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Exploring physiological and biochemical responses of different wheat genotypes under heat stress conditions

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Abstract

Wheat (*Triticum aestivum*) is a vital global cereal crop, playing a pivotal role in food security. In India, it is crucial for sustenance and economic stability. However, heat stress poses a significant threat to wheat production, especially during terminal growth stages. This study examined the responses of six wheat genotypes to terminal heat stress. Physiological and biochemical traits were evaluated, including chlorophyll content, relative water content, injury % of cellular membrane stability, proline accumulation, catalase activity, and peroxidase activity. Under heat stress, chlorophyll content, water retention, and membrane stability decreased, while proline, catalase, and peroxidase activities were increased. Variability in genotypic responses highlighted genetic diversity in heat stress adaptation. Genotypes like RAJ4083, PBW71 and DBW90 exhibited higher tolerance, making them potential candidates for heat-resistant varieties. These findings deepen our understanding of wheat's responses to heat stress, emphasizing the significance of genetic diversity. This knowledge could contribute to breeding strategies for heat-tolerant wheat, enhancing food security in a changing climate.

Keywords: Wheat, terminal heat stress, physiological responses, biochemical markers and heat-tolerant genotypes

Introduction

Wheat (*Triticum aestivum*) holds a pivotal role in global food security as a staple cereal crop, serving as a primary food source for a substantial portion of the world's population (FAOSTAT, 2021) [12]. In the context of agriculture, wheat's significance is equally pronounced in India, where it ranks among the primary cereal crops and contributes significantly to both sustenance and economic stability. Despite its crucial role, wheat production faces formidable challenges, with biotic and abiotic stresses posing substantial threats. Among these, heat stress emerges as a significant factor impacting wheat cultivation, particularly during the terminal stages of growth.

Heat stress, resulting from elevated temperatures during crucial growth stages, poses a substantial threat to wheat production. Terminal heat stress, characterized by elevated temperatures during the reproductive phase, has far-reaching consequences on wheat yield and quality. This stress scenario is particularly pronounced in arid and semi-arid regions, where the deleterious effects on grain development, pollen viability and reproductive success are often exacerbated by limited water availability (Wassmann *et al.*, 2009) [25]. Moreover, the mounting concerns of climate change further intensify the frequency and severity of heatwaves, amplifying the challenges imposed by terminal heat stress on agricultural systems (IPCC, 2021) [13].

Wheat cultivation in diverse agro-climatic zones exposes the crop to varying degrees of terminal heat stress. Northwest plain zones (NWPZ), which often coincide with wheat-growing regions, are especially vulnerable to the intensifying heat waves driven by climate change. As temperatures continue to rise, the frequency and severity of heat stress events are projected to increase, potentially jeopardizing global wheat production and exacerbating food insecurity.

In response to these challenges, an in-depth exploration of the physio-biochemical responses of different wheat genotypes to terminal heat stress becomes imperative. A comprehensive characterization of these responses, encompassing aspects such as chlorophyll content, relative water content, injury % of cell membrane stability, and the activity of biochemical markers like proline accumulation, catalase activity and peroxidase, is essential to decipher how wheat plants adapt to and mitigate the impacts of heat stress.

Such insights are invaluable for developing strategies that enhance the crop's resilience to terminal heat stress, thereby ensuring sustainable agricultural productivity.

The effects of terminal heat stress extend beyond immediate yield reductions, encompassing aspects such as altered biochemical pathways, modified metabolic processes and compromised cellular structures. To address these challenges, this study aims to unravel the physio-biochemical responses of different wheat genotypes under terminal heat stress conditions. By deciphering the complex mechanisms that underlie wheat's adaptation or susceptibility to heat stress, this research intends to provide a deeper understanding of the crop's responses to temperature extremes. This knowledge forms the foundation for developing targeted strategies, including breeding efforts aimed at selecting and developing heat-tolerant wheat varieties. These varieties have the potential to sustain agricultural productivity and contribute to global food security in the face of a changing climate.

