

Seed priming and thermal seed treatments alleviate yield losses in late-sown wheat across two field locations

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Abstract

Background

Wheat sowing is delayed in Rice-Wheat and Cotton-Wheat rotations. It exposes late-sown wheat to low and high temperature stresses at germination and grain development stages, respectively, affecting the growth and productivity of wheat. Therefore, this study evaluated the effect of late-sowing on wheat performance and the potential of seed treatments to alleviate yield losses in late-sown wheat. Field experiments were conducted during 2021–2022 at Bahawalpur (EL1) and Multan (EL2), Pakistan. A split-plot randomized complete block design with three replications was used at both locations, with sowing date as the main-plot factor and seed treatment as the subplot factor. Sowing was performed on 15 November 2021 for recommended sowing, and 15 December 2021 for delayed sowing at both experimental locations. Seeds were subjected to five treatments: heat treatment, cold treatment, zinc priming, proline priming, and an untreated control.

Results

Late sowing reduced grain yield of untreated plants by 38.6% at Bahawalpur and 67.5% at Multan and decreased their germination index by 21.4% and 25.8%, respectively. It also reduced plant growth, spike development, and yield-related traits, although the magnitude of these effects differed between locations. Heat treatment improved germination under late-sown conditions and produced the highest late-sown grain yield at Bahawalpur (5.11 t ha⁻¹). Zinc priming produced the highest late-sown grain yield at Multan (2.48 t ha⁻¹), while zinc and proline priming generally improved growth, spike traits, physiological performance, and grain yield. Zinc and proline priming also enhanced antioxidant responses, although treatment effects were location-dependent. Cold treatment provided no consistent benefit and resulted in poor crop establishment under delayed sowing at Multan.

Conclusions

Delayed sowing significantly affected germination, growth and yield of wheat. Based on this one season and two locations study, heat treatment, zinc priming and proline priming showed potential for improving late-sown wheat performance, whereas cold treatment was unreliable under the tested conditions. However, broader multi-season and multi-location validation is required before general recommendations are made.

1. Background

Wheat is the second most produced crop in Pakistan after cotton, contributing 4% in global wheat production. Pakistan is the 8th largest wheat producer in the world, with an average production of 26.53 million metric tons over the last ten years [1]. With an ever-increasing food demand, sustaining and improving wheat yield remains a major challenge in Pakistan. Wheat is mostly cultivated under Rice-Wheat or Cotton-Wheat rotations, where its sowing is delayed due to the elongated growth periods of rice and cotton [2]. Mid-November is regarded as the optimal time for wheat sowing in Pakistan, with a 1% yield reduction per day reported if sowing is delayed beyond 20 November [3].

Delayed sowing shortens the growing period of wheat, affecting growth and resource use efficiency [4]. Along with a shorter growing period, late sowing exposes the wheat plants to low-temperature stress during early establishment phase and terminal heat stress during reproductive stage, which together reduce the final yield [5]. Delayed sowing exposes seeds to cold stress at germination, leading to poor crop stand, and high temperature stress at reproductive stage that affects grain development, causing reduction in final yield [6]. Several researchers have reported the inhibitory

effect of late sowing on germination, plant height, number of spikelets and grains per spike, number of leaves, leaf area, fertility ratio, harvest index, biochemical activity, biological yield, and economical yield of wheat [7, 8].

To mitigate the adverse effects of late sowing, several genetic [9] and agronomic strategies have been recommended [10]. Suggested agronomic practices include increased seed rate, efficient use of macro [11] and micro nutrients [12], use of plant growth regulators [13], and different planting methods [5]. Both plant growth regulators (PGRs) and micro-nutrients help plants to combat oxidative stress under stress conditions. PGRs such as thiourea, salicylic acid [14], and gibberellic acid [13] have been reported to alleviate the adverse effects of late sowing by increasing antioxidant activity. Proline is also a PGR produced by plants and helps them adjust to osmotic stress. Exogenous application of proline in the form of seed priming can improve wheat germination [15] under late-sown conditions by improving stress tolerance at the early plant developmental stage. Application of micronutrients like Zinc (Zn) also boosts antioxidant activity in late-sown wheat to increase stress tolerance and yield. Zn is primarily applied in foliar form or incorporated in soil; however, seed priming with Zn can improve germination and antioxidant activity [16].

Besides seed priming, thermal seed treatments can also be used to improve wheat performance mitigate the effects of late sowing. Controlled exposure of seeds to heat or low temperature has been proposed to induce acclimation related responses that may improve endurance against stress tolerance. Although heat and cold treatment of seeds have helped improve stress tolerance, yet very limited studies have been conducted on its effects and causes [17, 18]. Therefore, this field experiment was conducted at two locations with aim of evaluating the potential of low cost and easily accessible priming and seed treatment techniques in mitigating the adverse effects of late sowing. We hypothesized that thermal seed treatment and seed priming would improve wheat performance under delayed sowing by improving germination, growth, yield, and biochemical activity.

2. Methods

Experiments were conducted in Bahawalpur and Multan to evaluate the potential of physiological enhancements for the productivity of late-sown wheat. The experimental locations and experiment layout are given in Fig. 1. In Bahawalpur, the experiment was conducted at the Arid Zone Research Institute, while the research area of Bahauddin Zakariya University was selected for the experiment in Multan. An RCBD split-plot design was used, with two sowing dates (normal and late) in the main plots and five treatments in the subplots. Normal sowing at both locations was performed on 15 November, and late sowing on 15 December. Five treatments included heat and cold-treated seeds, zinc and proline-primed seeds, and control plants grown under normal and late sowing conditions. The dimensions of each experimental unit were 5 m × 4 m with a 25 cm R × R distance. The experiment was replicated three times.

In experiment, *Triticum aestivum* L. cv. *Galaxy* (source: AZRI, Bahawalpur) was used at both locations. Seeds were divided into five groups of heat-treated (exposed to 40°C for 24 hours [18]), cold-treated (exposed to -20 ± 2°C for 24 hours [18]), zinc primed (priming with 0.5 M ZnSO₄ for 12 hours [19]), proline-primed (priming with a solution of 2 mM proline for 12 hours [20]) and control (untreated). Sowing was performed at a rate of 125 kg ha⁻¹ using a single-row man-pulled drill on pre-determined dates. All recommended agronomic practices (fertilization, irrigation, weeding, and pest control) were performed to ensure healthy environment. Meteorological data during growth period were obtained from the Department of Environmental Sciences, BZU, Multan, and AZRI, Bahawalpur (Fig. 2).

2.1. Data collection

Data of interest included germination, physiological, yield, and biochemical parameters. Mean germination time (MGT) and germination index (GI) were calculated by using equations 1 and 2, respectively.

$$MGT = \sum D_n / \sum n \quad [1]$$

Where n = total number of seeds germinated, D = total number of days taken for complete germination

$$GI = \left(\frac{G_{DAG1}}{DAG} \right) + \left(\frac{G_{DAG2}}{DAG} \right) + \dots + \left(\frac{G_{DAGn}}{DAG} \right) [2]$$

Where, DAG = days after first seeds germination, G_{DAG1} = seeds germinated on 1 DAG, G_{DAG2} = seeds germinated on 2 DAG, G_{DAGn} = seeds germinated on final DAG

Physiological data included growth and yield data. At physiological maturity, plants were harvested from an area of 1 m² per replication. Plant height (cm) and spike length (cm), spikelet count per spike, and grain count per spike were recorded. Fertility ratio (FR) and harvest index (HI) were calculated using equations 3 and 4, respectively. Biological yield and grain yield were measured in (g m⁻²) and then converted to t ha⁻¹ using Eq. 5.

FR=Numbers of grain per spike / Numbers of spikelet per spike × 100	[3]
HI=Grain yield / Biological Yield × 100	[4]
Yield (t ha⁻¹)=Yield (gm⁻²) / 100	[5]

2.2. Biochemical analysis

Leaf samples for biochemical analysis were collected at booting stage. Bradford assay [21] was used to determine total soluble protein. Collected leaf samples (500 mg) were extracted in 50 mM phosphate buffer (pH 7.2), then centrifuged at 12000 rpm for 5 min, and the supernatant was used to measure TSP. Coomassie Brilliant Blue G-250 dye, distilled water, and bovine serum albumin (reaction mixture) were used to construct the standard curve. Spectrophotometer (UV-4000, O.R.I., Germany) was used to measure absorbance of the reaction mixture and the sample at 595 nm [22] (Fig. 3a). 500 mg of leaf was homogenized in 80% acetone, followed by centrifugation to collect the supernatant. The absorbance of supernatant was recorded at 663 nm and 645 nm using a spectrophotometer (Fig. 3b) to quantify chlorophyll content using the equation described by Arnon [23].

Leaf sample (500 mg) was ground in the presence of phosphate buffer (5 ml). The ground material was centrifuged at room temperature (15,000 rpm for 20 minutes). The supernatant, hereafter referred to as the enzyme extract, was used to measure antioxidant activity (SOD, CAT) (Fig. 3c).

SOD activity was determined by using method described by Giannopolitis and Ries [24]. The reaction mixture consisted of 75 mM EDTA (500 µL), 50 µM NBT (1 mL), 1.3 µM riboflavin (1 mL), 50 mM phosphate buffer (950 µL), and 13 mM methionine (500 µL). Glass test tubes with reaction mixture were placed under a 30 W fluorescent lamp for 5 minutes, followed by absorbance measurement at 560 nm using a Spectrophotometer (UV-4000, O.R.I., Germany). The glass test tubes containing only the reaction mixture were used as blanks (ABS_{blank}), while the glass tube containing the reaction mixture and the enzyme extract was used to measure the sample absorbance (ABS_{sample}). Eq. 6 was used to calculate SOD activity (Fig. 3d).

Method described by Maehly [25] was used to measure catalase activity. The absorbance at 240 nm of the reaction mixture (5.9 mM H₂O₂ (900 µL), and 50 mM phosphate buffer (2 mL)) was used as a blank, and the enzyme extract + reaction mixture was used to measure sample absorbance. Ten readings were taken in a span of 5 minutes (30 seconds apart), and Eq. 7 was used to measure catalase activity (Fig. 3e).

$$SOD = \frac{(Abs_{blank} - Abs_{sample}) \times V_{Total} \times D}{0.5 \times Abs_{blank} \times V_{enzyme} \times Protein\ conc.} [6]$$

$$CAT = \frac{\Delta A_{240} \times 1000}{43.6 \times Protein\ conc.} [7]$$

Where: A_{blank} is Abs at 560 nm of blank, A_{sample} is Abs at 560 nm of sample, V_{total} is total reaction volume (4 mL), D = Dilution factor, P = Protein concentration (mg/mL), and ΔA_{240} is change in absorption of sample at 240 nm.

2.3. Statistical analysis

Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Data were first tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene’s test. Because environmental conditions and treatment responses differed markedly between the experimental sites, data were analyzed separately for each location using a two-factor split-plot ANOVA with sowing date and seed treatment as fixed factors. When significant differences were detected, means were separated using Tukey’s honestly significant difference (HSD) post hoc test. Statistical significance was considered at $p < 0.05$. Data are presented as mean $\pm$ standard error (SE). Graphical presentation and Pearson correlation analysis were performed using Python 3.11.15 with pandas, NumPy, SciPy, and Matplotlib packages.

