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Exploring the impact of biogenic zinc oxide nanoparticles derived from Lantana camara L. on alleviating heat stress in wheat

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Abstract

Wheat faces escalating vulnerability to heat stress as a consequence of climate change, resulting in a substantial annual loss of up to 30% in global wheat productivity. Nanotechnology emerges as a promising frontier, with the potential to revolutionize agriculture across various stages, from seed priming to post-harvest storage. In this study, biogenic zinc oxide nanoparticles (ZnO-NPs) synthesized from *Lantana camara* leaf extracts was evaluated on growth, morphological, bio physiochemical and molecular parameters in four wheat genotypes. The application of ZnO-NPs, particularly at a concentration of 100 ppm, significantly improved various seed growth parameters and total protein content. Genetic analyses unveiled substantial variability among wheat genotypes under both non-stress and heat-stress conditions. Heritability and genetic advance estimates underscored spike length and grain yield as promising traits. Moreover, genotypic and phenotypic correlations revealed intricate relationships between agronomic traits, providing valuable insights for targeted breeding strategies. Path analysis elucidated the direct and indirect contributions of traits to grain yield, emphasizing the significance of biomass, effective tillers per plant, and spike length. Physiological and biochemical responses indicated that ZnO-NPs alleviated the adverse effects of heat stress on chlorophyll content, photosynthetic rate, relative water content, and cellular membrane stability. Simultaneously, ZnO-NPs enhanced proline, catalase, peroxidase and superoxide dismutase activities. Gene expression analysis of heat shock proteins (HSP17, Hsp23, and Hsp70) in heat-tolerant and heat-sensitive genotypes under various heat shock treatments revealed distinct responses, with HSP17 exhibiting the highest expression in PBW226 under stress conditions. Overall, this research showcases the potential of biogenic ZnO-NPs to enhance wheat resilience in the face of climate change, highlighting nanotechnology's pivotal role in modern agriculture and its contribution to global food security.

Introduction

Wheat (*Triticum aestivum* L.) stands as a crucial staple food crop globally, constituting approximately 30% of the world's total grain production within the Poaceae family. Addressing the calorie needs of the global population, wheat faces a pressing challenge due to the imminent scarcity of food and fiber as populations rapidly expand. Rising global temperatures pose a significant threat to food security, impacting plants through physiological, biochemical, and molecular processes. Wheat, in particular, is highly susceptible to heat stress, with a 6% average decline in yield for every 1°C increase in atmospheric temperature (Asseng et al., 2015). Projections indicate a potential rise of 1.4 to 3.1°C in the world's average temperature by the end of the twenty-first century. The impact of elevated temperatures is particularly concerning for wheat production in South Asian countries like India, Pakistan, Nepal, and Bangladesh (Mondala et al., 2013). Increased temperatures lead to growth retardation, reduced cell division and elongation, resulting in dwarf plants, diminished root growth, and damage to cellular and protein structures (Rahman 2004). Photosynthetic enzymes become deactivated, generating reactive oxygen species (ROS) that hinder growth, development, and yield, marked by reduced grain size and number. Efforts to counteract high-temperature effects on wheat include hybridization (Zhao et al., 2008; Semenov and Halford 2009), genetic engineering (Barnabas et al., 2008), molecular marker-assisted selection, and QTL mapping (Vinh and Paterson 2005; Yusuf et al., 2010). However, these approaches face operational, cost, and technical challenges, prompting a continual search for practical, affordable, and feasible techniques. Nanotechnology, manipulating matter at the atomic or molecular level, holds promise for significant improvements in agricultural technologies. Some metal oxide nanoparticles, such as zinc nanoparticles (Zn-NP), have shown potential in enhancing crop productivity without causing metabolic imbalances. Studies in agriculture have highlighted the role of ZnO-NPs in protecting plants from heat stress by boosting antioxidant enzyme activity and accumulating osmolytes, free amino acids, and nutrients. Numerous studies have demonstrated the positive impact of ZnO-NPs, and their application to stressed plants has the potential to amplify the process of reactive oxygen species (ROS) generation, ultimately resulting in oxidative damage. Previous research findings indicate that nanoparticles synthesized through biological means exhibit lower toxicity when compared to those produced through chemical or physical synthesis. Biosynthesis of nanoparticles involves the use of microorganisms and plants, presenting an environmentally friendly, cost-effective, biocompatible, safe, and green approach. Consequently, the objective of this study was to employ zinc oxide nanoparticles on wheat genotypes within molecular breeding programs, aiming to develop superior wheat cultivars in the future.

Materials and Methodology

Green synthesis, optimization and characterization of zinc oxide nanoparticles were carried out in the Division of Plant Biotechnology, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India (Agrawal et al., 2023).

Collection of seed Material

Seed material from four wheat genotypes, comprising two heat-tolerant (PBW 226 and DBW 187) and two heat-susceptible varieties (DBW 173 and HD 3226) were obtained from the seed distribution center at the old campus of SVPUA&T, Meerut (Table 1). These four wheat genotypes were chosen for the screening process to assess seed germination.

Table 1 Details of wheat genotype used in present investigation

Genotype	Pedigree	Origin	Grown	Feature
HD3226	GRACKLE/HD2894	IARI, New Delhi	NWPZ	Irrigated, Timely Sown
PBW 226	C591/RN//JN/3/CHR/HD1941	PAU, Ludhiana Punjab	NWPZ	Irrigated, late sown
DBW187	NAC/TH.AC//3*PVN/3/MIRLO /BUC/4/2*PASTOR/5/KACHU/6/KACHUKACHU/SAUAL/8/ATTILA*2/PBW65/6/PVN//CAR422/ANA/ 5/BOW/CROW//	IIWBR, Karnal	NWPZ	Timely sown, irrigated conditions
DBW-173	KAUZ/AA//KAUZ/PBW602	IIWBR, Karnal	NWPZ	Irrigated, late sown

Evaluation of biogenic ZnO-NPs on germination parameters in wheat under laboratory conditions

Seed priming method

To study nanoparticle effects on seed priming, two methods were employed: hydropriming (water alone) and nanopriming (ZnO NPs in water). Untreated seeds served as the control. Wheat seeds were sterilized with 0.1% sodium hypochlorite for 2 min, then washed with deionized water. For priming, seeds were exposed to 100ml of ZnO-NPs at 50, 100, 150, and 200 ppm. Freshly prepared zinc oxide nano suspensions were used. Seeds immersed in NPs for 2 hrs at 25 °C with agitation. Primed seeds were washed (3-4 times, 3 min each) and dried. Seven seeds were placed in each petri dish on filter paper, and 2ml water added following guidelines. Post-treatment, seeds rinsed and germinated at 25 °C in an incubator.