Materials and Methods

Table 1: List of Six wheat genotype selected for physio-biochemical characterization under timely and late sowing condition

Sr. No.	Genotypes	Pedigree	Recommended zone for cultivation	Response towards heat stress
1.	PBW 343	ND/VG1944//KAL//BB/3/YACO's/4/VEE5's'	NWPZ	Thermo-sensitive
2.	HD 2967	ALD/CUC//URES/HD2160/HD2278	NWPZ/NEPZ	-----do-----
3.	DBW 303	WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/SHAMA*2/5/PBW343*2/ KUKUNA*2//FRTL/PIFED	NWPZ	-----do-----
4.	DBW 90	HUW-468/WH-730	NWPZ	Thermo-tolerant
5.	RAJ 4083	PBW-343/UP-2442//WR-258/UP-2425	NWPZ/PZ	-----do-----
6.	PBW 71	PRINIA/UP2425	NWPZ	-----do-----

Studies on physio-biochemical responses of wheat genotypes under timely and late sowing condition
Chlorophyll content

In the current study, the determination of chlorophyll content was conducted using a portable Minolta Chlorophyll Meter SPAD-502 (Soil Plant Analysis Development), following the methodology outlined by Dhyani *et al.*, (2013) [11].

Experimental details

Physio-biochemical characterization studies on wheat genotypes were conducted to investigate their responses to heat stress conditions. The experiments took place at the Crop Research Center, SVPUAT, in Meerut, Uttar Pradesh, India, from November 2022 to April 2023. The study involved two different sowing dates: One under timely sown conditions (November 18, 2022) and the other under late sown conditions (December 28, 2023), with a gap of 40 days from the timely sown date condition to create heat stress environment in field. The experimental design employed a Randomized Block Design with three replications. Among a pool of 30 wheat genotypes, six were selected based on their response to heat stress. Three genotypes exhibited heat tolerance (DBW 90, RAJ 4083, and PBW 71), while the other three were heat-sensitive (PBW 343, HD 2967, and DBW 303), (Table 1). The distance was maintained in plant to plant (10 cm) and row to row (22.5cm) and the row length was maintained as 3.5-m.

Specifically, chlorophyll content measurements were taken during the vegetative stage for both timely and late sowing conditions, utilizing the SPAD Chlorophyll meter. To ensure accuracy, triplicate readings were obtained for each measurement, focusing on the third upper expanded flag leaflet.

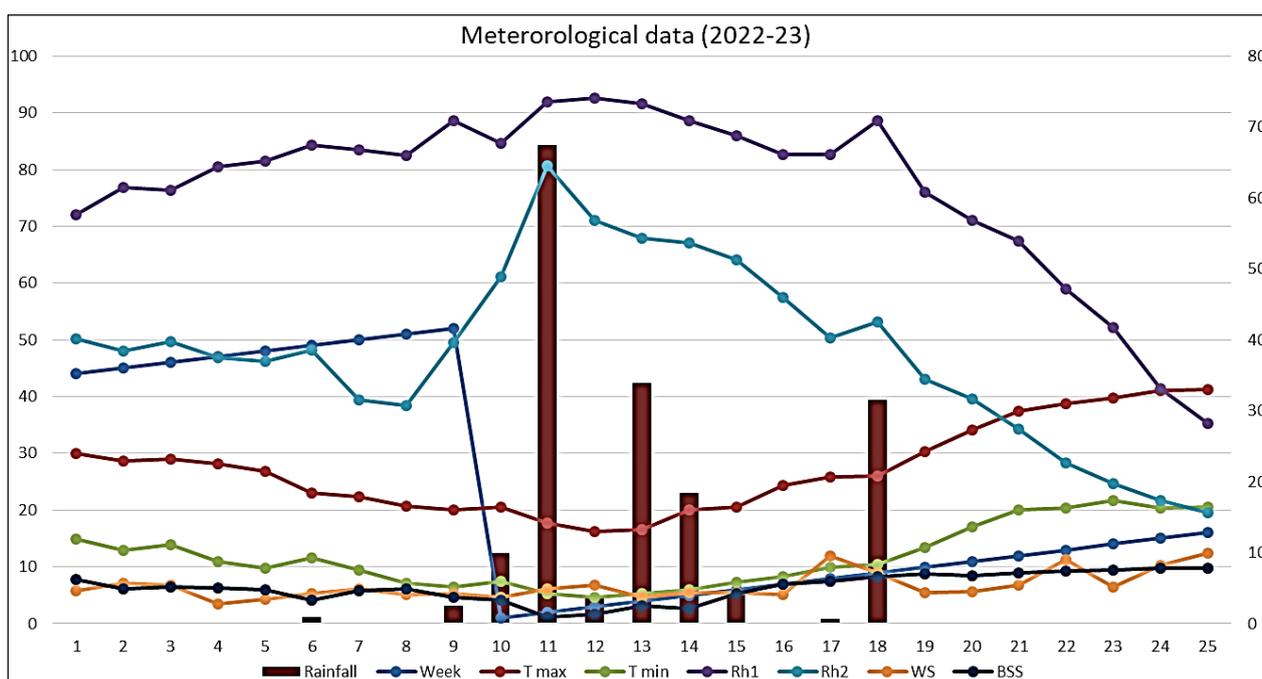


Fig 1: Weekly Agro-meteorological data during the crop growing *Rabi* season (2022-23) (November to April)