3. Results

3.1. Individual effects of late sowing and treatments on germination, growth, and yield of wheat

Analysis of variance for Bahawalpur and Multan are presented in Table 1 and Table 2 respectively. It revealed that late sowing significantly affected wheat germination, growth, and yield. At both experimental locations, germination index (GI) was significantly affected by late sowing; however, mean germination time (MGT) significantly differed between sowing dates only at Multan (EL2), whereas no significant difference was observed at Bahawalpur (EL1). In the control treatment (T5), GI under recommended sowing at EL2 was 28.02, whereas late sowing reduced GI to 20.80, representing a 25.8% decline. Similarly, at EL1, GI in the control treatment decreased from 32.84 under recommended sowing to 25.80 under late sowing, corresponding to a 21.44% reduction.

Late sowing significantly affected plant growth at EL2, where plant height in the control treatment decreased from 105.3 cm under recommended sowing to 72.0 cm under late sowing, representing a 31.64% reduction. In comparison, no significant effect of late sowing was observed on plant height at EL1. However, spike length was reduced by 11.4% and 11.2% at EL1 and EL2, respectively, under late sowing. At EL1, control plants under recommended sowing produced 12.2 cm long spikes, with 52.8 spikelets and 43.4 grains per spike. Under late sowing, these values declined to 10.8 cm spike length, 40.8 spikelets per spike, and 30.2 grains per spike, corresponding to reductions of 11.4%, 22.7%, and 30.2%, respectively. At EL2, spike length in the control treatment was 12.01 cm under recommended sowing, with 52.06 spikelets and 41.9 grains per spike. Under late sowing, these values decreased to 10.67 cm, 50.26 spikelets per spike, and 36.8 grains per spike, corresponding to reductions of 11.2%, 3.5%, and 12.25%, respectively. Fertility ratio was also reduced under late sowing, with reductions of 10.11% and 8.8% observed at EL1 and EL2, respectively.

Besides germination and growth, biological yield and grain yield were severely affected by late sowing. In the control treatment, biological yield under recommended sowing was 17.08 t ha⁻¹ and 9.20 t ha⁻¹ at EL1 and EL2, respectively. Under late sowing, biological yield declined to 11.89 t ha⁻¹ at EL1 and 3.53 t ha⁻¹ at EL2, corresponding to reductions of 30.37% and 61.59%, respectively. Similarly, grain yield under recommended sowing was 6.82 t ha⁻¹ and 2.99 t ha⁻¹ at EL1

and EL2, respectively, whereas late sowing reduced grain yield to 4.19 t ha⁻¹ and 0.97 t ha⁻¹ at EL1 and EL2, respectively. These reductions corresponded to 38.56% at EL1 and 67.48% at EL2.

Table 1

Variance analysis and mean comparison of germination, growth, and yield parameters of wheat affected by sowing dates, treatments, and their interaction at Bahawalpur location

RS	MGT	GI	PH	SL	SC	GC	BY	GY	FR	HI
	10.6a	26.9a	80.0a	13.24a	59.64a	49.28a	1560.9a	623.96a	82.44a	40.50a
LS	9.9a	31.4b	79.0a	11.82b	45.64b	35.96b	1119.7b	433.53b	78.53b	38.81a
SE	0.622	1.69*	3.59	0.30**	3.10**	2.95**	79.27**	20.67**	1.56*	1.25
T1	11.32a	31.24ab	77.66a	12.26bc	52.96a	42.80a	1466.8a	580.00a	80.49a	39.63a
T2	11.42a	25.76b	76.50a	13.10ab	55.13a	44.63a	1050.0b	445.40c	80.81a	41.93a
T3	10.03ab	25.75b	76.50a	13.33a	54.90a	45.63a	1302.0ab	496.50bc	82.25a	38.30a
T4	8.9b	33.75a	80.50a	12.46a-c	53.33a	43.20a	1434.5a	571.33a	80.83a	39.76a
T5	9.8ab	29.31ab	86.33a	11.50c	46.86a	36.83a	1448.3a	550.50ab	78.06a	38.64a
SE	0.98	2.68*	5.67	0.47**	4.90	4.66	125.34*	32.67**	2.48	1.97
T*S	1.39**	3.79**	8.03	0.67	6.93	6.59	177.26	46.213	3.50	2.79

Values represent mean comparisons for sowing date and seed treatments. Within each factor, means followed by the same letter in a column are not significantly different according to Tukey's HSD test at $p < 0.05$. RS indicates recommended sowing date, and LS indicates late sowing. T1, T2, T3, T4, and T5 indicate heat seed treatment, cold seed treatment, zinc priming, proline priming, and control, respectively. SE indicates standard error, and S × T indicates the interaction between sowing date and treatment. * and ** indicate significance at the 5% and 1% probability levels, respectively. MGT, mean germination time; GI, germination index; PH, plant height; SL, spike length; SC, spikelet count per spike; GC, grain count per spike; BY, biological yield; GY, grain yield; FR, fertility ratio; HI, harvest index

Seed-treatment effects were evaluated separately at each location and differed among the measured traits. At EL1, treatment effects were significant for GI, spike length, biological yield, and grain yield, whereas other traits showed non-significant differences among treatments. Proline-primed seeds produced the highest GI at both experimental locations. At EL2, proline priming resulted in the highest spike length, number of spikelets per spike, and number of grains per spike. Zinc-primed seeds produced the highest grain yield and harvest index at EL2, while fertility ratio was statistically similar among most treatments except cold seed treatment. At EL1, zinc priming produced the maximum spike length, whereas heat-treated seeds produced the highest biological and grain yields and were statistically comparable with proline priming and the control treatment.

Table 2
Variance analysis and mean comparison of germination, growth, and yield parameters of wheat affected by sowing dates, treatments, and their interaction at Multan location

RS	MGT	GI	PH	SL	SC	GC	BY	GY	FR	HI
	12.2a	26.37a	102.47a	11.54a	48.88a	39.43a	725.33a	293.73a	80.55a	41.41a
LS	7.2b	19.24b	60.53b	8.45b	37.025b	25.80b	290.67b	117.11b	56.49b	33.13b
SE	0.71**	1.88**	2.54**	0.26**	1.58**	1.42**	51.57**	18.86**	3.86**	1.26**
T1	12.50a	23.06b	89.83a	10.33c	41.60c	29.50c	510.0ab	181.33bc	71.69a	35.36c
T2	7.44c	10.67c	51.67b	5.44d	22.03d	19.0d	366.67b	155.17c	43.26b	21.16e
T3	10.49ab	21.60b	88.83a	10.75bc	46.23bc	34.36b	486.67ab	256.93a	75.62a	56.93a
T4	9.41bc	34.30a	88.50a	12.13a	53.73a	40.83a	540.0a	235.33ab	74.76a	42.65b
T5	8.94bc	24.41b	88.66a	11.34ab	51.16ab	39.36a	636.67a	198.33a-c	77.24a	30.22d
SE	1.12**	2.97**	4.02**	0.41**	2.50**	2.24**	81.54	29.82*	6.10**	1.99**
S × T	1.58**	4.20**	5.69**	0.58**	3.54**	3.16**	115.32*	41.18**	8.63**	2.81**

Values represent mean comparisons for sowing date and seed treatments. Within each factor, means followed by the same letter in a column are not significantly different according to Tukey's HSD test at $p < 0.05$. RS indicates recommended sowing date, and LS indicates late sowing. T1, T2, T3, T4, and T5 indicate heat seed treatment, cold seed treatment, zinc priming, proline priming, and control, respectively. SE indicates standard error, and S × T indicates the interaction between sowing date and treatment. * and ** indicate significance at the 5% and 1% probability levels, respectively. MGT, mean germination time; GI, germination index; PH, plant height; SL, spike length; SC, spikelet count per spike; GC, grain count per spike; BY, biological yield; GY, grain yield; FR, fertility ratio; HI, harvest index

3.2. Interactive effects of late sowing and treatments on germination, growth, and yield of wheat

Sowing date × treatment significantly affected wheat germination at both ELs. The lowest mean germination time in RSW and LSW at both ELs was observed for proline-primed seeds, which were statistically at par with the control treatment. The highest germination index was observed for proline-primed seeds in RSW and heat-treated seeds in LSW. Cold-treated seeds showed poor germination in LSW at EL2, with only a few plants germinating and reaching maturity (Fig. 4).

The tallest plants under late sowing conditions at EL1 were observed for proline-primed seeds, while at EL2 for heat-treated seeds. For both sowing dates, zinc-primed seeds produced the tallest spikes at EL1 and proline-primed seeds at EL2. Maximum spikelet count and grain count per spike at EL1 were observed for proline-primed seeds in RSW and for zinc-primed seeds in LSW. Similar results were observed for RSW at EL2; however, control plants produced maximum spikelet and grain count per spike in LSW (Fig. 5).

Under normal sowing conditions, control plants produced the highest biological yield; however, under late sowing conditions, heat-treated seeds produced the highest biological yield at both ELs, followed by proline-primed seeds. Maximum grain yield for both sowing dates at EL1 was observed for proline-primed seeds. At EL2, proline-primed seeds produced the highest grain yield under normal sowing conditions, while zinc-primed seeds produced the highest grain

yield under late sowing conditions. A significantly higher harvest index was also observed for zinc-primed seeds in late sown wheat at EL2 (Fig. 6).

3.3. Interactive effects of late sowing and treatments on biochemical and antioxidant activity of wheat

Sowing dates, treatments, and their interaction also significantly affected the antioxidant activity in wheat plants at both experimental locations. At EL1, all seed treatments produced significantly higher SOD and CAT activity than the control under late-sowing conditions; however, at EL2, only heat-treated and zinc-primed seeds showed significantly higher SOD and CAT activity under stressed conditions. Zinc-primed seeds also produced significantly higher protein content under late-sowing conditions at EL1, while at EL2, it was statistically at par with the control treatment. Proline-primed seeds produced significantly higher protein content than control in late sown wheat at EL2, while the two treatments were statistically at par under late sowing conditions at EL1 for protein production. Zinc and proline-primed seeds also produced non-significantly higher chlorophyll content than the control under late sowing conditions at both ELs (Fig. 7).

3.4. Correlation analysis among germination, growth, and yield-related traits

Pearson correlation analysis showed positive associations among most germination, growth, and yield-related traits (Fig. 8). Grain yield was very strongly correlated with biological yield ($r = 0.96, p < 0.001$), indicating that yield improvement was closely associated with biomass accumulation. Grain yield also showed significant positive correlations with number of grains per spike ($r = 0.67, p < 0.001$), spike length ($r = 0.62, p < 0.001$), number of spikelets per spike ($r = 0.58, p < 0.001$), and fertility ratio ($r = 0.56, p < 0.001$). Spike-related traits were strongly interrelated, particularly number of spikelets per spike and number of grains per spike ($r = 0.95, p < 0.001$), and spike length was strongly associated with spikelets per spike ($r = 0.90, p < 0.001$), grains per spike ($r = 0.88, p < 0.001$), and fertility ratio ($r = 0.89, p < 0.001$). Germination index was positively associated with several growth and yield traits, including spike length, plant height, biological yield, and grain yield, whereas mean germination time showed a weak non-significant correlation with germination index.

