thereby requiring the advancement of crops that are tolerant to drought. In India, where several wheat zones experience high-temperature stress, the development and adoption of heat-tolerant wheat varieties are critical for ensuring food security. By integrating genetic improvements and agronomic practices, such as seed priming, with advancements in crop management, India can enhance wheat productivity and resilience to changing climatic conditions. The objective of this study was to investigate the effect of seed priming with high temperature on physiological and yield-related aspects of wheat varieties under heat stress, contributing to the development of sustainable agricultural practices in the face of global climate change.

Material and methods

Seed priming and sowing

High-temperature tolerant RAJ3765 (RAJ) (Azameti *et al.,* 2022) and sensitive HD2967 (HD) wheat seeds were selected, screened for purity, sterilized, heat-primed (HP) with 60-65 seeds soaked in 25ml water overnight at 22^{o}C, heat-stressed at 42^{o}C for 2 hours, then sown in 10-inch pots with autoclaved 8 kg soil and 1 kg vermicompost mix at greenhouse facility of IARI, New Delhi, The experiment was laid out in CRD.

Acute high temperature heat stress treatment

All plants grew together in the net house until anthesis. Post-anthesis, except control plants both primed and nonprimed plants were moved to a heat trap chamber at 11.00 am. Temperatures were recorded hourly using thermometers inside the chamber, outside, and within the net house until 3.00 pm. After recording temperatures, pots were returned to the net house with respective control plants, repeating this for three days. Following heat stress, flag leaf samples were collected, frozen in liquid nitrogen, and stored at -80^{o}C for analysis of physiological parameters.

Table 1. Analysis of heat stress effects on physiological parameters in "RAJ"(RAJ3765) and "HD"(HD2967) plant varieties with and without heat priming (HP).

Parameters	Treatments	Mean ± S. Em	% Change over Control	SD	t-stat	P ≤ 0.05
Chlorophyll content (SPAD value)	RAJ	47.20 ± 0.53	-7.39	1.07	4.919	0.014
	RAJ+HS	43.71 ± 0.24		0.48		
	HP+RAJ	48.09 ± 1.01	-5.37	2.03	4.187	0.020
	HP+RAJ+HS	45.50 ± 0.16		0.32		
	HD	46.18 ± 0.53	-9.47	1.04	4.511	0.010
	HD+HS	41.81 ± 0.25		0.51		
	HP+HD	47.17 ± 0.64	-5.13	1.29	3.645	0.011
	HP+HD+HS	44.75 ± 0.14		0.69		
Chlorophyll content index	RAJ	34.57 ± 0.03	-10.49	0.07	3.693	0.010
	RAJ+HS	30.94 ± 0.10		0.20		
	HP+RAJ	33.25 ± 0.41	-5.56	0.83	3.56	0.012
	HP+RAJ+HS	31.40 ± 0.31		0.62		
	HD	35.71 ± 0.33	-9.49	0.66	6.152	0.001
	HD+HS	32.32 ± 0.43		0.87		
	HP+HD	35.75 ± 0.27	-5.13	0.55	3.456	0.034
	HP+HD+HS	33.92 ± 0.48		0.86		
Membrane stability index (MSI)	RAJ	79.15 ± 1.31	-15.24	2.62	5.922	0.001
	RAJ+HS	67.09 ± 1.76		1.93		
	HP+RAJ	76.78 ± 0.10	-11.29	0.17	6.98	0.003
	HP+RAJ+HS	68.11 ± 0.45		0.91		
	HD	75.27 ± 0.56	-15.77	1.12	5.322	0.005
	HD+HS	63.40 ± 0.23		1.42		
	HP+HD	80.65 ± 0.80	-10.66	1.61	8.19	0.001
	HP+HD+HS	72.05 ± 0.66		1.33		

Analysis conducted on the physiological impact of heat stress (HS) in "RAJ" and "HD" plant varieties, exploring the potential of heat priming in improving including chlorophyll content, chlorophyll content index (CCI), and membrane stability index (MSI). The analysis was performed using independent sample t-tests with a significance level of $p < 0.05$.