Relative water content

The Leaf Relative Water Content (RWC) % was assessed following the method outlined by Barrs and Weatherley in 1962 [6]. Leaves were sampled from both timely and late sown field conditions during the vegetative stage. Uniform leaf samples, approximately 1.5 cm in diameter, were obtained by cutting. The fresh weight of these leaf samples was promptly recorded. Subsequently, the leaf samples were placed in petri plates with distilled water and kept under constant light for a 4-hour period. The turgid weight of the soaked leaf samples was then determined. The leaf samples were later dried in an incubator at 80 °C for 24 hours and the total dry weight was measured the following day. RWC was calculated by given formula-

$$\text{RWC}\% = \frac{\text{Fresh weight- Dry weight}}{\text{Turgid weight- Dry weight}} \times 100$$

Injury % Cellular Membrane Stability Index

Cell membrane stability assessment was conducted based on the electrolyte leakage method outlined by Blum and Ebercon (1981) [8]. Six leaves of approximately equal size were placed in distilled water for a 12-hour period, after which the electrical conductivity (EC1) of the solution was measured using an EC meter. Subsequently, samples immersed in water were subjected to autoclaving at 50 °C for 60 minutes and then allowed to cool to room temperature. The conductivity of killed tissues (EC2) was again measured. Cell membrane stability was calculated as the ratio between EC1 and EC2.

Proline estimation

The proline concentration was determined following the procedure outlined by Bates *et al.* (1973) [7] with minor modifications. The method involved the use of various reagents and stock solutions, including aqueous sulfosalicylic acid (3%), glacial acetic acid, toluene and acid ninhydrin.

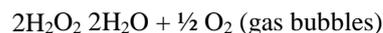
For proline estimation, approximately 0.5 g of leaf samples were ground and homogenized with 10 ml of 3% sulfosalicylic acid. The resulting homogenate was then filtered, and 2 ml of the filtrate was mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin reagent. The mixture was thoroughly mixed and incubated in a boiling water bath at 100 °C for 1 hour. Subsequently, the reaction was terminated by cooling the mixture in an ice bath and then allowing it to warm to room temperature (28 °C). In order to extract the proline, 4 ml of toluene was added to the mixture and thoroughly shaken. The upper toluene layer, which exhibited a distinct pink color, was carefully separated and used as the sample, while the lower layer was discarded. The color intensity of the sample was measured at 520 nm using a UV 42 Spectrophotometer (Perkin Elmer UV/VIS spectrometer Lambda 25). The Proline was calculated by given formula-

$$\mu\text{moles per gram tissue} = [(\mu\text{g proline/ml}) \times \text{ml toluene}] / [115.5 \mu\text{g}/\mu\text{mole}] / [(\text{g sample})/5]$$

Catalase estimation

Catalase activity was assayed according to Sinha *et al.*, (1972) [24]. In this method various reagents and stocks solution *viz.* Phosphate buffer 0.1 M (pH-7.0), Potassium dichomate acetic acid, 5% (294.19 mw) K₂Cr₂O₇ + glacial acetic acid in 1:3 ratios and H₂O₂ 2% were used. Catalase facilitates the

distribution of H₂O₂ to water and O₂ according to the reaction



100mg leave sample crushed with 10ml of PO₄ buffer and Centrifuged at 10,000 rpm for 20 min at 4 °C. The 1ml of supernatant was extracted by homogenized filtrate and 1 ml H₂O₂, 3ml of PO₄ Buffer was added in test tube mixture. The test tube was incubated at 37 °C for 3min in water bath. 2ml of aliquot was with 4 ml K₂Cr₂O₇/CH₃COOH solution and incubated in water bath for 10 min at 37 °C. Finally observation was recorded at 570nm at spectrophotometer (Perkin Elmer UV/VIS spectrometer Lambda 25).