4. Discussion

Wheat is among the most produced crops in the world, mainly rotated with rice or cotton. Delay in harvesting of rice and cotton pushes the sowing date of wheat, exposing its monocots to cold stress at germination. A poor crop stand is the first step toward a decrease in yield, followed by shortened growing season and terminal heat stress at the reproductive stage. Reduction in the yield of one of the most widely produced crops in the world poses a serious threat to global food security. Pakistan is a major wheat producer, and delayed sowing is an inevitable problem in its wheat-producing regions. Current study showed that late sowing significantly affected wheat germination at both locations. Seed germination is temperature-dependent, and optimum temperature for wheat seed germination is 20–22°C [26]. Mean air temperature at both locations was in favorable range for recommended sown wheat. However, a 5 °C drop in temperature significantly affected germination of late-sown wheat at both locations. A decrease in temperature reduces amylase activity, thereby reducing seed storage degradation and leading to poor germination [27, 28]. Germination of late-sown wheat was severely affected. The germination index of the untreated control decreased by 25.8% at Multan and 21.4% at Bahawalpur. Bahawalpur had more favorable meteorological conditions (17 °C temperature and 60% humidity) than Multan (15 °C temperature and 67% humidity). Therefore, late-sown wheat showed better germination (mean germination time and germination index) at Bahawalpur than at Multan. Heat-treated seeds mitigated the adverse effect of late sowing by significantly improving the germination index at both locations. Pre-sowing exposure of

seeds to heat improves the production of small heat shock proteins [29], which helps plants tolerate abiotic stress during germination [30]. Therefore, heat treatment appears promising for improving the establishment of late-sown wheat, whereas cold treatment should be used carefully under colder sowing conditions and it should be validated across additional seasons and cultivars.

Although cold-treated seeds improved germination index in late-sown wheat at Bahawalpur by imparting stress memory, in Multan, germination of cold-treated late-sown wheat was severely affected, producing few plants only. Based on these observations, heat-treatment of seeds showed high potential to mitigate the adverse effects of late sowing and improve germination. However, cold-treatment should be applied cautiously under colder field conditions and verified across various seasons and cultivars particularly when the temperature falls below 17°C. Delay in sowing also significantly affected the biological yield of wheat at both locations. Biological yield positively correlated with germination index, plant height, and spike length. Therefore, any decrease in germination directly affected biomass production. Delay in sowing reduced the key phenological phases, leading to reduced biomass production [31]. We observed a significant decrease in plant height and spike length of late-sown wheat at both locations. Antioxidant activity also significantly decreased in late-sown wheat at both locations. Under stress conditions, reactive oxygen species (ROS) are produced in mitochondria, chloroplasts, apoplasts, and peroxisomes. Overaccumulation of ROS causes osmotic stress and protein inactivation that affects plant normal growth [32]. Plants combat ROS by producing enzymatic and non-enzymatic antioxidants. In this study, we observed a significant increase in antioxidant activity in treated seeds (zinc-primed, proline-primed, and heat-treated) of late-sown wheat at both locations compared with the control. Increased antioxidant activity helps plants to cope with stress by scavenging ROS [33]. An increase in antioxidant activity translated into higher biological and grain yields in zinc-primed and heat-treated seeds of late-sown wheat. Ali et al. [34] also reported similar results where zinc priming significantly improved biological and grain yield of wheat.

Grain (kernel) is the economic part of wheat, and its yield is of the highest importance. Late sowing significantly reduced grain yield at both locations. Grain yield positively correlated with germination index, plant height, biological yield, spikelets, and grain count per spike. Besides germination and biological yield, late-sowing significantly affected spikelet count per spike and grain count per spike. Late-sowing is reported to reduce spikelet count by delaying the initiation of spikelet primordia and shortening the vegetative phase [35]. Spikelet count per spike decreases proportionally with the delay in sowing [31]. Late-sowing also exposed wheat plants to terminal heat stress at the grain-filling stage, which increases oxidative damage, accelerates maturity, and reduces grain yield [7]. The effects of terminal heat stress were evident by a decrease in grain count per spike. Overall, the results indicate that poor establishment, reduced vegetative growth, shortened crop duration, and adverse conditions during grain filling collectively contributed to the grain-yield penalty under delayed sowing.

Pre-sowing seed treatments improved the grain yield of late-sown wheat, although their effectiveness differed between locations. At Bahawalpur, the highest grain yield in late-sown wheat was recorded for heat-treated seeds, followed by proline-primed seeds. Heat-treated seeds improved grain yield under stress conditions by mitigating cold stress at germination, thereby improving the germination index, and by mitigating oxidative stress through improved antioxidant activity. Grain yield positively correlated with germination index, biological yield, and grain count. Proline-priming also improved germination, biological yield, and grain count in late-sown wheat at Bahawalpur, which translated into higher yield. Seed priming with proline improves mean germination time by reducing lag time of imbibition and improving biochemical modification, like enzyme activation and hydrolysis, to improve germination [36]. Proline-priming also improves the defense mechanism of plants by scavenging ROS under stress conditions [15]. Similar results were observed in the present study, leading to improved biological and grain yield. In Multan, zinc-primed seeds significantly improved the grain yield of late-sown wheat by improving germination and higher SOD and CAT activities than the untreated control. Zinc-priming has been reported to improve 31–47% grain yield in maize [37] by improving antioxidant activity and nitrogen absorption [38].

The present study confirmed that late sowing significantly reduced wheat yield at both locations. It is highly dependent on the optimal temperature for crop establishment. Delay in sowing shortens the growth period of wheat and exposes it to multiple stresses, including cold-stress at germination, terminal heat stress at grain filling, and oxidative stress. Physiological enhancements were successful in mitigating the adverse effects of cold stress at germination and oxidative stress; however, a shortened life cycle and terminal heat stress during grain filling also affected the yield of treated plants. The results support that wheat germination was more favorable when temperatures were closer to the reported optimum range of 20–22°C, whereas lower temperatures under delayed sowing reduced germination and subsequent yield performance. Heat-treatment of seeds and priming with zinc or proline therefore appear promising for late sown wheat, although their effectiveness should be evaluated for multiple-locations and multiple-season. Moreover, a combination of seed treatment and foliar application of plant growth regulators or micronutrients should be studied to mitigate the effect of late-sowing on germination and grain filling stage to produce higher yield in cases where timely sowing of wheat is not possible.

5. Conclusion

Late-sowing significantly affects wheat germination, growth, and yield. Delay in sowing results in cold stress at germination, a shortened growing period, terminal heat stress during grain filling, and oxidative stress. 20–22 °C is the optimal temperature for wheat germination; 15–20 °C negatively affects germination, with severe effects observed at ≤ 15 °C. Heat seed treatment, zinc priming, and proline priming improved germination; antioxidant enzyme, growth, yield traits, although their effectiveness differed between locations. However, the shortened crop duration continued to limit biomass production and grain yield. Therefore, timely sowing remains the most reliable strategy for maintaining wheat productivity, while heat treatment and zinc or proline priming may provide practical options where delayed sowing is unavoidable. The longer the delay in sowing, the shorter the growth period for wheat, which consequently has a greater impact on wheat yield. Therefore, wheat should be sown on recommended time (Temp. 20–22 °C). Heat seed treatment, zinc-priming, and proline priming are recommended to improve germination, growth, and yield of wheat in areas where late-sowing is inevitable. Moreover, a combination of pre-sowing seed treatment and foliar application at the reproductive stage should be explored to further enhance wheat production under late-sowing conditions.

Declarations

1. Ethics approval and consent to participate

Not applicable.

2. Consent for publication

Not applicable.

3. Availability of data and materials

The datasets and Python notebooks used for data analysis and figure preparation are available in the Zenodo repository at [39].

4. Competing interests

The authors declare that they have no competing interests.

5. Funding

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6. Clinical Trial Number

Not applicable.

7. Authors' contributions

Conceptualization: MWN, MHJ, AY; Data curation: MWN, MHJ; Formal analysis: MA, MWN, AY; Methodology: MHJ, MA; Project administration and Resources: AY, ZT; Software: CT, AY; Supervision: AY; Validation: CT, AY, ZT; Visualization: MHJ, MWN; Writing – original draft: MWN, MHJ, MA; Writing – review and editing: CT, AY, ZT

8. Acknowledgements

Not applicable.

References

1. USDA. Wheat | USDA Foreign Agricultural Service. 2025. <https://www.fas.usda.gov/data/production/commodity/0410000>. Accessed 3 Jan 2026.
2. Shahzad M, Hussain M, Jabran K, Farooq M, Farooq S, Gašparovič K, et al. The Impact of Different Crop Rotations by Weed Management Strategies' Interactions on Weed Infestation and Productivity of Wheat (*Triticum aestivum* L). *Agronomy*. 2021;11. <https://doi.org/10.3390/agronomy11102088>.
3. Mujeeb-ur-Rehman H, Ali S, Akram MM. Resource conservation strategy for enhancing wheat productivity in Pakistan. *Mycopath*. 2011;9:79–85.
4. Dubey R, Pathak H, Singh S, Chakravarti B, Thakur AK, Fagodia RK. Impact of Sowing Dates on Terminal Heat Tolerance of Different Wheat (*Triticum aestivum* L.) Cultivars. *Natl Acad Sci Lett*. 2019;42:445–9. <https://doi.org/10.1007/s40009-019-0786-7>.
5. Meleha AMI, Hassan AF, El-Bialy MA, El-Mansoury MAM. Effect of Planting Dates and Planting Methods on Water Relations of Wheat. *Int J Agron*. 2020;2020:8864143. <https://doi.org/10.1155/2020/8864143>.
6. Sihag P, Kumar U, Sagwal V, Kapoor P, Singh Y, Mehla S, et al. Effect of terminal heat stress on osmolyte accumulation and gene expression during grain filling in bread wheat (*Triticum aestivum* L). *Plant Genome*. 2024;17:e20307. <https://doi.org/10.1002/tpg2.20307>.
7. Awad-Allah SM, Amer SM, Abdel-Moneim MH. Assessment of Spring wheat responses to late season heat stress under Mediterranean conditions. *Egypt Sci Mag*. 2026;13:70–85. <https://doi.org/10.21608/esm.2026.503230>.
8. Kumar H, Chugh V, Kumar M, Gupta V, Prasad S, Kumar S, et al. Investigating the impact of terminal heat stress on contrasting wheat cultivars: a comprehensive analysis of phenological, physiological, and biochemical traits. *Front Plant Sci*. 2023;14:1189005. <https://doi.org/10.3389/fpls.2023.1189005>.
9. Rehman Hur, Tariq A, Ashraf I, Ahmed M, Muscolo A, Basra SMA, et al. Evaluation of Physiological and Morphological Traits for Improving Spring Wheat Adaptation to Terminal Heat Stress. *Plants*. 2021;10:455. <https://doi.org/10.3390/plants10030455>.
10. Yadav MR, Choudhary M, Singh J, Lal MK, Jha PK, Udawat P, et al. Impacts, Tolerance, Adaptation, and Mitigation of Heat Stress on Wheat under Changing Climates. *Int J Mol Sci*. 2022;23:2838. <https://doi.org/10.3390/ijms23052838>.