Seed germination parameters

Selected germination parameters were recorded to assess the impact of zinc oxide nanoparticles on wheat seeds. These parameters included germination percentage (sprouted seeds divided by total seeds), root length (from seed base to tip), coleoptile length (shoot length up to tip), seedling length (sum of shoot and root lengths), fresh weight (weighing detached shoots and roots), and dry weight (weighing after overnight drying at 37 °C). The Seed Vigor Index (SVI) was calculated as Germination % multiplied by (Root length + Shoot Length) (ISTA, 1987).

Total protein content

Weigh 1 gram of seeds. In a cold mortar, add 1 ml of protein extraction buffer per gram of leaf. Grind the tissue with fine sand or liquid nitrogen until a thick paste forms. Centrifuge for 20 minutes at 12,000 rpm at 4°C, then transfer the supernatant to another tube. Take about 100 µl of the supernatant for protein quantification using the Bradford method (1976).

Evaluation of biogenic ZnO-NPs in wheat genotypes under field conditions.

Experimental site and weather conditions

The study was conducted during the Rabi seasons of 2021-2022 and 2022-2023 at the Crop Research Centre, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250110 (U.P.). The experimental field had fertile, sandy loam soil. Meteorological data, including weekly rainfall distribution, max/min temperature, relative humidity, wind velocity, mean evaporation rate, and sunshine hours, were recorded from the SVPUA&T meteorological observatory in Modipuram, Meerut. Total rainfall for the crop period was 160.70 mm in both 2021-22 and 2022-23. The minimum temperature was 4.6 ºC in the third week of January, and the maximum temperature was 37.2 ºC in the 16th week of April in both years. The highest relative humidity (97.9%) occurred in the first week of January for both 2022 and 2023.

Experimental material and design

Wheat genotypes from timely and late sowings during Rabi 2021-22 and Rabi 2022-23 were obtained from the seed distribution center at the old campus of SVPUAT. The experiment followed a Randomized Block Design (RBD) with three replications for each sowing. Regular irrigation was applied per the recommended schedule. Treatment involved spraying 100 ppm ZnO nanoparticles (both biologically synthesized and synthetic) before pre-anthesis.

Observation recorded

Field observations were recorded from five randomly chosen plants in both years. The average values of these plants were used for statistical analysis. Various morphological attributes, including Days to 50% heading, Days to 50% flowering, Plant height (cm), Spike length with awn (cm), Number of spikelets per spike, Number of grains per spike, Number of effective tillers per plant, 1000-grain weight (g), Biological yield per plant (g), Grain yield per plant (g), Straw yield per plant (g), and Harvest index (%), were examined.

Statistical Analysis

Data on different traits were statistically analyzed following Panse and Sukhatme's (1985) procedure. Replication-wise mean values underwent statistical analysis, including (Fisher's 1954) analysis of variance, mean, range, and coefficient of variation by Burton and de Vane's (1953). Additionally, heritability

(Hanson et al., 1956), genetic advance (Johnson et al., 1955), correlation coefficient (Searle, 1961), and path coefficient (Wright, 1921; Dewey and Lu, 1959) were employed to test the significance of differences among genotypes.

Evaluation of zinc oxide nanoparticles on physiochemical parameters

Physiological parameters, including Chlorophyll content (Dhyani et al., 2013), Relative Water Content (Barrs and Weatherley 1962), and cell membrane stability (Blum and Ebercon 1981), were recorded. In the current investigation, Biochemical parameters such as Proline content (Bates et al., 1973), Catalase activity (Sinha et al., 1972), Peroxidase activity (Malik and Singh 1980), and Superoxide dismutase activity (Dhindsa et al., 1981) were also analyzed for the rabi seasons of 2021-22 and 2022-23 under both timely and late sowing conditions.

Evaluation of zinc oxide nanoparticles on heat stress responsible genes in wheat

Sample preparation for gene expression analysis

Gene expression studies were conducted in the lab. Four wheat genotypes were grown in thermocol pots with normal soil for 20 days at 25˚C under a 16-hour light period in an incubator. To induce heat stress, 20-day-old plants were transferred to temperatures of 34˚C and 37˚C for 90 minutes. Leaf samples were collected from different temperatures and stored at (-80˚C) for subsequent use.

Total plant RNA extraction

Aerial parts' total RNA was isolated using trizol reagent. Frozen wheat tissue was crushed in liquid nitrogen, and 1000µl of Trizol reagent was added to 0.5 g of leaf tissues. After vortexing and incubating in ice, 200µl of chloroform was added, vortexed, and incubated in ice. Centrifugation at 11,000 rpm for 15 minutes at 40ºC separated the supernatant. To the supernatant, 500µl of isopropanol was added, incubated at (-20ºC) for 1 hour, and then centrifuged at 12,000 rpm for 10 minutes at 40ºC. After discarding the supernatant, the pellet was washed in 500µl of 75% ethanol, centrifuged at 11,000 rpm for 3 minutes, and dried at room temperature for 10 minutes. The RNA pellet was re-suspended in 20µl of DEPC-treated water and stored at (-80ºC) for future use.

Synthesis of cDNA using reverse transcriptase

First strand cDNA was synthesized by Thermo Scientific RevertAid First Strand cDNA Synthesis Kit obtained from Thermo Fisher Scientific Inc.

Designing of primers for Quantitative (Real-Time) RT-PCR

A GenBank search identified four *Triticum aestivum* sequences encoding, HSP17 (JN572711.1), HSP23 (AF104108.1), and HSP70 (JN129258.1), along with one for the endogenous control GAPDH (EF592180). Specific primers for Quantitative (Real-Time) RT-PCR were designed based on their alignment, using Primer 3 plus software (Table 2).

Real time PCR for gene expression analysis

Gene expression analysis utilized real-time PCR (Biorad) with Maxima SYBR Green dye (Invitrogen). Protocols followed the supplier's instructions. Triplicate reactions for target genes (HSP17, HSP23, and HSP70) at various temperature conditions and the endogenous control GAPDH were conducted for each sample. Average threshold cycle (CT) was determined, and a triplicate Non-template Control (NTC) served as a negative control, containing all components except the template.

Table 2. List of Primers used in Real Time PCR

Sr. No	Primer name	Forward Primers (5'-3')	Reverse Primers (5'-3')
1	HSP17	AGAAGGAGGACGCCAAGTAC	CTCGCTACTCTCTGCTTCGA
2	HSP23	CAAGGCGAGATGAAGAACG	GACCCGACACGACAAACAAA
3	HSP70	CCCTCCCACCAGATCTGTTT	GCAGGAATACGACCGAAACC
4	GAPDH	TCAAGGAGGAGTCTGAGGGA	CTGTGCATGTGACGGATGAG

Results and discussion

Effects of biogenic ZnO-NPs on growth parameters of wheat genotypes

The results revealed significant improvement in all growth parameters under treatment with ZnO-NPs compared to control conditions. The data pertaining to seed priming has been presented in Table 3 and Figure 1 is showing germination percentage and length of seedlings.