Peroxidase estimation

Peroxidase activity was determined calorimetrically following the method outlined by Malik and Singh (1980) [18], with specific modifications. The enzymatic reaction involved the catalysis of substrate oxidation and the removal of hydrogen, which subsequently combined with H₂O₂. The experimental procedure utilized various reagents and stock solutions, including a 0.1 M phosphate buffer (pH 6.1) and pyrogallol (0.01 M) with a molecular weight of 126.11 g/mol. The preparation of the H₂O₂ solution (2%) involved the calculation: Pyrogallol = 0.01 x 126.11 x 100/1000 = 0.12611 g/100 ml.

Leaves (1 g) were crushed and homogenized with 2 ml of phosphate buffer, followed by further homogenization with 10 ml of phosphate buffer. The resulting homogenate underwent centrifugation at 1000 rpm at 4 °C for 15 minutes. The supernatant was carefully collected and stored for future use at 4 °C. For the subsequent analysis, 1 ml of the extracted solution was mixed with 1.5 ml of 0.01 M pyrogallol and 2 ml of H₂O₂. The final volume of the mixture was adjusted to 6 ml by adding distilled water. The absorbance was recorded at 430 nm using a spectrophotometer (Perkin Elmer UV/VIS spectrometer Lambda 25).

Result and discussion

The results (Table 2) revealed that Physiological characters as Chlorophyll content, Photosynthetic rate, Relative water content and Injury % of Cellular membrane stability were investigated and found to be decreased under heat stress condition while Biochemical characters as Proline, Catalase and Peroxidase activities were found to be increased in heat treatment condition.

Chlorophyll content is a pivotal indicator of plant health and photosynthetic activity, particularly in response to heat stress. Our study revealed a significant reduction in chlorophyll content under heat stress conditions, with genotypes PBW71 and DBW303 exhibiting chlorophyll content ranging from 34.1 µg/cm² to 44.3 µg/cm² under timely sown conditions. Late sown conditions further exacerbated the decline, with chlorophyll content varying from 29.83 µg/cm² to 36.43 µg/cm². Notably, the average chlorophyll content decreased from 39.06 µg/cm² under timely sowing to 32.94 µg/cm² in heat-stress treatment. (Fig 2). These findings resonate with previous studies by Lehari *et al.* (2018) [16], Almeselmani *et al.*, (2012) [2] and Saeidi *et al.* (2015) [21], highlighting the sensitivity of chlorophyll content to heat stress and its subsequent implications for crop biomass and yield.

Relative Water Content (RWC) serves as a robust indicator of plant water status under stress conditions. Our results

demonstrated a substantial decline in RWC due to heat stress, indicating heightened cellular water loss. Timely sown conditions exhibited RWC ranging from 82.13% to 89.04%, whereas late sown conditions saw a decrease to 79.88% to 85.73%. The mean RWC declined from 83.93% to 79.93%, reflecting the adverse effect of heat stress on water availability (Fig 3). Similar trends have been reported by Saeidi *et al.* (2015) [21], Lugojan and Ciulca (2011) [17], Saleem *et al.*, (2017) [22] and Singh *et al.* (2014) [23], aligning with the concept that decreased RWC negatively impacts plant physiological processes and crop productivity.

Injury % of Cell Membrane Stability is a crucial parameter for assessing plant cell membrane integrity and tolerance to heat stress. Our investigation revealed a marked increase in injury % under heat stress conditions. Genotype DBW303 displayed the highest injury % (90.23%) under timely sown conditions, while genotype HD2967 exhibited the lowest (76.42%). Late sown conditions further amplified injury %, with values ranging from 66.34% to 78.74%. (Fig 4). These findings corroborate the susceptibility of cell membranes to heat stress, as reported by Khan *et al.* (2013) [14] and Dhindsa *et al.* (1991) [10]. The heightened injury % underscores the vulnerability of plant cell membranes to oxidative damage caused by heat-induced lipid peroxidation.

Proline accumulation is a well-documented response to various abiotic stresses, including heat stress. Our study confirmed increased proline content in genotypes subjected to heat stress. Under timely sown conditions, proline content ranged from 0.42 µg/gfw to 0.89 µg/gfw, whereas under late sown conditions, it varied from 1.28 µg/gfw to 2.42 µg/gfw. Notably, genotype RAJ4083 exhibited the highest proline content (0.89 µg/gfw) under timely sowing, and this increased to 2.42 µg/gfw after heat treatment (Fig 5). These findings align with the studies of Amirjani and Mahdiyeh (2013) [4], Ashgan *et al.*, (2014) [5] and Ahmed and Hasan (2011), underscoring proline's role as an osmotic regulator and protective agent against heat-induced cellular damage.