Physiological parameters

Chlorophyll Content was determined using standard protocols (Ling *et al*., 2011). Membrane Stability Index (MSI) was determined by adopting the method suggested by Blum, A. and Ebercon, A. (1981). Root phenotyping using root scanner by adopting the method suggested by Hobson *et al*. (2023).

Yield parameters

The yield traits such as spike length (cm), spike weight (gm), number of spikelets per spike, and thousand-grain weight (gm) will be recorded (Swamy *et al*., 2021)

Results and discussion

Impact of heat stress and heat priming on plant physiology

Heat stress led to significant reductions in chlorophyll content, chlorophyll content index (CCI), and membrane stability index (MSI) in both "RAJ" and "HD" varieties. Without heat priming, chlorophyll content decreased by 7.39% (p = 0.014) and 9.47% (p = 0.01) for "RAJ" and "HD" respectively, while with heat priming, decreases were 5.37% (p = 0.02) and 5.13% (p = 0.011) respectively. Similarly, CCI decreased by 10.49% (p = 0.01) and 9.49% (p = 0.001) respectively without heat priming, and by 5.56% (p = 0.012) and 5.13% (p = 0.034) respectively with heat priming. MSI reductions were also notable, with decreases of 15.24% (p = 0.001) and 15.77% (p = 0.005) without heat priming, and 11.29% (p = 0.003) and 10.66% (p = 0.001) with heat priming for "RAJ" and "HD" respectively

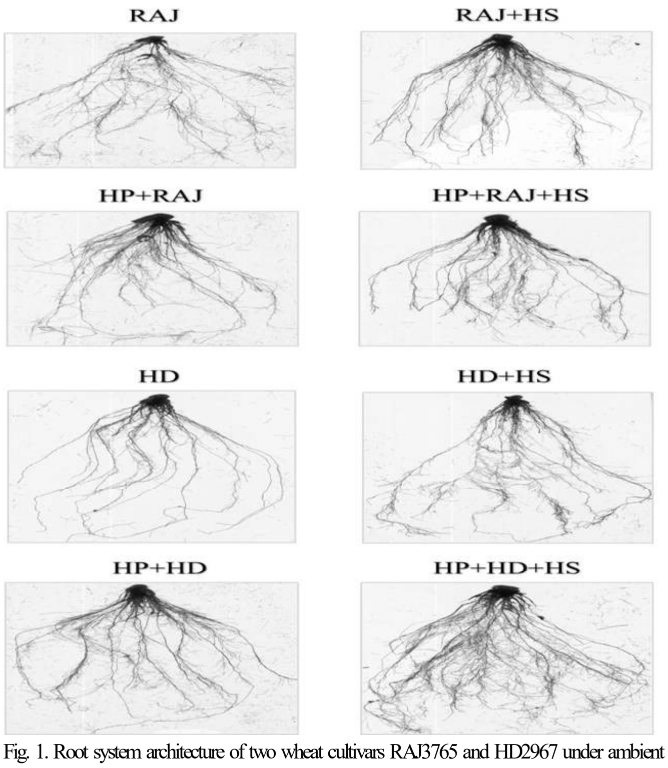

Fig. 1. Root system architecture of two wheat cultivars RAJ3765 and HD2967 under ambient and heat stress condition. RAJ: RAJ3776 and HP+RAJ: heat primed RAJ3765 both grown under ambient temperature. RAJ+HS: RAJ3765 exposed to heat stress, HP+RAJ+HS: heat primed RAJ3765 exposed to heat stress. HD: HD2967 and HP+HD: heat primed HD2967 both grown in ambient temperatue. HD+HS: HD2967 exposed to heat stress, HP+HD+HS: Heat primed HD2967 exposed to heat stress.

Table 2. Comparative analysis of root morphological parameters under heat stress conditions with and without heat priming, analyzed using independent sample t-Test ($p < 0.05$) in SPSS software.