Germination percentage

Seeds treated with ZnO-NPs demonstrated maximum wheat germination percentages (95.6% in PBW 226, followed by 95.4%, 93.8%, and 93.7% in DBW 187, HD3226, and DBW 173 at 100 ppm concentration) (Table 3). Improved germination, a key indicator of plant vigor and adaptability to environmental changes, was observed, particularly at the medium concentration (100 ppm) of either nanoparticle compared to the control. These findings align with Babar (2013) and Baddar (2016), indicating enhanced germination speed, mean germination time, and increased zinc nutrition in wheat through ZnO-NP treatment.

Shoot and root length

In the experiment, wheat seedling shoot lengths significantly varied with ZnO NPs concentration, ranging from 6 cm to 12.6 cm. ZnO-NPs from *L. camara* showed maximum shoot length (12.6 cm in PBW 226 at 100 ppm) (Table 3). The lowest (8.7 cm) occurred in HD3226 at 200 ppm. Nanoparticle exposure elongated shoot length, aligning with literature indicating improved seedling vigor to withstand climate changes (Shafea et al., 2017). ZnO-NPs at 100 ppm, outperformed in enhancing shoot length across wheat genotypes, potentially due to smaller nanoparticle size facilitating penetration into seeds. These findings mirror those of Narendhran et al. (2016), Munir et al. (2018), and Neto et al. (2020). In this experiment, the lowest root length (7.1 cm) occurred in wheat genotype HD 3226 under control conditions, while in treatment conditions, root length ranged from 7.2 cm to 13.3 cm. ZnO-NPs treated seedlings demonstrated maximum root length (13.3 cm in PBW 226 at 100 ppm). Root length increased up to 100 ppm and then decreased at 150 ppm and 200 ppm in all genotypes, mirroring the pattern observed in shoot length. Genotype PBW226 showed the maximum root length, and genotype HD3226 exhibited the minimum root length in the treatment with ZnO-NPs from *L. camara*. These findings align with results from Raliya and Tarafdar (2013), Jayarambabu et al. (2014), and Munir et al. (2018).

Seedling length

The lowest seedling length (13.1 cm) occurred in wheat genotype HD 3226 under control conditions, while in treatment conditions, seedling length ranged from 14 cm to 25.9 cm (Table 3 and Figure 1). ZnO-NPs treated seedlings demonstrated maximum seedling length (25.9 cm in PBW 226 at 100 ppm). Genotype PBW 226 exhibited both the maximum (25.9 cm) and minimum (13.1 cm) seedling lengths, indicating the significant role of ZnO-NPs in enhancing root growth. These findings align with results from Adhikari et al. (2015) in maize and Pandey et al. (2010) in chickpea, emphasizing the positive impact of ZnO-NPs on seedling length.

Seed vigor index

The lowest seed vigor index (985.12) occurred in wheat genotype HD 3226 under control conditions, while in treatment conditions, the index ranged from 1099 to 2476.04 (Table 3). ZnO-NPs treatment showed the maximum seed vigor index (2476.04 in PBW 226 at 100 ppm). The enhancement in vigor index, linked to increased water uptake capacity and the creation of new pores on the seed coat by nanoparticles, was observed to be maximum at 100 ppm concentration of ZnO-NPs and minimum in the control, aligning with findings from Zahra et al. (2019).

Fresh and Dry weight of seedlings

The seedling fresh weight, a combination of root and shoot weights, varied significantly with ZnO NPs concentration. The lowest fresh weight (1.03 grams) occurred in wheat genotype HD 3226 under control conditions, while under ZnO-NPs treatment, it ranged from 1.11 to 1.39 grams (Table 3). ZnO-NPs treatment demonstrated the maximum fresh weight (1.39 grams in PBW 226 at 100 ppm). Similarly, the lowest dry weight (0.06 grams) was observed in HD 3226 under control conditions, while under ZnO-NPs treatment, it ranged from 0.12 to 0.39 grams. ZnO-NPs treated seedling showed the maximum dry weight (0.39 grams in PBW 226 at 100 ppm). The experiment highlighted the positive impact of ZnO-NPs on seedling fresh and dry weights, particularly in PBW 226 at 100 ppm concentration, aligning with findings from Singh et al. (2019) on the potential of ZnO nanoparticles as nanofertilizers. It's noted that high concentrations of nanometals can induce plant toxicity, but at optimal concentrations, the effect is positive for plant growth and biomass production (Kanwar et al., 2019).

Total protein content

The lowest protein content concentration (22.92 mg/g FW) occurred in wheat genotype HD 3226 under control conditions, while under different ZnO-NPs treatments, concentrations ranged from 23.15 mg/g FW to 32.68 mg/g FW. Treatment of ZnO-NPs demonstrated the maximum total protein content concentration (32.68 mg/g FW in PBW 226 at 100 ppm) (Table 3). Protein synthesis, a crucial mechanism indicating plant physiological state, was influenced by ZnO-NPs, with PBW 226 at 100 ppm showing the highest concentration. These findings align with research by Kapoor et al. (2013) and Pasandi-Pour et al. (2014), emphasizing the impact of environmental factors on gene expression, protein patterns, and soluble protein content during plant development. Iqbal et al. (2019) conducted similar research confirming the influence of ZnO-NPs on protein content.

Table 3. Effects of biogenic ZnO-NPs on different growth parameters of wheat genotypes.

Growth parameters	Concentration of treatment	Wheat genotypes			
		DBW173	HD3226	DBW187	PBW226
Germination percentage (%)	(Control) 0 ppm	75.6	75.2	76.4	76.8
	50 ppm	86.2	86.7	87.5	87.3
	100 ppm	93.7	93.8	95.4	95.6
	150 ppm	84.4	84.6	85.3	85.5
	200 ppm	79.1	79.5	80.6	80.7
Shoot length(cm)	(Control) 0 ppm	6.5	6	7.1	9
	50 ppm	10.6	9.6	11.2	11.3
	100 ppm	11.5	11.7	12.4	12.6
	150 ppm	10.1	10.2	10.5	10.6
	200 ppm	9.8	8.7	9.1	9.4
Root length (cm)	(Control) 0 ppm	7.3	7.1	8.1	10.1
	50 ppm	11.2	10.6	12.1	12.4
	100 ppm	12.3	12.5	13.2	13.3
	150 ppm	11.7	11.4	11.5	11.6
	200 ppm	10.1	9.3	10.4	10.6
Seedling Length (cm) at 7^{th} day	(Control) 0 ppm	13.8	13.1	15.2	19.1
	50 ppm	21.8	20.2	23.3	23.7
	100 ppm	23.8	24.2	25.6	25.9
	150 ppm	21.8	21.6	22	22.2
	200 ppm	19.9	18	19.5	20
Seed vigor index	(Control) 0 ppm	1043.28	985.12	1161.28	1466.88
	50 ppm	1879.16	1751.34	2038.75	2069.01
	100 ppm	2230.06	2269.96	2442.24	2476.04
	150 ppm	1839.92	1827.36	1876.6	1898.1
	200 ppm	1574.09	1431	1571.7	1614
Fresh weight of seedlings (grams)	(Control) 0 ppm	1.05	1.03	1.06	1.08
	50 ppm	1.26	1.27	1.28	1.29
	100 ppm	1.35	1.33	1.37	1.39
	150 ppm	1.25	1.24	1.27	1.29
	200 ppm	1.16	1.14	1.17	1.18
Dry weight of seedlings (grams)	(Control) 0 ppm	0.07	0.06	0.08	0.09
	50 ppm	0.27	0.26	0.25	0.28
	100 ppm	0.36	0.34	0.38	0.39
	150 ppm	0.26	0.24	0.27	0.28
	200 ppm	0.17	0.16	0.15	0.18
Total protein content (mg/g FW)	(Control) 0 ppm	22.99	22.92	23.05	23.14
	50 ppm	28.79	28.64	29.62	29.81
	100 ppm	31.75	31.56	32.41	32.68
	150 ppm	26.53	26.24	27.11	27.63
	200 ppm	24.95	24.86	25.43	25.66