11. Javaid MH, Ali B, Neelam A, Bukhari SAH, Munir R, Haider Z, et al. Green-engineered calcium-doped carbon nanospheres enhance salt tolerance and growth in maize by modulating gene expressions and antioxidant defense mechanisms. *J Environ Chem Eng*. 2025;117061. <https://doi.org/10.1016/j.jece.2025.117061>.
12. Ijaz M, Ul-Allah S, Sher A, Sattar A, Mahmood K, Alamri S, et al. Boron Fertilization Alleviates the Adverse Effects of Late Sowing in Wheat under Different Tillage Systems. *Agriculture*. 2023;13:1229. <https://doi.org/10.3390/agriculture13061229>.
13. Zhang X, Ma M, Wu C, Huang S, Danish S. Mitigation of heat stress in wheat (*Triticum aestivum* L.) via regulation of physiological attributes using sodium nitroprusside and gibberellic acid. *BMC Plant Biol*. 2023;23:302. <https://doi.org/10.1186/s12870-023-04321-9>.
14. Nagre SP, Tiwari G, Anand KJ, Thakur S, Hote S. Mitigation of terminal heat stress in wheat through physiological and biochemical responses induced by salicylic acid and thiourea. *Int J Adv Biochem Res*. 2025;9:1073–81. <https://doi.org/10.33545/26174693.2025.v9.i11Sn.6380>.
15. Ambreen S, Athar H-R, Khan A, Zafar ZU, Ayyaz A, Kalaji HM. Seed priming with proline improved photosystem II efficiency and growth of wheat (*Triticum aestivum* L). *BMC Plant Biol*. 2021;21:502. <https://doi.org/10.1186/s12870-021-03273-2>.
16. Chatti D. Effect of Zinc Nutrition in Mitigating Late Sowing Induced Heat Stress in Wheat Crop. *Int J Curr Microbiol Appl Sci*. 2020;9:2415–21. <https://doi.org/10.20546/IJCMAS.2020.901.275>.
17. Basra SMA, Farooq M, Khaliq A. Comparative Study of Pre-Sowing Seed Enhancement Treatments in Fine Rice. *Pakistan J Life Social Sci*. 2003;1:21–5.
18. Farooq M, Basra S, Rehman H. Seed priming enhances emergence, yield, and quality of direct-seeded rice. *Int Rice Res Notes*. 2009;31. <https://doi.org/10.3860/irrn.v31i2.1132>.
19. Rehman A, Farooq M, Ahmad R, Basra SMA. Seed priming with zinc improves the germination and early seedling growth of wheat. *Seed Sci Technol*. 2015;43:262–8. <https://doi.org/10.15258/sst.2015.43.2.15>.
20. Perveen S, Nazir M. Proline treatment induces salt stress tolerance in maize (*Zea mays* L. Cv. Safaid Afgoi). *Pak J Bot*. 2018;50:1265–71.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–54. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
22. Yasmeen A, Basra SMA, Wahid A, Nouman W, Rehman HU. Exploring the potential of *Moringa oleifera* leaf extract (MLE) as a seed priming agent in improving wheat performance. *Turk J Bot*. 2013;37:512–20. <https://doi.org/10.3906/bot-1205-19>.
23. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*. 1949;24:1–15. <https://doi.org/10.1104/pp.24.1.1>.
24. Giannopolitis CN, Ries SK. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol*. 1977;59:309–14. <https://doi.org/10.1104/pp.59.2.309>.
25. Maehly AC. The Assay of Catalases and Peroxidases. *Methods of Biochemical Analysis*. John Wiley & Sons, Ltd; 1954. pp. 357–424. <https://doi.org/10.1002/9780470110171.ch14>.
26. Khaeim H, Kende Z, Balla I, Gyuricza C, Eser A, Tarnawa Á, et al. The Effect of Temperature and Water Stresses on Seed Germination and Seedling Growth of Wheat (*Triticum aestivum* L). *Sustainability*. 2022;14:3887. <https://doi.org/10.3390/su14073887>.
27. Li X, Jiang H, Liu F, Cai J, Dai T, Cao W, et al. Induction of chilling tolerance in wheat during germination by pre-soaking seed with nitric oxide and gibberellin. *Plant Growth Regul*. 2013;71:31–40. <https://doi.org/10.1007/s10725-013-9805-8>.

28. Zhang H, Liu L, Wang Z, Feng G, Gao Q, Li X. Induction of Low Temperature Tolerance in Wheat by Pre-Soaking and Parental Treatment with Melatonin. *Molecules*. 2021;26:1192. <https://doi.org/10.3390/molecules26041192>.
29. Toda-Matsunaga S, Toda Y, Mega R, Tadano S, Alyza M, Yamasaki Y, et al. Wheat seeds exposed to heat during formation can germinate at high temperatures. *Front Plant Sci*. 2025;16:1539926. <https://doi.org/10.3389/fpls.2025.1539926>.
30. Waters ER, Vierling E. Plant small heat shock proteins - evolutionary and functional diversity. *New Phytol*. 2020;227:24–37. <https://doi.org/10.1111/nph.16536>.
31. Shah F, Coulter JA, Ye C, Wu W. Yield penalty due to delayed sowing of winter wheat and the mitigatory role of increased seeding rate. *Eur J Agron*. 2020;119:126120. <https://doi.org/10.1016/j.eja.2020.126120>.
32. Bao L, Liu J, Mao T, Zhao L, Wang D, Zhai Y. Nanobiotechnology-mediated regulation of reactive oxygen species homeostasis under heat and drought stress in plants. *Front Plant Sci*. 2024;15:1418515. <https://doi.org/10.3389/fpls.2024.1418515>.
33. del Río LA. Plant Peroxisomes and Their Metabolism of ROS, RNS, and RSS. In: Cánovas FM, Lüttge U, Risueño M-C, Pretzsch H, editors. *Progress in Botany*. Volume 82. Cham: Springer International Publishing; 2021. pp. 171–209. https://doi.org/10.1007/124_2020_37.
34. Ali N, Farooq M, Hassan MA, Arshad MS, Saleem MK, Faran M. Micronutrient seed priming improves stand establishment, grain yield and biofortification of bread wheat. *Crop Pasture Sci*. 2018;69:479–87. <https://doi.org/10.1071/CP18042>.
35. Arduini I, Pellegrino E, Ercoli L. Contribution of main culm and tillers to grain yield of durum wheat: Influence of sowing date and plant traits. *Ital J Agron*. 2018;13:1115. <https://doi.org/10.4081/ija.2018.1115>.
36. Faisal S, Muhammad S, Luqman M, Hasnain M, Rasool A, Awan MUF, et al. Effects of Priming on Seed Germination, Physico-Chemistry and Yield of Late Sown Wheat Crop (*Triticum aestivum* L). *Pol J Environ Stud*. 2023;32:1113–24. <https://doi.org/10.15244/pjoes/155970>.
37. Choukri M, Abouabdillah A, Bouabid R, Abd-Elkader OH, Pacioglu O, Boufahja F, et al. Zn application through seed priming improves productivity and grain nutritional quality of silage corn. *Saudi J Biol Sci*. 2022;29:103456. <https://doi.org/10.1016/j.sjbs.2022.103456>.
38. Ranazai SK, Sadiq M, Baloch MS, Qureshi H, Anwar T, Alarfaj AA, et al. Impact of different priming and sowing techniques in combination with different seed rates on wheat growth and yield. *Sci Rep*. 2024;14:26726. <https://doi.org/10.1038/s41598-024-78256-y>.
39. Nasir MW, Javaid MH, Junaid MD, Rabia M, Mehmood A, Tan C et al. Dataset and Python notebooks for seed priming and thermal seed treatments in late-sown wheat. 2026. <https://doi.org/10.5281/zenodo.21100637>

Figures

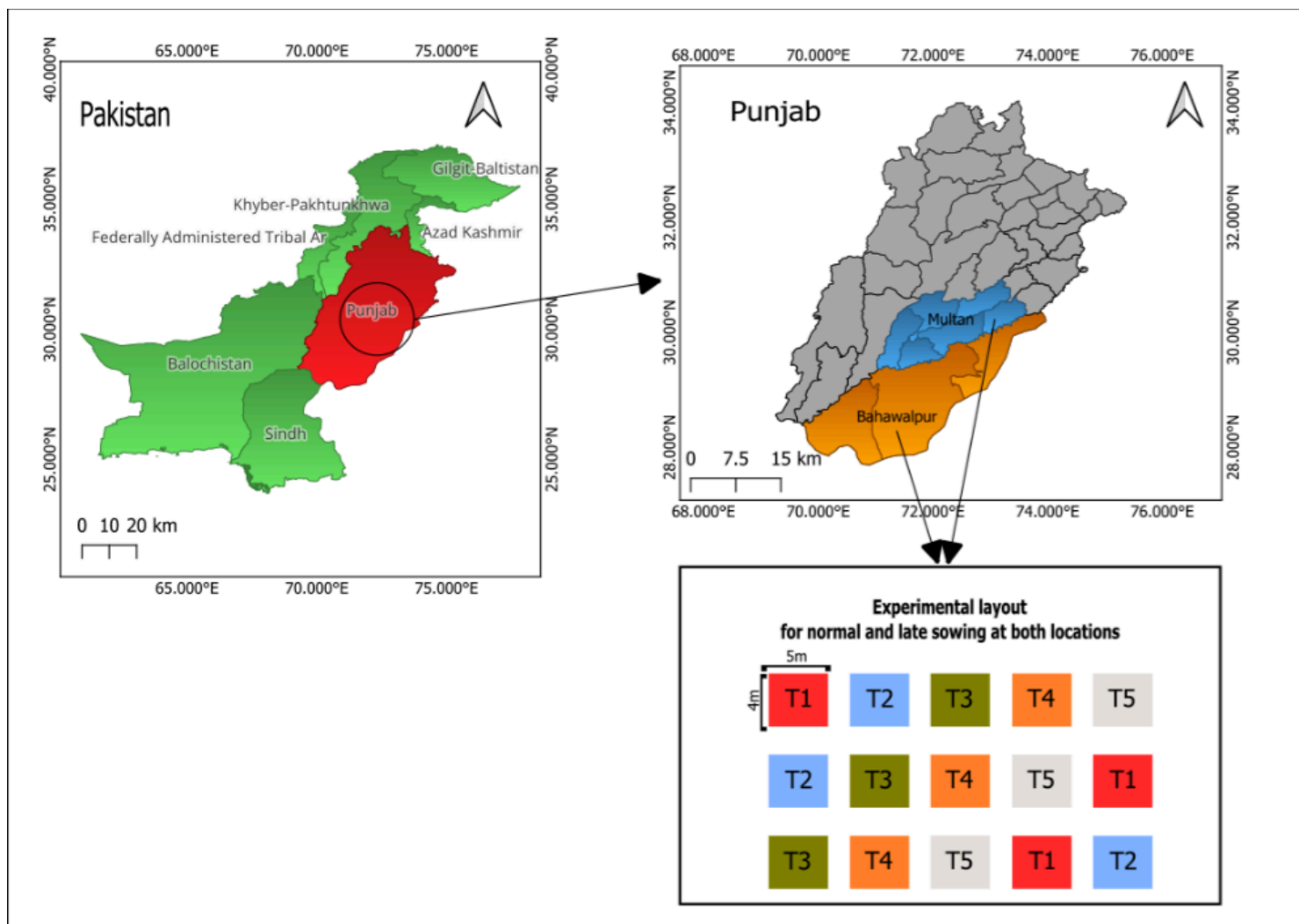


Figure 1

Study area and experimental layout. (a) Map of Pakistan showing Punjab province, (b) map of Punjab showing the experimental locations Bahawalpur and Multan, and (c) schematic representation of the randomized complete block design used for treatment arrangement. The same experimental layout was followed under normal and late sowing conditions at both locations