Catalase, an antioxidant enzyme, plays a critical role in mitigating oxidative damage induced by heat stress. Our research demonstrated an elevation in catalase content under heat stress conditions. Genotype RAJ4083 exhibited the highest catalase content (15.21 µmole/gfw) under late sown conditions, while genotype HD2967 showed the lowest (7.67 µmole/gfw) under timely sown conditions. Late sown conditions increased catalase content in the range of 8.96 µmole/gfw to 15.21 µmole/gfw (Fig 6). These results are

consistent with Kumar *et al.* (2012b) [15] Amarshettiwar *et al.*, (2018) [13] and Mansoor and Naqvi (2013) [19], highlighting the role of catalase in antioxidant defense against heat-induced oxidative stress.

Peroxidase is another enzyme involved in detoxifying reactive oxygen species under stress conditions. Our investigation demonstrated an increase in peroxidase activity with rising temperatures. Under timely sown conditions, peroxidase activity ranged from 2.49 µmole/gfw to 4.26 µmole/gfw, while under late sown conditions, it varied from 3.36 µmole/gfw to 5.30 µmole/gfw. Genotype RAJ4083 exhibited the highest peroxidase activity (5.30 µmole/gfw) after heat treatment (Fig 7). These findings align with Chakraborty and Pradhan (2012), indicating peroxidase's role in managing oxidative stress induced by heat.

Conclusion

The physiological and biochemical characterizations shed light on the complex interplay between wheat genotypes and heat stress. The observed reductions in chlorophyll content, RWC and cell membrane stability underscore the vulnerability of plants to heat stress-induced physiological changes. The accumulation of proline, catalase, and peroxidase suggests the activation of protective mechanisms to counteract heat-induced oxidative damage. Our findings resonate with prior studies, emphasizing the importance of these physiological and biochemical responses in conferring heat stress tolerance. The contrasting responses of different genotypes indicate genetic diversity in heat stress adaptation. Genotypes such as RAJ4083, PBW71 and DBW90 exhibited higher proline, catalase and peroxidase activities, suggesting their potential as heat-tolerant candidates. Further investigations into the genetic basis of these responses could facilitate the development of heat-resistant wheat varieties.

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Table 2: Comparison of Physio-Biochemical traits of six wheat genotypes

Genotype	Chlorophyll content (µg/cm ²)		Relative water Content (%)		Injury (%) of CMS		Proline (µmole/gfw)		Catalase (µmole/gfw)		Peroxidase (µmole/gfw)	
	Timely	Late	Timely	Late	Timely	Late	Timely	Late	Timely	Late	Timely	Late
PBW 343	41.47	34.29	82.13	78.07	76.42	67.92	0.58	1.31	7.86	9.22	2.68	3.88
HD 2967	42.3	35.76	80.25	77.82	78.67	66.34	0.68	1.28	7.67	8.96	2.49	3.36
DBW 303	44.3	36.43	79.88	76.01	76.82	68.76	0.42	1.36	8.72	9.42	2.81	3.85
DBW 90	36.5	30.23	85.4	82.98	89.81	77.62	0.89	2.42	13.86	15.21	4.26	5.3
RAJ 4083	34.1	29.83	89.04	85.73	87.76	75.23	0.69	2.1	11.1	12.99	3.26	4.89
PBW 71	35.7	31.12	86.88	79.01	90.23	78.74	0.78	2.21	13.01	14.66	3.87	5.08
Mean	39.0617	32.9433	83.93	79.9367	83.285	72.435	0.67	1.78	10.37	11.74	3.23	4.39
C.D.	2.342	1.973	5.012	4.78	4.99	4.331	0.042	0.11	0.637	0.719	0.197	0.265
SE(m)	0.734	0.618	1.57	1.497	1.563	1.357	0.013	0.035	0.199	0.225	0.062	0.083
SE(d)	1.038	0.874	2.221	2.118	2.211	1.919	0.018	0.049	0.282	0.319	0.087	0.118
C.V.	3.254	3.249	3.24	3.245	3.251	3.245	3.354	3.358	3.332	3.324	3.31	3.277