Effects of biogenic ZnO-NPs in wheat genotypes under field conditions

Analysis of variance

Analysis of variance demonstrated highly significant differences among genotypes for various characteristics, including days to 50% heading, days to 50% flowering, days to maturity, plant height (cm), number of effective tillers per plant, spike length with awns (cm), number of spikelets per spike, number of grains per spike, 1000-grain weight (g), biological yield per plant (g), harvest index (%), grain yield per plant (g), and straw yield per plant (g) (Table 4 and 5). The significant variability observed among genotypes in both timely and late-sown conditions during the years 2021-22 and 2022-23 suggests potential for improvement in these traits. These results align with earlier findings in wheat by Arya et al. (2017), Thapa et al. (2019), Prasad et al. (2021), and Yadav et al. (2021).

Mean performance of various traits under timely and late sown conditions during 2021-22 to 2022-2023.

Table 6 and 7 present the mean and range performance of four wheat genotypes under control and ZnO NP treatment in both timely and late sowing during the crop years 2021-22 and 2022-23. The genotypes exhibited wide variation in mean performance across 13 studied characters. Under timely sown conditions, days to heading, flowering, and maturity ranged from 85.00 to 98.00 days, 88.00 to 101.00 days, and 118.00 to 130.00 days, respectively. Various traits such as plant height, spike length, spikelets, effective tillers, grains per spike, 1000 grain weight, biomass per plant, straw yield, harvest index, and grain yield also showed significant variation. Similar trends were observed under late sown conditions, aligning with earlier reports in wheat by Arya et al. (2018) and Thapa et al. (2019).

Genotypic and phenotypic coefficient of variation

Genetic variability information is crucial for breeding programs, ensuring better chances of developing desired plant types. Heritability, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and genetic advance estimates guide trait-specific selection methods. In the current study (Table 8 and 9), phenotypic coefficients of variance (PCV) were higher than genotypic coefficients of variance (GCV) for all traits, suggesting less environmental influence. In the 2021-22 timely sown conditions, effective tillers exhibited the highest PCV and GCV, followed by spike length with awns, straw yield, 1000 grain weight, and harvest index. Similarly, in late sown conditions, grain yield per plant had the highest PCV and GCV. In the 2022-23 season, straw yield had the highest PCV and GCV under timely sown conditions, while grain yield per plant had the highest values under late sown conditions. Days to heading consistently showed the lowest PCV and GCV. Comparable results were reported by Bhushan et al. (2013), Wolde et al. (2016), Bhushal and Sarial (2016), and Negasa and Chauhan (2016), indicating potential for improvement through intermating and selective breeding for these traits.

Heritability and genetic advance

Genetic parameters like heritability play a crucial role in assessing the genetic control and transmission of traits to progeny, impacting selection efficiency (Table 8 and 9). Johnson et al. (1955) recommended considering both heritability and genetic advance for effective selection. In the 2021-22 timely sown conditions, high heritability and genetic advance were observed for various traits, including straw yield, harvest index, grain yield per plant, effective tillers, spike length with awns, and 1000 grain weight. Similarly, in late sown conditions, high heritability and genetic advance were noted for grain yield per plant, 1000 grain weight, number of grains per spike, biomass per plant, straw yield, spike length with awns, harvest index, number of spikelets, and number of effective tillers. In the 2022-23 season, traits such as spike length with awns, number of spikelets, straw yield, 1000 grain weight, number of grains per spike, and harvest index exhibited high heritability and genetic advance under timely sown conditions. In late sown conditions, grain yield per plant, 1000 grain weight, number of grains per spike, biomass per plant, harvest index, spike length with awns, number of effective tillers per plant, and straw yield showed high heritability and genetic advance. These traits with high heritability and genetic advance suggest effective selection due to additive gene action with minimal environmental influence. Similar findings were reported by Nukasani et al. (2013), Wolde et al. (2016), Singh et al. (2012), and Veeresha and Naik (2016). Additionally, in 2021-22, high heritability with moderate genetic advance was observed for traits like number of spikelets, biomass per plant, plant height, days to heading, and under late sown conditions, high heritability with moderate genetic advance was observed for days to maturity, days to flowering, and plant height. In 2022-23, high heritability with moderate genetic advance was observed for grain yield per plant, days to heading, days to flowering, and number of effective tillers per plant under timely sown conditions, while under late sown conditions, it was observed for days to maturity, number of spikelets, and plant height. Similar trends were reported by Jan and Kashyap (2013), Bhushan et al. (2013), and Kumar et al. (2014).

Correlation coefficient analysis

The correlation coefficient assesses the linear association between variables, aiding in understanding the relationship between yield and its components. Genotypic correlations (Table 10 and 11) generally exceeded phenotypic correlations (Table 14 and 15), and negative correlations at the genotypic level also appeared at the phenotypic level. In timely sown conditions (2021-22), grain yield showed positive significant genotypic correlations with various traits, including Harvest Index, Numbers of effective tillers, Days to Heading, Biomass per plant, Plant Height, Numbers of spikelets, Days to flowering, Spike length with awns, and Straw yield. Conversely, negative and significant genotypic correlations were observed with Days to Maturity and Numbers of spikelets. Similar patterns were observed in late sown conditions (2021-22) and timely sown conditions (2022-23), with positive significant genotypic correlations for certain traits and negative correlations for others. Phenotypic correlations generally mirrored genotypic correlations. For instance, in timely sown conditions (2022-23), grain yield had positive significant phenotypic correlations with Harvest Index, Plant Height, Days to Heading, Days to flowering, Numbers of effective tillers per plant, Numbers of grains per spike, and Straw yield, while negative correlations were observed with Days to Maturity and 1000 grain weight. Similar agreement was observed in late sown conditions (2022-23). Both genotypic and phenotypic correlation results align with studies by El-Mohsen et al. (2012), Singh et al. (2012), Mohammadi et al. (2014) and Avinashe et al. (2015).