Parameters	Treatments	Mean ± S. Em	% Change over Control	SD	t-stat	P ≤ 0.05
Root length (cm)	RAJ	1825.79 ± 17.06	4.89	34.13	4.029	0.007
	RAJ+HS	1915.05 ± 14.12		28.25		
	HP+RAJ	1908.66 ± 31.85	10.42	63.71	5.112	0.003
	HP+RAJ+HS	2107.62 ± 22.35		44.71		
	HD	1742.25 ± 27.53	3.50	55.07	1.745	0.132
	HD+HS	1803.25 ± 21.53		43.06		
	HP+HD	1768.72 ± 24.41	8.55	48.83	3.071	0.022
	HP+HD+HS	1919.95 ± 42.76		85.52		
Surface area (cm^2)	RAJ	147.42 ± 1.54	5.60	3.09	2.54	0.044
	RAJ+HS	155.68 ± 2.84		5.69		
	HP+RAJ	178.59 ± 2.23	12.15	4.47	3.82	0.018
	HP+RAJ+HS	200.29 ± 5.20		10.41		
	HD	160.93 ± 0.69	4.47	1.39	4.52	0.054
	HD+HS	168.13 ± 2.20		4.40		
	HP+HD	175.99 ± 1.40	12.63	2.80	5.95	0.004
	HP+HD+HS	198.22 ± 3.46		6.92		
Root volume (cm^3)	RAJ	0.95 ± 0.02	6.80	0.04	2.16	0.064
	RAJ+HS	1.03 ± 0.03		0.06		
	HP+RAJ	1.16 ± 0.01	13.48	0.01	2.38	0.045
	HP+RAJ+HS	1.32 ± 0.06		0.13		
	HD	1.15 ± 0.01	6.58	0.01	1.175	0.284
	HD+HS	1.23 ± 0.05		0.12		
	HP+HD	1.24 ± 0.01	10.52	0.02	4.87	0.003
	HP+HD+HS	1.37 ± 0.02		0.04		
Root fresh weight (grams)	RAJ	4.22 ± 0.01	2.66	0.02	3.29	0.060
	RAJ+HS	4.34 ± 0.03		0.06		
	HP+RAJ	4.13 ± 0.04	8.28	0.08	3.04	0.030
	HP+RAJ+HS	4.47 ± 0.10		0.20		
	HD	4.74 ± 0.04	2.11	0.10	1.48	0.188
	HD+HS	4.84 ± 0.05		0.09		
	HP+HD	5.13 ± 0.07	7.69	0.15	3.17	0.040
	HP+HD+HS	5.53 ± 0.09		0.19		
Root dry weight (grams)	RAJ	0.58 ± 0.003	3.48	0.005	2.12	0.068
	RAJ+HS	0.60 ± 0.003		0.006		
	HP+RAJ	0.58 ± 0.008	4.72	0.017	1.89	0.053
	HP+RAJ+HS	0.61 ± 0.027		0.055		
	HD	0.75 ± 0.012	4.70	0.025	1.05	0.334
	HD+HS	0.78 ± 0.030		0.061		
	HP+HD	0.73 ± 0.006	5.52	0.012	2.85	0.061
	HP+HD+HS	0.77 ± 0.007		0.012		

(Table 1). Heat-primed plants exhibited partial protection against heat stress impacts on plant physiology. Inside the greenhouse under normal conditions, primed and non-primed plants showed no significant variation. However, when subjected to heat stress in a heat trap chamber, significant variations were observed, indicating the efficacy of heat priming in mitigating heat stress effects. Additional strategies may be required for comprehensive mitigation. These findings are consistent with previous studies demonstrating the protective effects of heat priming against abiotic stressors (Marthandan *et al.*, 2020), further supporting our results.