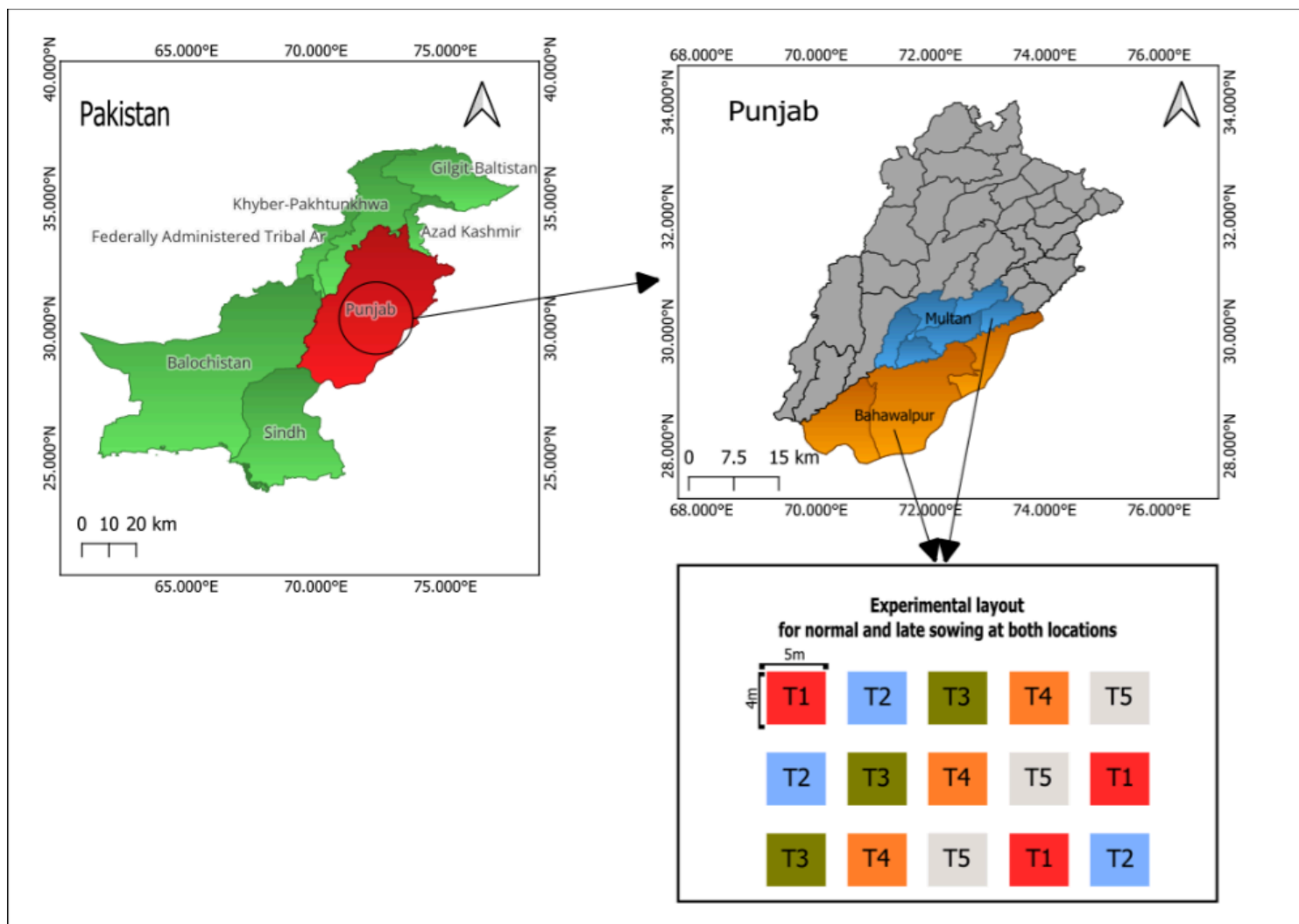


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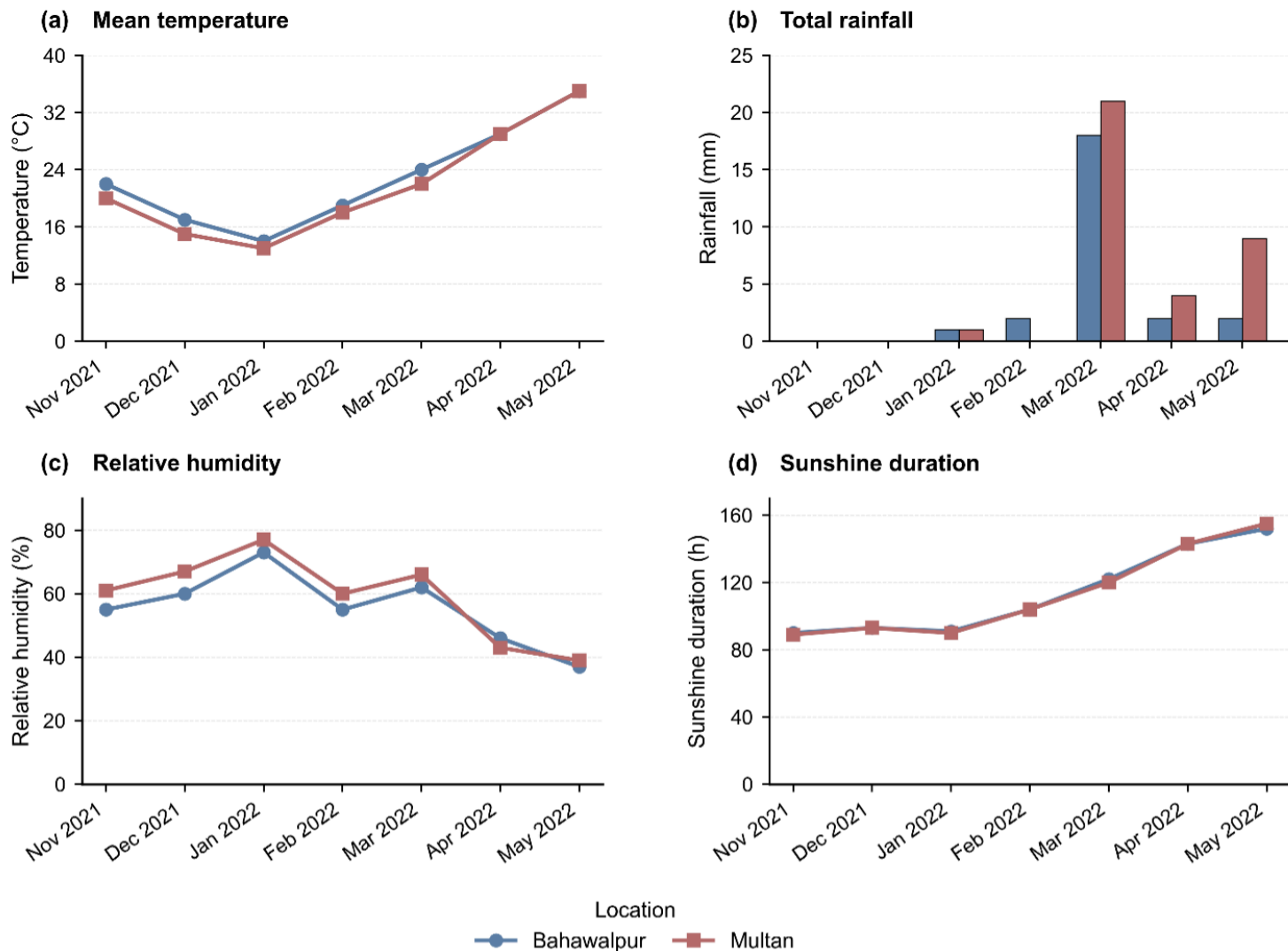


Figure 2

Monthly weather conditions at Bahawalpur and Multan during the experimental period. Panels show (a) mean temperature, (b) total rainfall, (c) relative humidity, and (d) sunshine duration from November 2021 to May 2022