Path coefficient analysis

Genotypic path coefficient analysis under timely and late sown condition during 2021-22 and 2022-23

Path coefficient analysis of genotypic direct effects (Table 12 and 13) highlighted that, under timely sown conditions (2021-22), Numbers of effective tillers exhibited the highest positive direct effect on grain yield, followed by Straw yield, Numbers of spikelets, Plant Height, Days to flowering, Days to Maturity, Numbers of grains per spike, and Days to Heading. In late sown conditions (2021-22), Numbers of spikelets had the highest positive direct effect on grain yield, followed by 1000 grain weight, Spike length with awns, Days to heading, Days to flowering, and Biomass per plant. Negative direct effects were recorded for Harvest Index, Spike length with awns, 1000 grain weight (-0.7142), and Biomass per plant under timely sown conditions (2021-22). In late sown conditions (2021-22), negative direct effects were recorded for Harvest Index, Days to Maturity, Numbers of effective tillers, Plant Height, Numbers of grains per spike, and Straw yield. Under timely sown conditions (2022-23), Numbers of grains per spike had the highest positive direct effect on grain yield, followed by Numbers of effective tillers per plant, Numbers of spikelets, Biomass per plant, Days to Heading, and Days to Maturity. In late sown conditions (2022-23), biomass (g/plant) had the highest positive direct effect on grain yield, followed by Numbers of effective tillers per plant, Numbers of grains per spike, Harvest Index, and Days to Heading. Negative direct effects were recorded for Plant Height, followed by Days to flowering, Harvest Index, Spike length with awns, 1000 grain weight, and Straw yield under timely sown conditions (2022-23). In late sown conditions (2022-23), negative direct effects were recorded for Days to Maturity, Days to flowering, Plant Height, Numbers of spikelets, Spike length with awns, 1000 grain weight, and Straw yield. Similar findings were reported by Bhushan et al. (2013), Avinashe et al. (2015) and Rahman et al. (2016).

Phenotypic path coefficient analysis under timely and late sown condition during 2021-22 and 2022-23

A critical perusal of path coefficient analysis of phenotypic which diagonal values is direct effects revealed that Numbers of effective tillers had highest Phenotypic positive direct effect on grain yield followed by Numbers of grains per spike, Plant Height, Straw yield, Days to Maturity, Harvest Index, Days to flowering and Biomass per plant under timely sown condition (2021-22) (Table 16 and 17). While, in late sown condition (2021-22) 1000 grain weight had highest Phenotypic positive direct effect on grain yield followed by Spike length with own Straw yield,

Harvest Index, Days to Heading and Numbers of grains per spike. The negative direct effect was recorded for Days to Heading 1000 grain weight, Spike length with own and Numbers of spikelets under timely sown condition (2021-22). While, in late sown condition (2021-22) Biomass per plant followed by Numbers of effective tillers, Days to flowering, Plant Height, Days to Maturity and Numbers of spikelets. Biomass (g/plant) had highest phenotypic positive direct effect on grain yield followed by Numbers of effective tillers per plant, Numbers of grains per spike, Harvest Index and Days to Heading under timely sown condition (2022-23). While, in late sown condition (2022-23) Biomass per plant had highest Phenotypic positive direct effect on grain yield followed by Numbers of grains per spike, Numbers of effective tillers per plant, numbers of spikelets, Harvest Index, Days to Maturity and Days to Heading. The negative direct effect was recorded for Days to Maturity followed by Days to flowering, Plant Height, numbers of spikelets, Spike length with own, 1000 grain weight and Straw yield under timely sown condition (2022-23). While, in late sown condition (2022-23) Plant Height followed by Days to flowering, Spike length with own, 1000 grain weight and Straw yield. Similar findings were also observed by Nukasani et al., (2013), Bhushan et al., (2013), Avinashe et al., (2015). The residual factor values were 0.0410 and 0.0414 under timely and late sown conditions for genotypic path coefficient analysis in 2021-22, and 0.0410 under both conditions in 2022-23. For phenotypic path coefficient analysis in 2021-22, the residual factor values were 0.0320 and 0.0680 under timely and late sown conditions, and in 2022-23, the values were 0.0320 and 0.0047. These findings suggest that factors not considered in the study may influence grain yield. The study concludes that biomass, harvest index, and effective tillers per plant are crucial traits supporting grain yield in wheat, emphasizing their importance in genotype selection.

Table 4. Pooled data of mean sum squares genotypes for timely sown condition during year 2021-23

Source of variation	df	PlantHeight	Days to Maturity	Days to Heading	Days to flowering	Spike lengthwith own	Numbersof spikelets	Numbersof effective tillers	Biomassper plant	1000 grainweight	Numbersof grains per spike
Repl	2	0.78	1.03	0.79	0.85	0.02	0.03	0.09	1.31	0.22	0.17
Treat	11	28.65**	42.80**	56.09**	56.80**	5.73**	9.09**	4.79**	52.91**	47.59**	22.12**
Error	22	9.61	15.04	8.22	8.75	0.33	0.55	0.85	19.07	3.56	1.83
Total	35	15.09	22.96	22.84	23.40	2.01	3.21	2.05	28.69	17.21	8.11

*,**significantat5% and 1%level,respectively

Table 5. Pooled data of Mean sum squares genotypes for late sown condition duringyear2021-23

Source of variation	df	PlantHeight	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	numbers of spikelets	Numbers of effective tillers	Biomass per plant	1000 grainweight	Numbers of grains per spike	Strawyie
Repl	2	0.72	0.48	0.34	0.36	0.03	0.03	0.04	1.07	0.21	0.08	0.41
Treat	11	28.52**	62.43**	13.64**	18.80**	4.71**	1.73**	8.11**	287.40**	72.05**	29.59**	71.66**
Error	22	8.19	9.71	5.79	6.35	0.24	0.38	0.50	11.17	2.15	1.11	5.44
Total	35	14.15	25.75	7.95	9.92	1.63	0.78	2.86	97.41	24.01	10.00	25.96