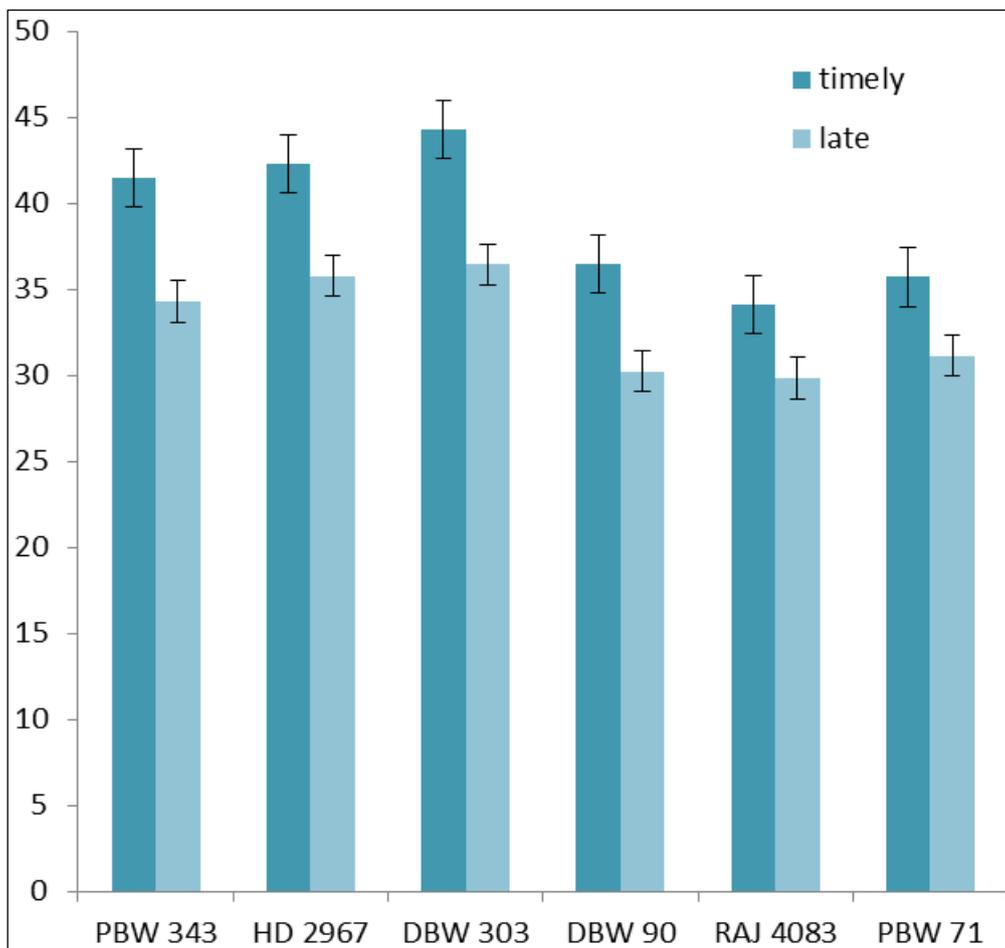


Fig 2: Comparison of Chlorophyll content ($\mu\text{g}/\text{cm}^2$) of Six Wheat genotypes under timely and late sown condition