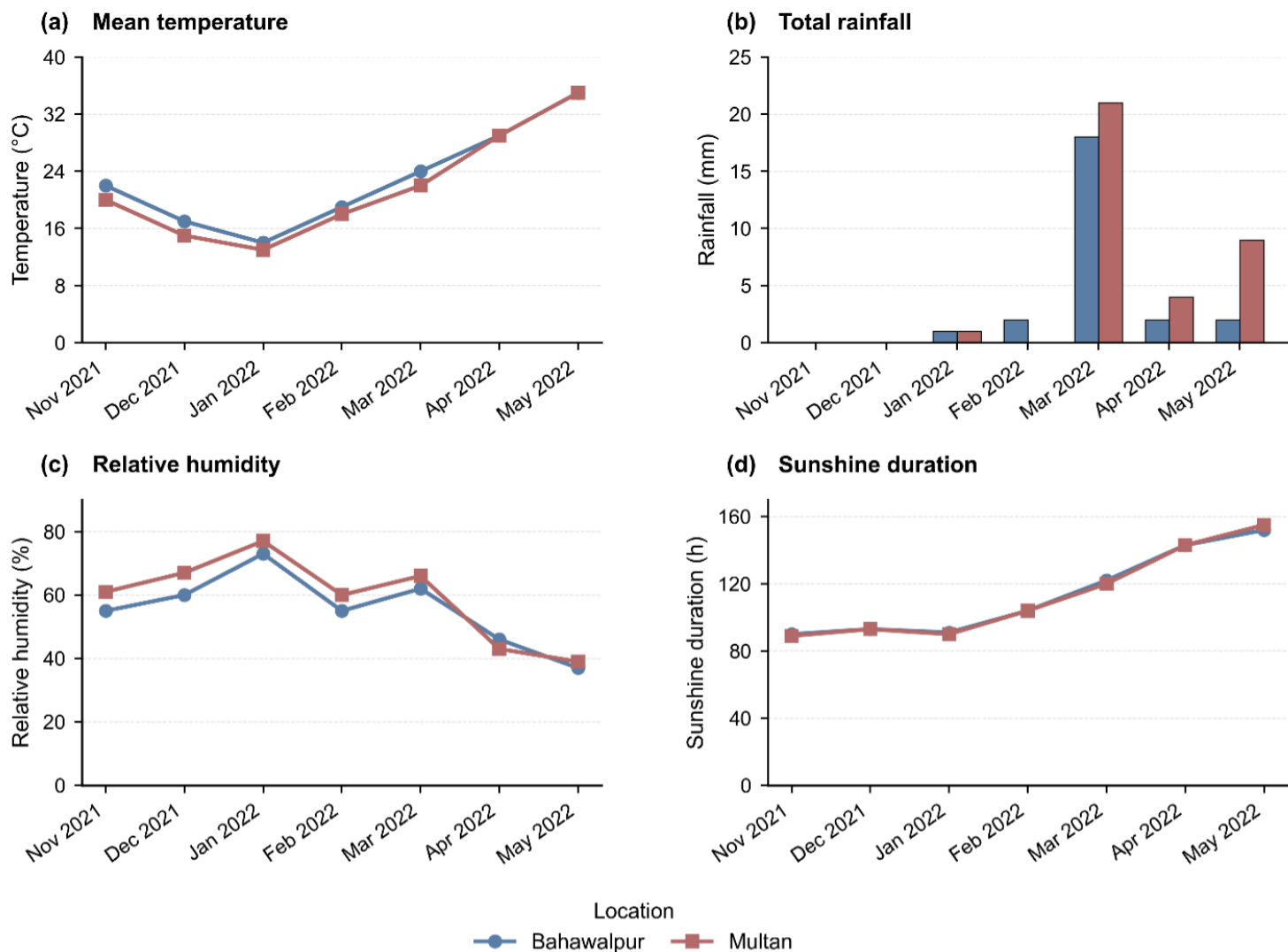


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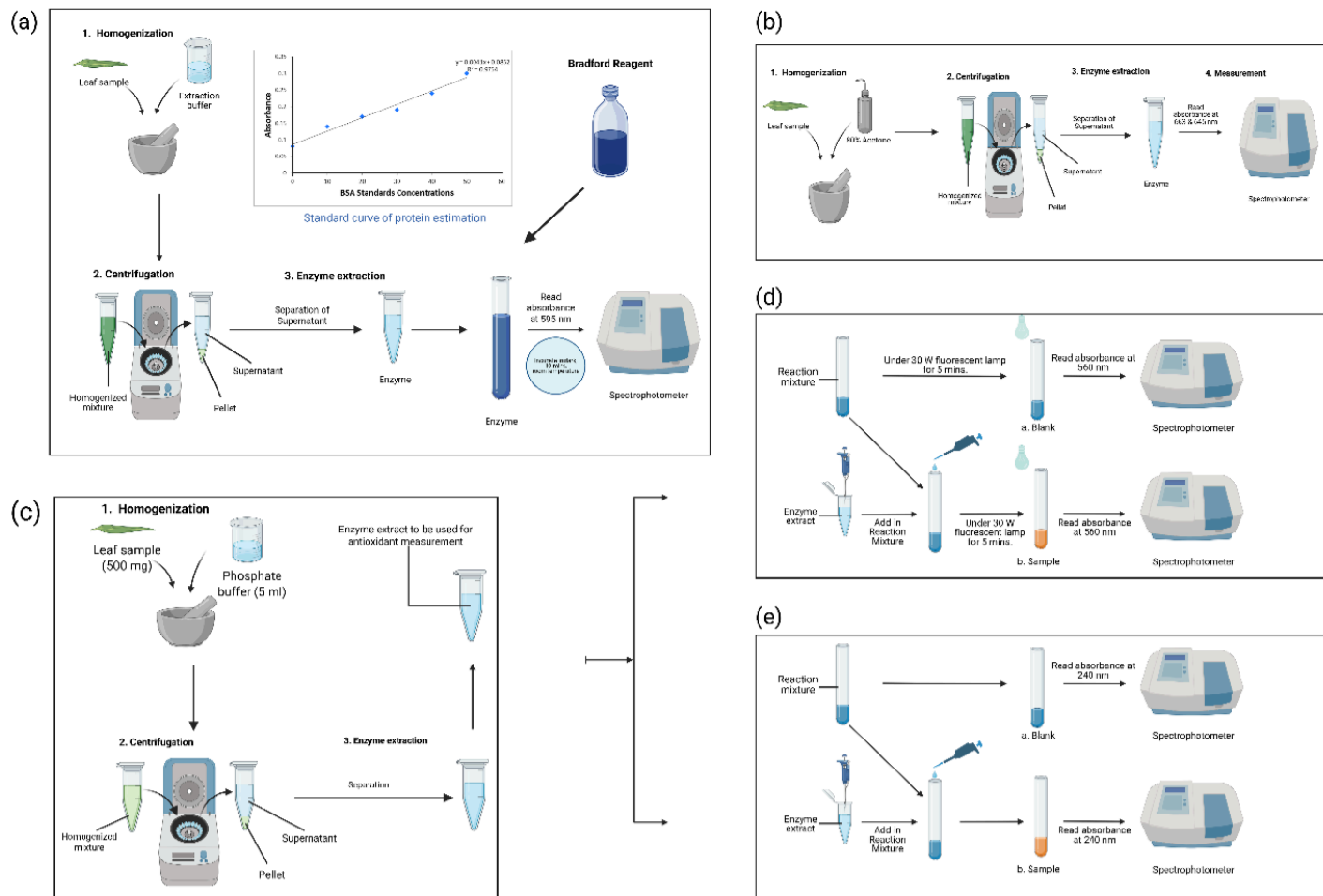


Figure 3

Schematic workflow for biochemical and enzymatic assays performed on leaf samples. The figure summarizes methods to measure (a) total soluble protein, (b) chlorophyll content, (c) enzyme extraction for antioxidant measurement, (d) SOD activity, and (e) catalase activity (created using (BioRender))

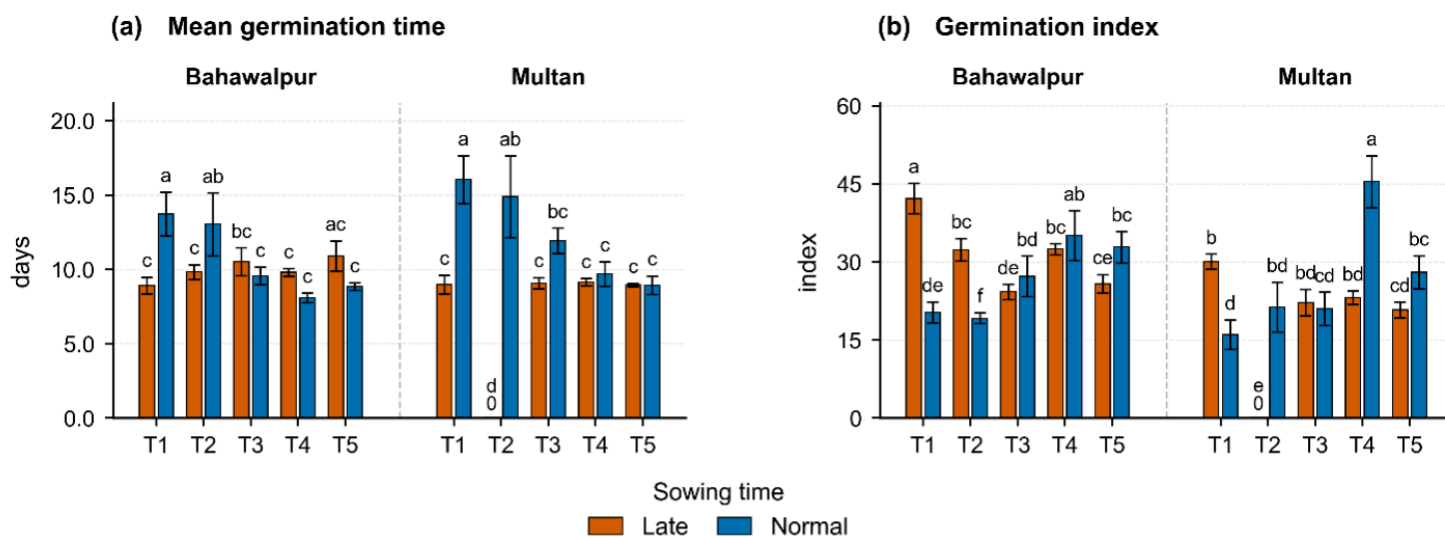


Figure 4

Effects of sowing time and treatment application on germination traits at Bahawalpur and Multan. Panels show (a) mean germination time and (b) germination index. Bars represent mean $\pm$ SE. Different letters above bars indicate significant differences among treatment combinations according to the applied post hoc test at $p < 0.05$. T1, T2, T3, T4, and T5 indicate heat seed treatment, cold seed treatment, zinc priming, proline priming, and control, respectively. Zero values indicate negligible or no measurable germination response and were retained as observed values

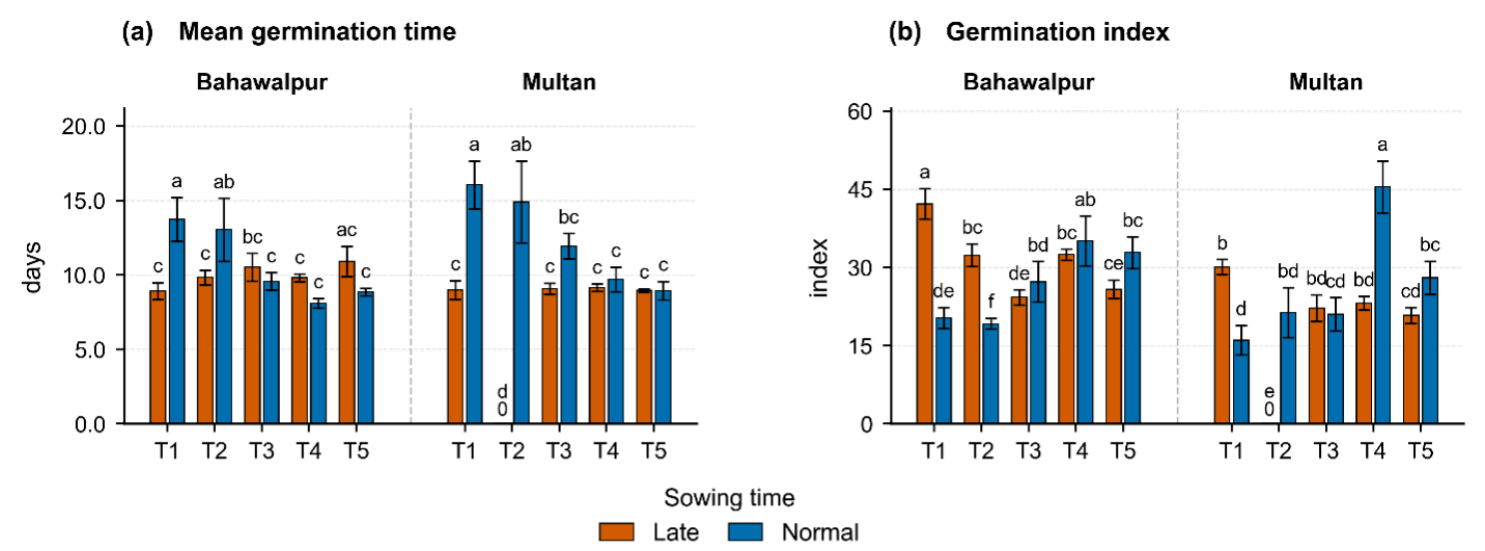


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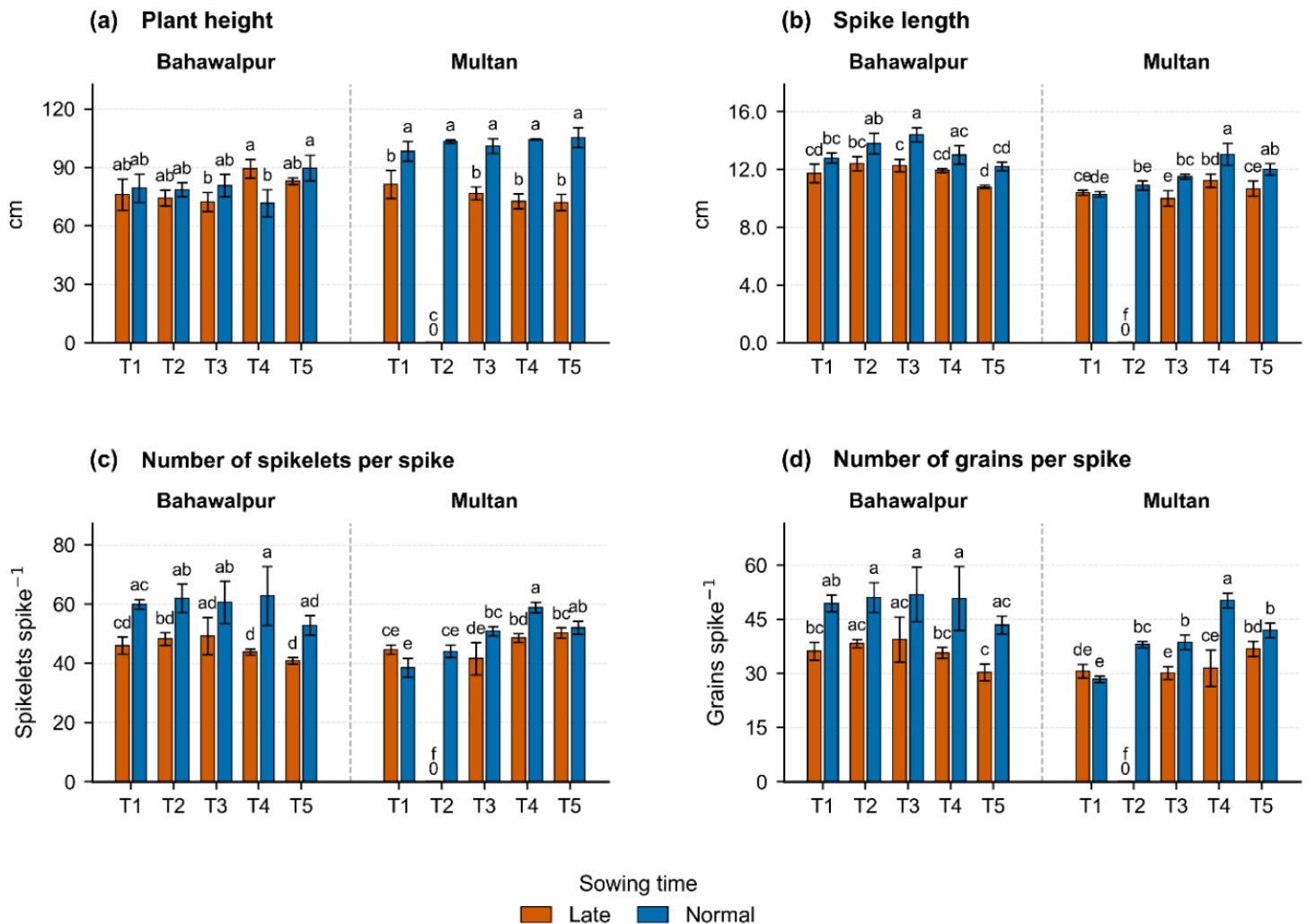