*,** significantat 5% and 1%level, respectively

Table 6. Pooled data for mean performance of timely sown condition during Year 2021-2023

s.no	Treatments	Genotype	PlantHeight	Days to Maturity	Days to Heading	Days to flowering	Spike lengthwith own	Numberof spikelets	Numbersof effective tillers per plant	Biomassper plant	1000 grain
1	Control	HD3226	100.90	128.00	97.00	100.00	15.92	21.70	28.63	132.00	53.5
2		DBW173	95.00	130.00	91.00	94.00	16.75	24.34	27.42	137.00	58.8
3		DBW187	98.40	124.00	87.00	90.00	18.92	24.60	29.12	140.00	63.3
4		PBW226	94.72	120.00	95.00	98.00	17.94	21.00	30.45	142.00	62.0
5	SyntheticZnONPs	HD3226	103.25	126.00	98.00	101.00	17.13	22.64	29.60	134.84	54.7
6		DBW173	97.35	127.00	90.00	93.00	17.96	25.28	28.39	139.84	60.0
7		DBW187	100.75	122.00	85.00	89.00	20.13	25.54	30.09	142.84	64.5
8		PBW226	97.07	118.00	92.00	95.00	19.15	21.94	31.42	144.84	63.2
9	BiogenicZnONPs	HD3226	104.50	125.00	96.00	99.00	17.48	22.93	29.87	135.12	54.9
10		DBW173	98.60	127.00	91.00	93.00	18.31	25.57	28.66	140.12	60.1
11		DBW187	102.00	123.00	86.00	88.00	20.48	25.83	30.36	143.12	64.6
12		PBW226	98.32	119.00	94.00	97.00	19.50	22.23	31.69	145.12	63.3
		Mean	99.24	124.08	91.83	94.75	18.31	23.63	29.64	139.74	60.2
		Min	94.72	118.00	85.00	88.00	15.92	21.00	27.42	132.00	53.5
		Max	104.50	130.00	98.00	101.00	20.48	25.83	31.69	145.12	64.6
		SE(d)±	2.53	3.17	2.34	2.42	0.47	0.61	0.76	3.57	1.54
		C.D.at5%	5.28	6.61	4.89	5.04	0.98	1.26	1.58	7.44	3.22
		C.V.(%)	3.12	3.13	3.12	3.12	3.12	3.14	3.12	3.13	3.13

Table 7. Pooled data for mean performance of late sown condition during Year 2021-2023

s.no	Treatments	Genotypes	PlantHeight	Days to Maturity	Days to Heading	Days to flowering	Spike lengthwith own	Numberof spikelets	Numbersof effective tillers per plant	Biomassper plant	100 grai
1	Control	HD3226	93.23	95.00	75.00	78.00	14.50	18.50	20.16	95.34	40.4
2		DBW173	86.78	104.00	80.00	84.00	13.40	19.40	21.14	96.00	42.6
3		DBW187	91.78	105.00	78.00	82.00	15.00	18.30	22.12	112.00	48.6
4		PBW226	88.00	100.00	77.00	80.00	16.40	19.50	24.21	116.00	52.1
5	SyntheticZnONPs	HD3226	95.17	95.00	74.00	77.00	15.44	19.38	21.00	97.99	41.5
6		DBW173	88.72	103.00	79.00	83.00	14.34	20.28	21.98	98.65	43.7
7		DBW187	93.89	102.00	76.00	80.00	15.94	19.18	22.96	114.65	49.8
8		PBW226	89.94	98.00	75.00	78.00	17.34	20.38	25.05	118.65	53.2
9	BiogenicZnONPs	HD3226	96.26	93.00	76.00	79.00	15.74	19.67	21.29	98.30	41.6
10		DBW173	89.81	103.00	81.00	85.00	14.64	20.57	22.27	98.96	43.9
11		DBW187	94.98	104.00	77.00	81.00	16.24	19.47	23.25	114.96	49.9
12		PBW226	91.03	93.00	76.00	80.00	17.64	20.67	25.34	118.96	53.3
		Mean	91.63	99.58	77.00	80.58	15.55	19.61	22.56	106.71	46.7
		Min	86.78	93.00	74.00	77.00	13.40	18.30	20.16	95.34	40.4
		Max	96.26	105.00	81.00	85.00	17.64	20.67	25.34	118.96	53.3
		SE(d)±	4.88	5.31	4.10	4.29	0.83	1.04	1.20	5.70	2.50
		C.D.at5%	1.65	1.80	1.39	1.46	0.28	0.35	0.41	1.93	0.85
		C.V.(%)	2.34	2.54	1.97	2.06	0.40	0.50	0.58	2.73	1.20

Table 8. Pooled data for genetic variability for timely sown condition during year2021-2023

Genotypes	Mean	Min	Max	var(g)	var(p)	Heritability (%)	GA	GA% mean	GCV (%)	PCV (%)
Plant Height	99.24	94.72	104.50	6.35	15.96	39.79	3.27	3.30	2.54	4.03
Days to Maturity	124.08	118.00	130.00	9.25	24.29	38.10	3.87	3.12	2.45	3.97
Days to Heading	91.83	85.00	98.00	15.96	24.18	65.99	6.68	7.28	4.35	5.35
Days to flowering	94.75	88.00	101.00	16.01	24.77	64.66	6.63	7.00	4.22	5.25
Spike length with own	18.31	15.92	20.48	1.80	2.13	84.63	2.54	13.89	7.33	7.97
Numbers of spikelets	23.63	21.00	25.83	2.85	3.40	83.81	3.18	13.47	7.14	7.80
Numbers of effective tillers per plant	29.64	27.42	31.69	1.31	2.16	60.54	1.83	6.19	3.86	4.96
Biomass per plant	139.74	132.00	145.12	11.28	30.35	37.16	4.22	3.02	2.40	3.94
1000 grain weight	60.27	53.54	64.66	14.68	18.24	80.47	7.08	11.75	6.36	7.09
Numbers of grains per spike	43.29	39.30	46.79	6.76	8.59	78.70	4.75	10.98	6.01	6.77
Straw yield	79.00	69.13	83.22	26.57	32.69	81.27	9.57	12.12	6.52	7.24
Harvest Index	43.51	40.74	47.63	6.58	8.42	78.07	4.67	10.73	5.89	6.67
Grain yield per plant(gm/plant)	60.74	55.89	64.13	8.10	11.69	69.27	4.88	8.03	4.68	5.63

Table 9. Pooled data for genetic variability for late sown condition during year2022-2023

Genotypes	Mean	Min	Max	var(g)	var(p)	Heritability (%)	GA	GA%mean	GCV(%)	PCV(%)
Plant Height	91.63	86.78	96.26	6.78	14.96	45.28	3.61	3.94	2.84	4.22
Days to Maturity	99.58	93.00	105.00	17.57	27.28	64.41	6.93	6.96	4.21	5.25
Days to Heading	77.00	74.00	81.00	2.61	8.41	31.09	1.86	2.41	2.10	3.77
Days to flowering	80.58	77.00	85.00	4.15	10.50	39.52	2.64	3.27	2.53	4.02
Spike length with own	15.55	13.40	17.64	1.49	1.73	86.34	2.34	15.04	7.86	8.45
Numbers of spikelets	19.61	18.30	20.67	0.45	0.83	54.60	1.02	5.22	3.43	4.64
Numbers of effective tillers per plant	22.56	20.16	25.34	2.54	3.04	83.56	3.00	13.29	7.06	7.72
Biomass per plant	106.71	95.34	118.96	92.08	103.25	89.18	18.67	17.49	8.99	9.52
1000 grain weight	46.76	40.42	53.37	23.30	25.45	91.56	9.51	20.35	10.32	10.79
Numbers of grains per spike	33.55	28.40	37.84	9.49	10.60	89.54	6.01	17.90	9.18	9.71
Straw yield	74.55	67.89	80.63	22.07	27.51	80.24	8.67	11.63	6.30	7.04
Harvest Index	29.96	26.83	33.91	6.01	6.89	87.25	4.72	15.75	8.18	8.76
Grain yield per plant(gm/plant)	32.16	25.76	40.34	29.19	30.22	96.59	10.94	34.02	16.80	17.10