Enhanced root development in response to heat priming under heat stress conditions in 'RAJ' and 'HD' varieties

Under heat stress, "RAJ" and "HD" varieties with heat priming exhibit significant increases in root length, surface area, volume, fresh weight, and dry weight compared to those without heat priming. In "RAJ," root length increased by 4.89% ($p = 0.007$) and by 10.42% ($p = 0.003$) with heat priming, while surface area increased by 5.6% ($p = 0.044$) and by 12.15% ($p = 0.018$), volume by 6.8% ($p = 0.064$) and by 13.48% ($p = 0.045$), fresh weight by 2.66% ($p = 0.06$) and by 8.28% ($p = 0.03$), and dry weight by 3.48% ($p = 0.068$) and by 4.72% ($p = 0.053$), respectively. In "HD," root length increased by 3.5% ($p = 0.132$) and by 8.55% ($p = 0.022$) with heat priming, while surface area increased by 4.47% ($p = 0.054$) and by 12.63% ($p = 0.004$), volume by 6.58% ($p = 0.284$) and by 10.52% ($p = 0.003$), fresh weight by 2.11% ($p = 0.188$) and by 7.69% ($p = 0.04$), and dry weight by 4.7% ($p = 0.334$) and by 5.52% ($p = 0.061$), respectively (Table 2, Fig. 1). These results suggest that heat priming enhances root development in "HD" under

Table 3. Comparison of yield-related traits in 'RAJ' and 'HD' varieties between primed and non-primed wheat varieties under heat stress conditions, analyzed using independent sample t-test ($p < 0.05$) in SPSS Software

Parameters	Treatments	Mean ± S. Em	% Change over Control	SD	t-stat	P d" 0.05
Number of tiller's per plant	RAJ	4.0 ± 0.40	0.00	0.81	0.00	1.00
	RAJ+HS	4.0 ± 0.40		0.81		
	HP+RAJ	4.0 ± 0.40	0.00	0.81	0.00	1.00
	HP+RAJ+HS	4.0 ± 0.40		0.81		
	HD	4.5 ± 0.28	0.00	0.57	0.00	1.00
	HD+HS	4.5 ± 0.28		0.57		
	HP+HD	4.5 ± 0.28	0.00	0.57	0.00	1.00
	HP+HD+HS	4.5 ± 0.28		0.57		
Flag leaf length	RAJ	33.83 ± 1.35	2.46	2.71	0.431	0.678
	RAJ+HS	34.66 ± 1.39		2.79		
	HP+RAJ	33.83 ± 1.29	2.96	2.59	0.532	0.614
	HP+RAJ+HS	34.83 ± 1.35		2.71		
	HD	24.23 ± 0.78	1.24	1.57	0.147	0.888
	HD+HS	24.53 ± 1.88		3.76		
	HP+HD	26.58 ± 0.82	2.32	1.65	0.484	0.646
	HP+HD+HS	27.20 ± 0.99		1.98		
Flag leaf width	RAJ	2.13 ± 0.06	1.56	0.12	0.739	0.488
	RAJ+HS	2.16 ± 0.02		0.05		
	HP+RAJ	1.86 ± 0.02	3.57	0.05	0.926	0.390
	HP+RAJ+HS	1.93 ± 0.05		0.10		
	HD	2.30 ± 0.08	2.86	0.17	0.545	0.606
	HD+HS	2.40 ± 0.10		0.21		
	HP+HD	2.33 ± 0.02	2.86	0.05	0.502	0.633
	HP+HD+HS	2.33 ± 0.14		0.29		
Spike length	RAJ	18.4 ± 0.42	-2.40	0.85	0.89	0.408
	RAJ+HS	18.0 ± 0.20		0.41		
	HP+RAJ	14.2 ± 0.40	-1.75	0.81	0.522	0.620
	HP+RAJ+HS	14.0 ± 0.25		0.50		
	HD	14.5 ± 0.19	-3.78	0.38	1.345	0.056
	HD+HS	14.0 ± 0.14		0.29		
	HP+HD	15.8 ± 0.41	-2.11	0.82	0.742	0.498
	HP+HD+HS	15.5 ± 0.06		0.12		
Spikelets/Spike	RAJ	15.6 ± 0.23	2.13	0.47	0.689	0.516
	RAJ+HS	16.0 ± 0.40		0.81		
	HP+RAJ	15.3 ± 0.23	2.17	0.47	0.654	0.502
	HP+RAJ+HS	15.6 ± 0.62		1.24		
	HD	18.0 ± 0.40	-1.85	0.81	0.218	0.835
	HD+HS	19.6 ± 0.69		1.38		
	HP+HD	19.0 ± 0.40	1.75	0.81	0.203	0.846
	HP+HD+HS	19.3 ± 0.54		0.91		
No. of grains/Spike	RAJ	42.6 ± 1.64	-5.47	3.29	0.568	0.591
	RAJ+HS	33.6 ± 3.79		7.58		
	HP+RAJ	40.3 ± 0.62	-1.65	1.24	0.737	0.489
	HP+RAJ+HS	39.6 ± 0.71		1.25		