Figure 5

Effects of sowing time and treatment application on plant growth traits at Bahawalpur and Multan. Panels show (a) plant height, (b) spike length, (c) number of spikelets per spike, and (d) number of grains per spike. Bars represent mean $\pm$ SE. Different letters above bars indicate significant differences among treatment combinations at $p < 0.05$. T1, T2, T3, T4, and T5 indicate heat seed treatment, cold seed treatment, zinc priming, proline priming, and control, respectively. Zero values indicate negligible or no measurable growth and were retained as observed values

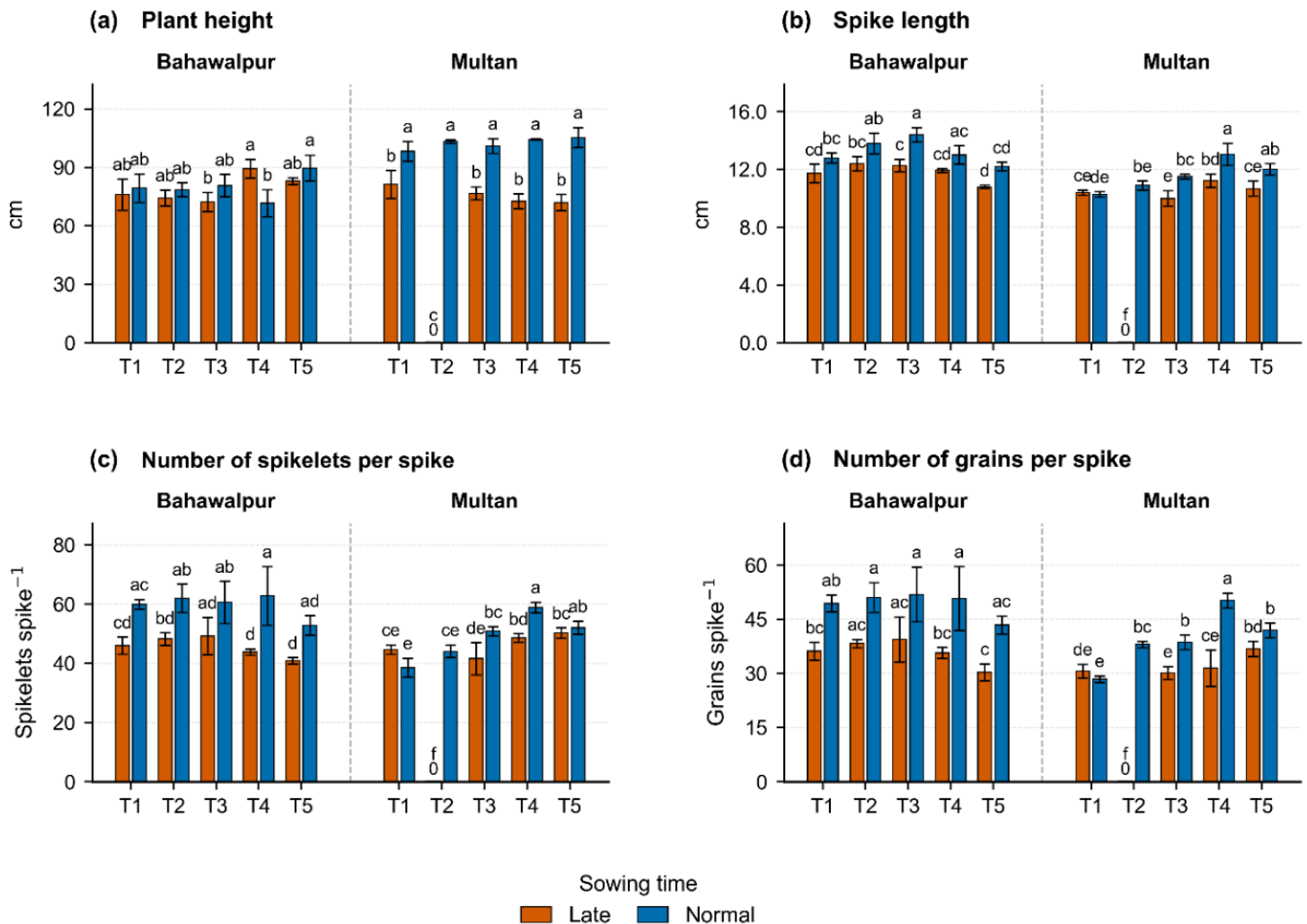


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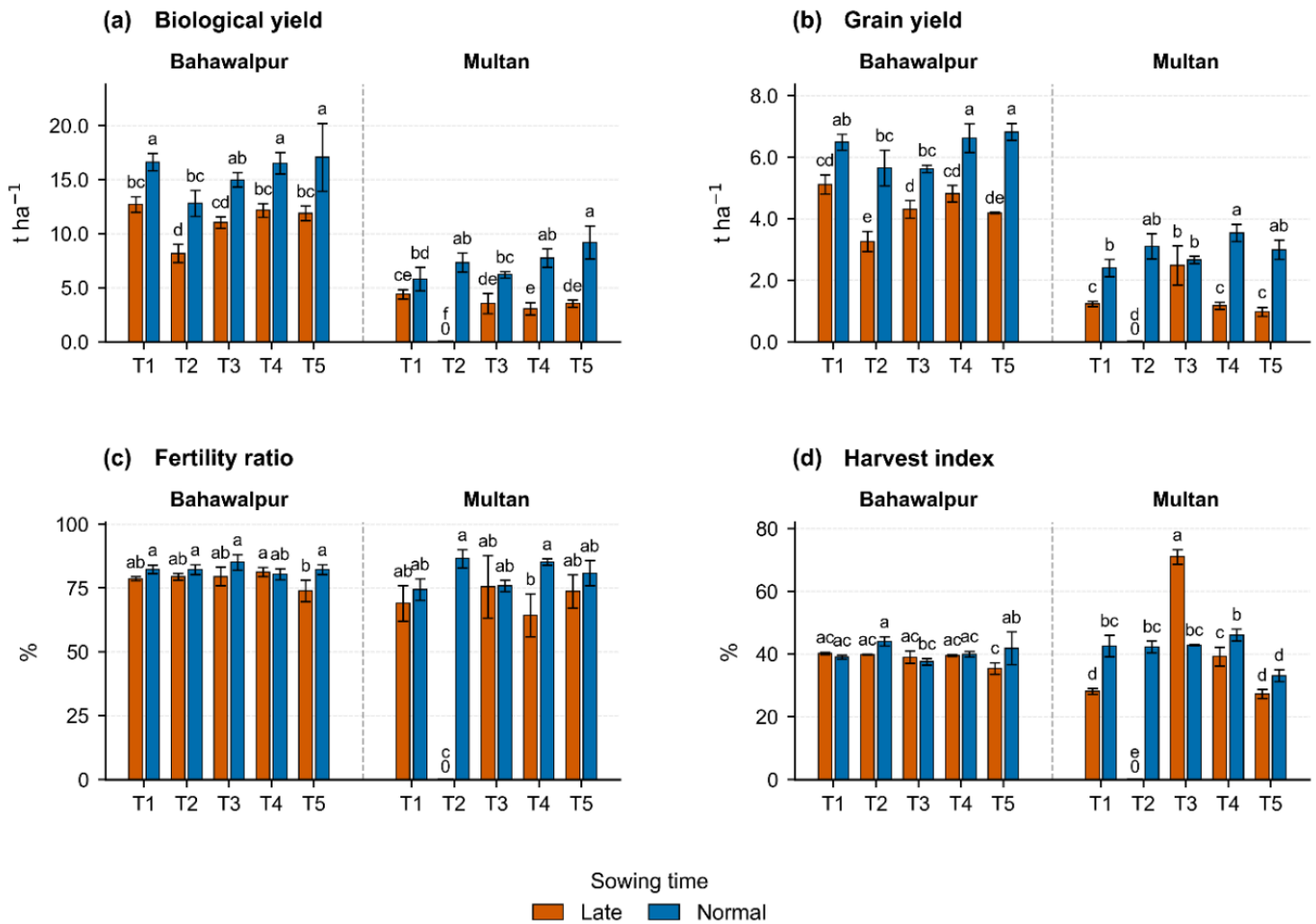


Figure 6

Effects of sowing time and treatment application on yield-related traits at Bahawalpur and Multan. Panels show (a) biological yield, (b) grain yield, (c) fertility ratio, and (d) harvest index. Bars represent mean $\pm$ SE. Different letters above bars indicate significant differences among treatment combinations at $p < 0.05$. T1, T2, T3, T4, and T5 indicate heat seed treatment, cold seed treatment, zinc priming, proline priming, and control, respectively. Zero values indicate negligible or no measurable yield response and were retained as observed values.