Table 10.Pooled data for genotypic correlations for timely sown condition during year 2021-2023

Characters	PlantHeight	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Numberof spikelets	No of effective tillers	Biomassper plant	1000 grainweight	Numbersof grains per spike	S
Plant Height	1.000	-0.304	-0.068	-0.082	-0.135	-0.029	-0.145	-1.134**	-0.719**	-0.258	-(
Days to Maturity			-0.161	-0.200	-1.038**	0.279	-1.640**	-1.765**	-1.037**	-1.461**	-(
Days to Heading				0.992**	-0.909**	-1.001**	-0.133	-1.057**	-0.985**	-0.424**	-1
Days to flowering					-0.917**	-1.032**	-0.125	-1.069**	-0.987**	-0.405*	-1
Spike length with own						0.395*	0.588**	0.932**	0.886**	0.753**	0
Numbers of spikelets							-0.578**	0.026	0.313	-0.321	0
No of effective tillers								0.553**	0.406*	0.913**	0
Biomass per plant									1.003**	0.789**	0
1000 grain weight										0.694**	0
No of grains per spike											0
Straw yield											
Harvest Index											
Grain yield per plant											

Table 11. Pooled data for genotypic correlations for late sown condition during year 2021-2023

Characters	PlantHeight	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Numberof spikelets	No of effective tillers	Biomassper plant	1000 grainweight	Numbersof grains per spike	St
Plant Height	1.000	-0.817**	-1.500**	-1.287**	0.145	-0.758**	-0.487**	-0.176	-0.361*	0.553**	-0.
Days to Maturity			0.651**	0.683**	-0.594**	-0.439**	-0.146	-0.007	0.053	-0.288	0.
Days to Heading				0.987**	-0.974**	-0.125	-0.376*	-0.528**	-0.349*	-0.237	-0.
Days to flowering					-0.841**	-0.075	-0.276	-0.392*	-0.238	-0.230	-0.
Spike length with own						0.314	0.829**	0.821**	0.786**	-0.650**	0.
Numbers of spikelets							0.508**	0.080	0.222	-0.404*	-0.
No of effective tillers per plant								0.897**	0.949**	-0.967**	0.
Biomass per plant									0.983**	-0.905**	0.
1000 grain weight										-0.972**	0.
Numbers of grains per spike											-0.
Straw yield											
Harvest Index											
Grain yield per plant(gm/plant)											

Table 12. Pooled data for genotypic path with grain yield (g/plant) for timely sown condition during year 2021-23

Characters	Plant Height	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Numbers of spikelets	No of effectivetillers	Biomassper plant	1000 grainweight	Numbersof grains per spike	Strawyi
Plant Height	**-0.0008**	0.0000	0.0000	0.0001	0.0215	-0.0027	-0.0178	-0.1458	0.1244	-0.0543	0.4024
Days to Maturity	0.0003	**0.0001**	-0.0001	0.0003	0.1647	0.0262	-0.2014	-0.8834	0.1795	-0.3072	0.0204
Days to Heading	0.0001	0.0000	**0.0003**	-0.0015	0.1443	-0.0940	-0.0164	-0.0673	0.1704	-0.0891	0.5493
Days to flowering	0.0001	0.0000	0.0003	**-0.0015**	0.1455	-0.0969	-0.0154	-0.0798	0.1708	-0.0851	0.5795
Spike length with own	0.0001	-0.0001	-0.0003	0.0014	**-0.1588**	0.0371	0.0723	0.0412	-0.2534	0.1583	-0.0615
Numbers of spikelets	0.0000	0.0000	-0.0003	0.0016	-0.0628	**0.0939**	-0.0710	0.0267	-0.0542	-0.0676	-0.8339
No of effective tiller per plant	0.0001	-0.0002	0.0000	0.0002	-0.0934	-0.0543	**0.1228**	0.5581	-0.0703	0.1919	0.0007
Biomass per plant	0.0010	-0.0002	-0.0003	0.0016	-0.1479	0.0025	0.0678	**0.0102**	-0.1737	0.1658	-0.3159
1000 grain weight	0.0006	-0.0001	-0.0003	0.0015	-0.1407	0.0294	0.0498	0.0136	**-0.1731**	0.1458	-0.3478
Numbers of grains per spike	0.0002	-0.0001	-0.0001	0.0006	-0.1196	-0.0302	0.1121	0.7970	-0.1201	**0.2102**	-0.4406
Straw yield	0.0008	-0.0001	-0.0003	0.0016	-0.1101	0.0511	-0.0001	0.0680	-0.1524	0.0605	**-0.7315**
Harvest Index	-0.0005	0.0000	0.0003	-0.0012	0.0944	-0.0805	0.0181	-0.0752	0.2464	-0.0203	0.6799

Table 13. Pooled data for genotypic path with grain yield (g/plant) for late sown condition during year 2021-23

Characters	Plant Height	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Number of spikelets	No of effectivetillers	Biomassper plant	1000 grainweight	Numbersof grains per spike	Straw
Plant Height	**-0.0077**	0.0004	-0.0011	0.0024	-0.0039	0.0121	-0.0494	-0.3292	0.0604	0.0056	0.171
Days to Maturity	0.0063	**-0.0004**	0.0005	-0.0013	0.0161	0.0070	-0.0148	-0.0140	-0.0088	-0.0029	-0.227
Days to Heading	0.0116	-0.0003	**0.0008**	-0.0018	0.0263	0.0020	-0.0381	-0.9891	0.0583	-0.0024	0.303
Days to flowering	0.0099	-0.0003	0.0007	**-0.0018**	0.0227	0.0012	-0.0279	-0.7339	0.0398	-0.0023	0.161
Spike length with own	-0.0011	0.0003	-0.0007	0.0015	**-0.0270**	-0.0050	0.5841	0.5386	-0.1313	-0.0066	-0.039
Numbers of spikelets	0.0059	0.0002	-0.0001	0.0001	-0.0085	**-0.0160**	0.0515	0.1506	-0.0371	-0.0041	0.071
No of effective tillers per plant	0.0038	0.0001	-0.0003	0.0005	-0.0224	-0.0081	**0.5013**	0.6801	-0.1586	-0.0098	-0.062
Biomass per plant	0.0014	0.0000	-0.0004	0.0007	-0.0222	-0.0013	0.8909	**0.8736**	-0.1642	-0.0092	-0.615
1000 grain weight	0.0028	0.0000	-0.0003	0.0004	-0.0213	-0.0036	0.5962	0.8418	**-0.1671**	-0.0099	-0.293
Numbers of grains per spike	-0.0043	0.0001	-0.0002	0.0004	0.0176	0.0065	-0.8980	-0.6952	0.1624	**0.0102**	0.519
Straw yield	0.0015	-0.0001	-0.0003	0.0003	-0.0169	0.0013	0.4775	0.7655	-0.1531	-0.0084	**-0.265**
Harvest Index	0.0014	0.0003	-0.0007	0.0015	-0.0248	-0.0030	0.8842	0.5250	-0.1357	-0.0084	-0.284