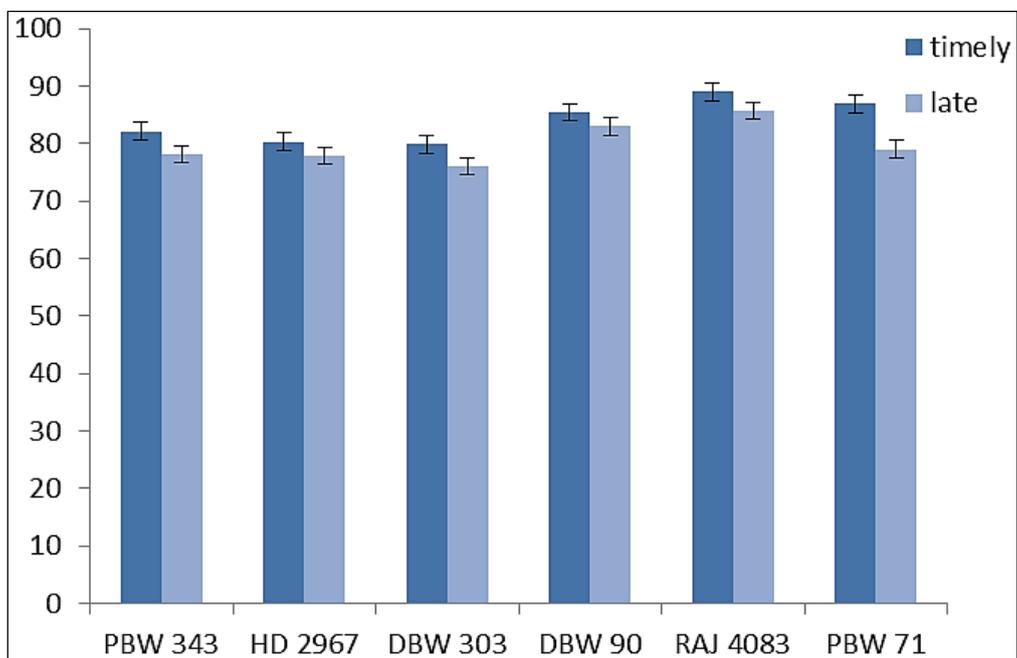


Fig 3: Comparison of Relative water Content (%) of Six Wheat genotypes under timely and late sown condition

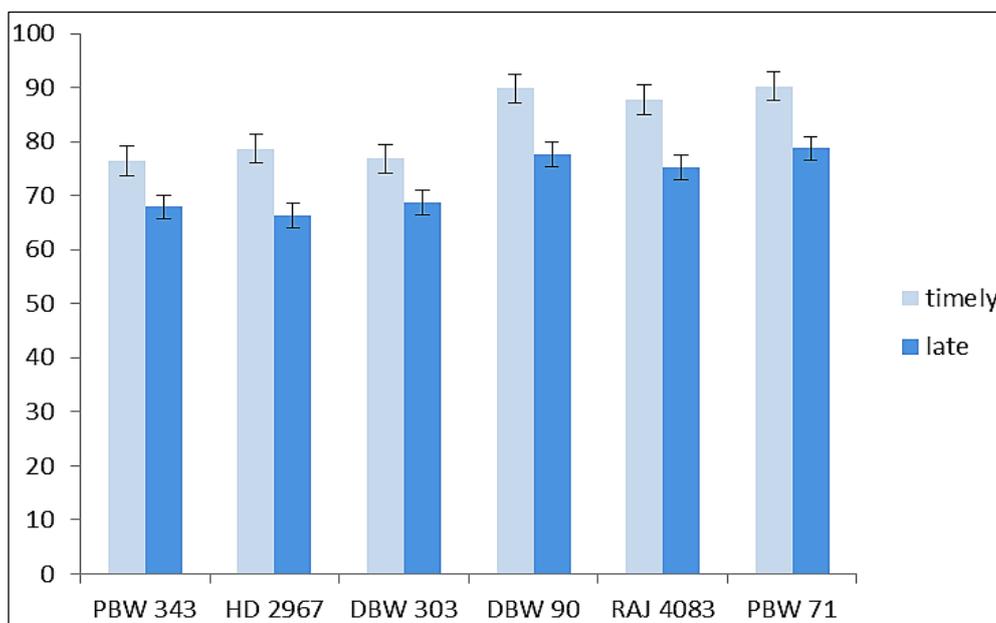


Fig 4: Comparison of Injury (%) of CMS of Six Wheat genotypes under timely and late sown condition

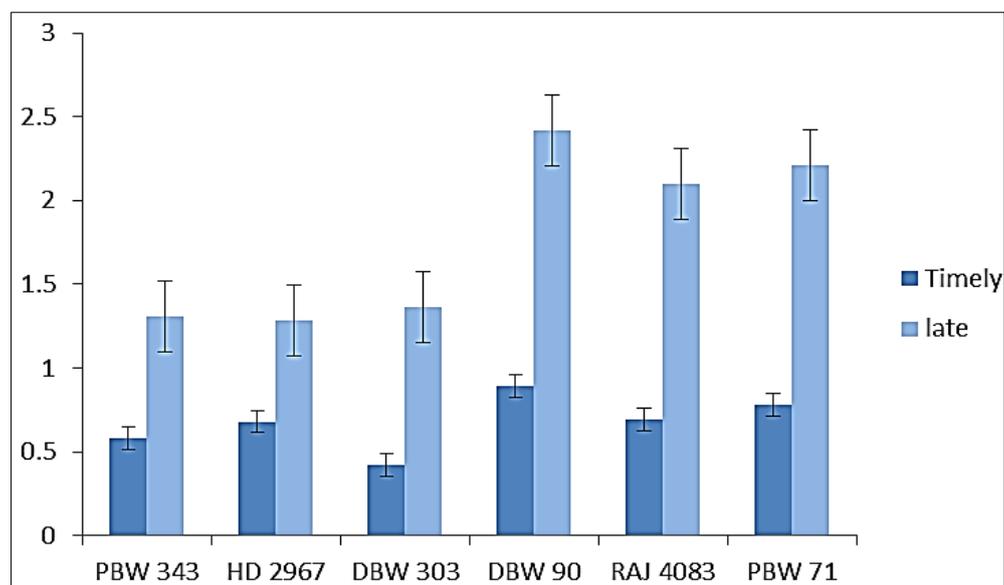


Fig 5: Comparison of Proline content (µmole/gfw) of Six Wheat genotypes under timely and late sown condition