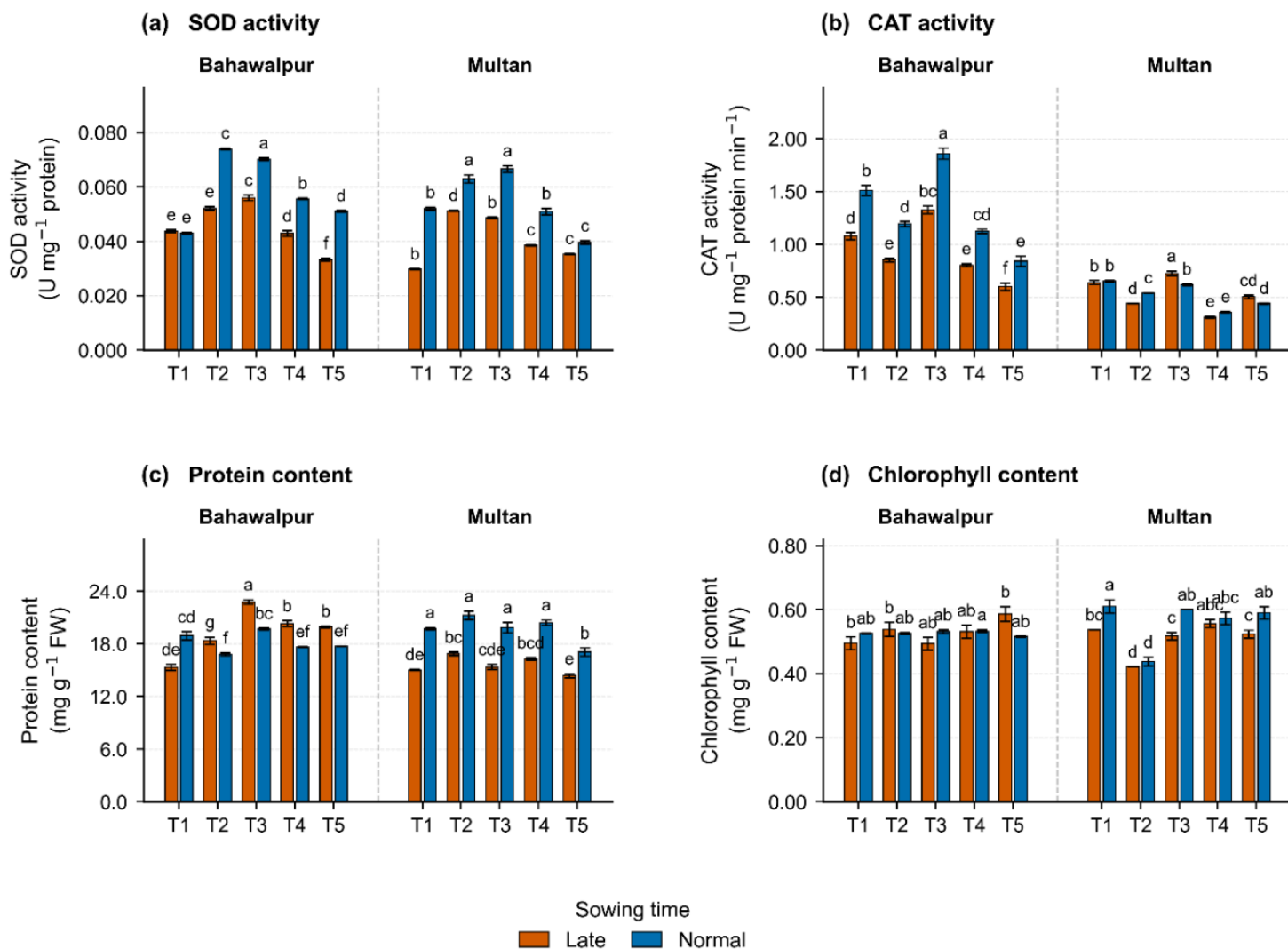


Figure 7

Effects of sowing time and seed treatments on biochemical and antioxidant-related traits at Bahawalpur and Multan. Panels show (a) superoxide dismutase activity, (b) catalase activity, (c) protein content, and (d) chlorophyll content. Bars represent mean $\pm$ SE. Different letters above bars indicate significant differences among treatment combinations according to Tukey's HSD test at $p < 0.05$. T1, T2, T3, T4, and T5 indicate heat seed treatment, cold seed treatment, zinc priming, proline priming, and control, respectively

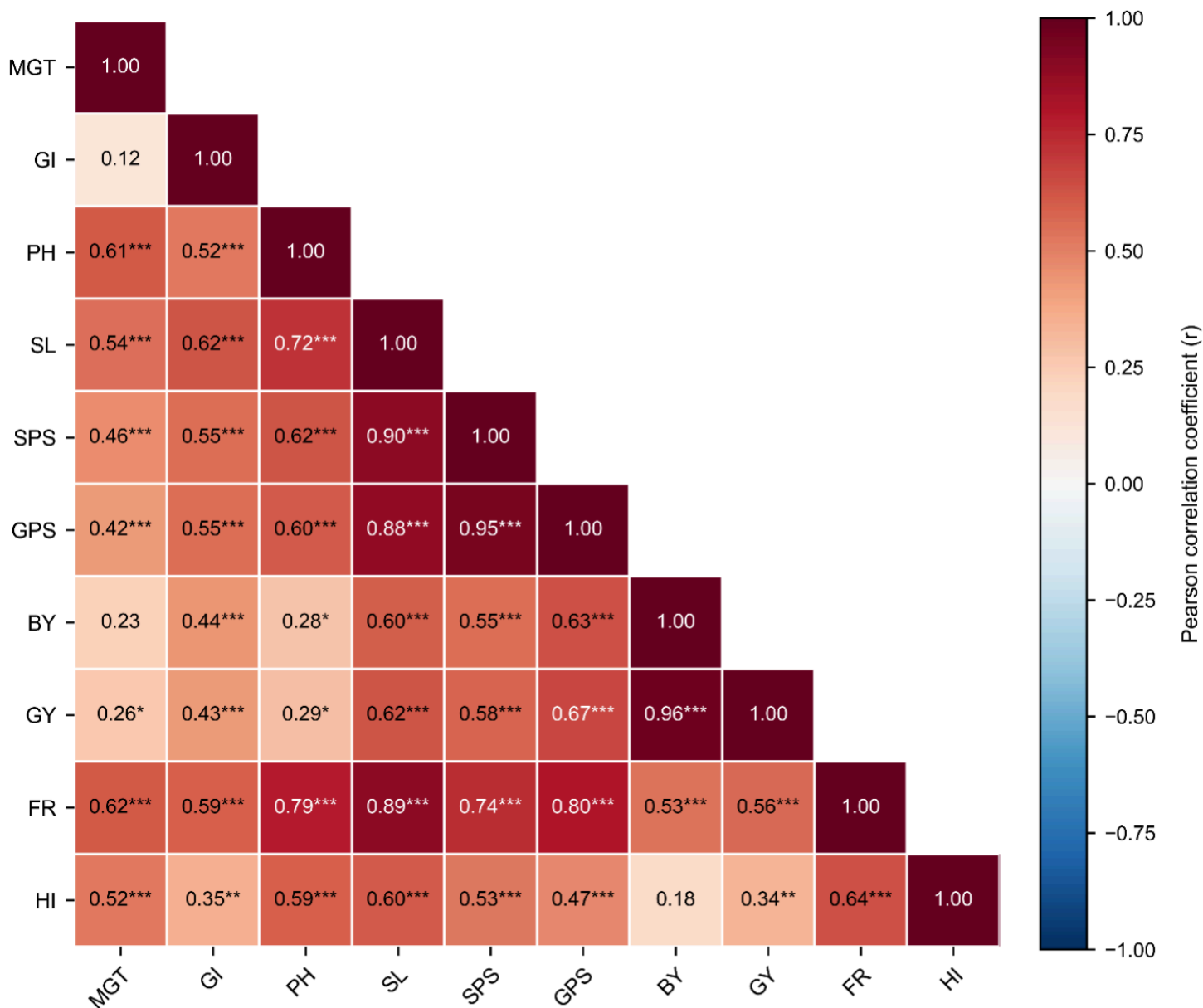


Figure 8

Pearson correlation heatmap among germination, growth, and yield-related traits across locations, sowing times, treatments, and replications. Values inside cells represent Pearson correlation coefficients. Asterisks indicate significance levels: $p < 0.05$, $p < 0.01$, and $p < 0.001$. MGT, mean germination time; GI, germination index; PH, plant height; SL, spike length; SPS, spikelets per spike; GPS, grains per spike; BY, biological yield; GY, grain yield; FR, fertility ratio; HI, harvest index

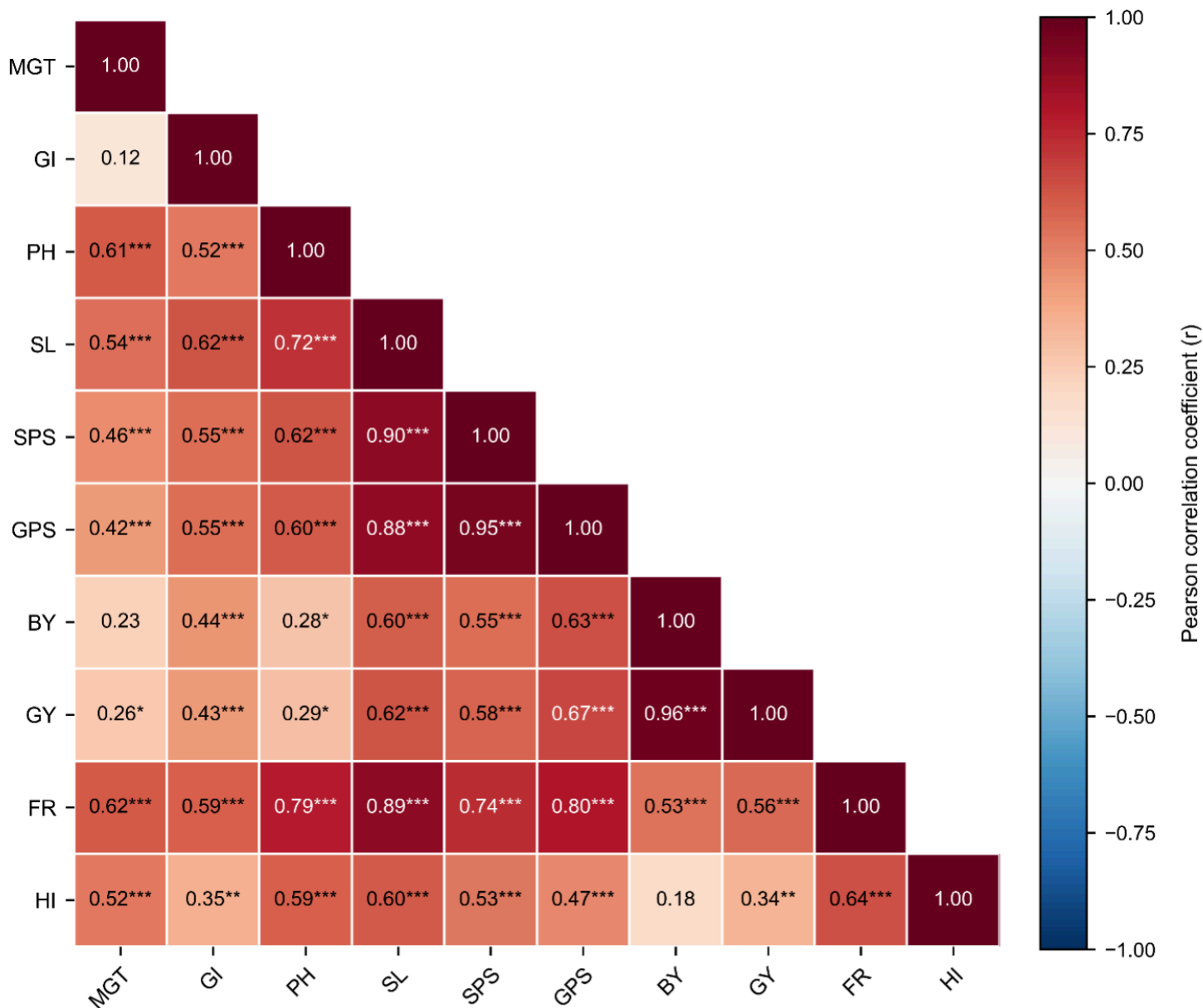


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