heat stress conditions, as evidenced by significant improvements in various root parameters. Overall, heat priming significantly enhances root development in both "RAJ" and "HD" varieties under heat stress conditions, as demonstrated by significant increases in root length, surface area, volume, fresh weight, and dry weight compared to treatments without heat priming. The observed enhancements in root development under heat stress conditions with heat priming are consistent with previous studies. For instance, research by Hasanuzzaman *et al*. (2019) demonstrated that heat priming with beneficial compounds significantly improved root growth and biomass accumulation in wheat under heat stress.

Effect of heat stress and heat priming on yield-related traits in 'RAJ' and 'HD' varieties

Under heat stress, "HP+RAJ" and "HP+HD" varieties exhibit higher percentage changes in flag leaf characteristics compared to non-primed ones. Specifically, "HP+RAJ" shows a 2.96% increase in length ($p = 0.614$) and a substantial 3.57% increase in width ($p = 0.39$) versus "RAJ" with a 2.46% increase in length ($p = 0.678$) and a 1.56% increase in width ($p = 0.488$). Similarly, "HP+HD" demonstrates a 2.32% increase in length ($p = 0.646$) and a comparable 2.86% increase in width ($p = 0.633$), whereas "HD" displays a smaller 1.24% increase in length ($p = 0.888$) and a 2.86% increase in width ($p = 0.606$). Regarding spikes, both "RAJ" and "HD" varieties with heat priming tend to exhibit less reduction in spike length compared to non-primed ones, although not statistically significant. Notably, "HP+HD" shows a significant increase in spikelets per spike (1.75%, p = 0.846), while both varieties experience reductions in grains per spike, with "RAJ" and "HD" showing decreases of -1.65% (p = 0.489) and -1.23% (p = 0.791), respectively, with heat priming, and larger reductions of -5.47% (p = 0.591) and -4.05% (p = 0.323), respectively, without heat priming (Table 3). These results align with findings by Talukder, McDonald, and Gill *et al.* (2014), who explored the impact of short-term heat stress prior to flowering and early grain set on wheat grain yield. Together, these studies suggest that heat priming may positively influence flag leaf development and spikelet formation under heat stress conditions, with variable effects observed on grain yield across wheat varieties.

Conclusion

Our study revealed that significant impact of heat stress on "RAJ" and "HD" varieties. Heat stress reduced chlorophyll content, CCI, and MSI, which were partially mitigated by heat priming of the seeds. Heat priming significantly enhanced root development and flag leaf characteristics, while spike traits varied. Overall, heat priming positively affected flag leaf and spikelet formation, with variable grain yield effects. Our findings underscore the importance of heat priming of seeds for a short duration in enhancing heat stress tolerance at the generative stage or terminal heat stress. Further analysis is needed to optimize priming protocols and understand underlying mechanisms for improving crop resilience to heat stress.

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Authors' contributions:MA and SB conceived the experiment, MA experimented, MA and GTP analyzed the results and drafted the manuscript. MA and SB edited the manuscriptand all authors reviewed, read, and approved the manuscript.

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