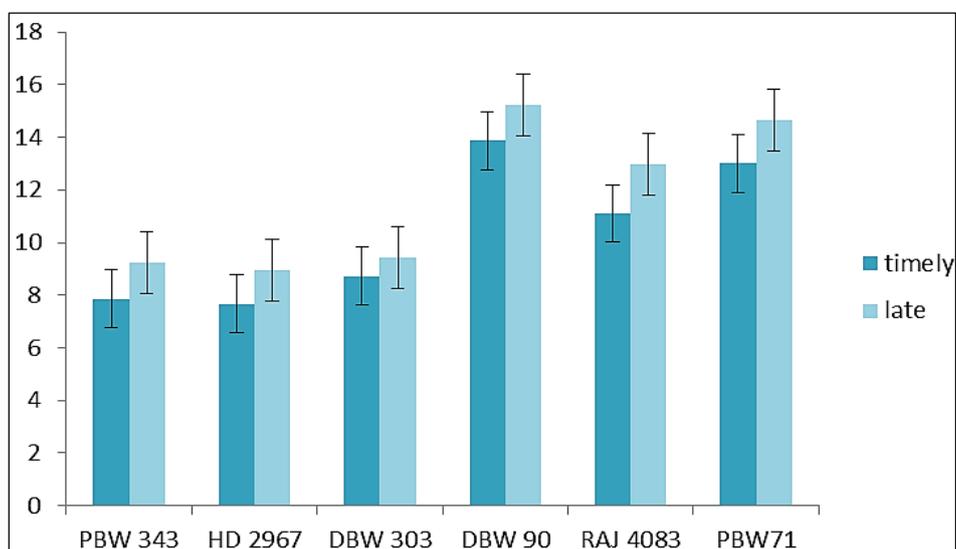


Fig 6: Comparison of Catalase activity (µmole/gfw) of Six Wheat genotypes under timely and late sown condition

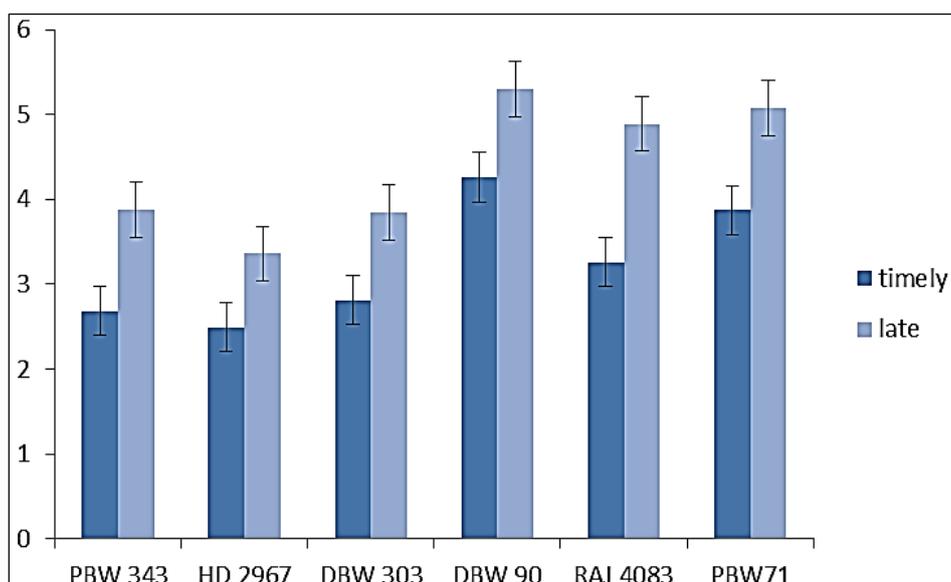


Fig 7: Comparison of Peroxidase activity ($\mu\text{mole/gfw}$) of Six Wheat genotypes under timely and late sown condition

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