Table 14. Pooled data for phenotypic correlations for timely sown condition during year 2021-2023

Characters	PlantHeight	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Numbers of spikelets	No of effective tillers	Biomassper plant	1000 grainweight	Numbersof grains per spike	S
Plant Height	1.000	0.469**	0.399*	0.401*	0.212	0.284	0.396*	0.153	-0.079	0.198	-
Days to Maturity			0.377*	0.368*	-0.282	0.473**	-0.294	-0.042	-0.228	-0.439**	(
Days to Heading				0.995**	-0.452**	-0.511**	0.282	-0.062	-0.461**	-0.037	-
Days o flowering					-0.446**	-0.522**	0.295	-0.054	-0.450**	-0.015	-
Spike length with own						0.490**	0.667**	0.833**	0.904**	0.795**	(
Numbers of spikelets							-0.160	0.333*	0.435**	-0.076	(
No of effective tillers per plant								0.760**	0.560**	0.920**	(
Biomass per plant									0.899**	0.792**	(
1000 grain weight										0.756**	(
Numbers of grains per spike											(
Straw yield											
Harvest Index											
Grain yield per plant(gm/plant)											

Table 15. Pooled data for phenotypic correlations for late sown condition during year 2021-2023

Characters	Plant Height	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Numbers of spikelets	No of effectivetillers	Biomassper plant	1000 grainweight	Numbersof grains per spike	St
Plant Height	1.000	-0.001	0.050	0.030	0.363*	0.121	-0.001	0.131	-0.019	0.591**	0.
Days to Maturity			0.786**	0.808**	-0.223	0.141	0.134	0.190	0.213	-0.027	0.
Days to Heading				0.992**	-0.199	0.508**	0.144	-0.006	0.054	0.142	0.
Days to flowering					-0.205	0.489**	0.156	0.022	0.082	0.114	0.
Spike length with own						0.464**	0.854**	0.842**	0.806**	-0.453**	0.
Numbers of spikelets							0.616**	0.277	0.352*	-0.066	0.
No of effective tillers per plant								0.907**	0.948**	-0.707**	0.
Biomass per plant									0.984**	-0.704**	0.
1000 grain weight										-0.788**	0.
Numbers of grains per spike											-0
Straw yield											
Harvest Index											
Grain yield per plant(gm/plant)											

Table 16. Pooled data for phenotypic path with grain yield (g/plant) for timely sown condition during year2021-23

Characters	Plant Height	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Numbers of spikelets	No of effectivetillers	Biomassper plant	1000 grainweight	Numbersof grain per spike	Strav
Plant Height	**-0.0010**	0.0003	0.0001	-0.0005	-0.0248	0.0199	0.0433	0.2092	0.0148	0.0409	0.27
Days to Maturity	-0.0005	**0.0007**	0.0001	-0.0004	0.0330	0.0333	-0.0321	-0.0572	0.0428	-0.0905	-0.00
Days to Heading	-0.0004	0.0003	**0.0004**	-0.0012	0.0529	-0.0359	0.0308	-0.0848	0.0867	-0.0077	0.67
Days to flowering	-0.0004	0.0003	0.0004	**-0.0012**	0.0522	-0.0367	0.0322	-0.0737	0.0847	-0.0032	0.67
Spike length with own	-0.0002	-0.0002	-0.0002	0.0005	**-0.1169**	0.0345	0.0729	0.1362	-0.1701	0.1642	-0.02
Numbers of spikelets	-0.0003	0.0003	-0.0002	0.0006	-0.0573	**0.0703**	-0.0175	0.4540	-0.0817	-0.0158	-0.85
No of effective tillers per plant	-0.0004	-0.0002	0.0001	-0.0004	-0.0780	-0.0112	**0.1093**	0.9364	-0.1054	0.2899	-0.37
Biomass per plant	-0.0002	0.0000	0.0000	0.0001	-0.0974	0.0234	0.0830	**0.3639**	-0.1690	0.1636	-0.11
1000 grain weight	0.0001	-0.0002	-0.0002	0.0005	-0.1057	0.0305	0.0612	0.2260	**-0.1880**	0.1561	-0.23
Numbers of grains per spike	-0.0002	-0.0003	0.0000	0.0000	-0.0930	-0.0054	0.1005	0.9807	-0.1421	**0.2065**	-0.48
Straw yield	0.0002	0.0000	-0.0002	0.0006	-0.0870	0.0438	0.0296	0.1118	-0.1698	0.0887	**-0.37**
Harves Index	-0.0007	0.0002	0.0003	-0.0010	0.0352	-0.0356	0.0431	-0.2049	0.0874	0.0288	0.92

Table 17. Pooled data for phenotypic path with grain yield (g/plant) for late sown condition during year2021-23

Characters	Plant Height	Days to Maturity	Days to Heading	Days to flowering	Spike length with own	Numbers of spikelets	No of effectivetillers	Biomassper plant	1000 grainweight	Numbersof grains per spike	Strav
Plant Height	**-0.0073**	0.0000	0.0000	-0.0001	-0.0042	-0.0018	-0.0001	0.2448	0.0018	0.0030	-0.19
Days to Maturity	0.0000	**-0.0005**	0.0007	-0.0019	0.0026	-0.0021	0.0088	0.3559	-0.0197	-0.0001	-0.42
Days to Heading	-0.0004	-0.0004	**0.0009**	-0.0023	0.0023	-0.0076	0.0094	-0.0115	-0.0050	0.0007	-0.18
Days to flowering	-0.0002	-0.0004	0.0009	**-0.0023**	0.0024	-0.0073	0.0102	0.0414	-0.0076	0.0006	-0.22
Spike length with own	-0.0026	0.0001	-0.0002	0.0005	**-0.0116**	-0.0069	0.6558	0.9790	-0.0748	-0.0023	-0.63
Numbers of spikelets	-0.0009	-0.0001	0.0005	-0.0011	-0.0054	**-0.0149**	0.0402	0.5192	-0.0327	-0.0003	-0.22
No of effective tillers per plant	0.0000	-0.0001	0.0001	-0.0004	-0.0099	-0.0092	**0.3653**	0.7015	-0.0879	-0.0036	-0.05
Biomass per plant	-0.0010	-0.0001	0.0000	-0.0001	-0.0098	-0.0041	0.2592	**0.8753**	-0.0912	-0.0036	-0.08
1000 grain weight	0.0001	-0.0001	0.0001	-0.0002	-0.0094	-0.0052	0.2619	0.8449	**-0.0927**	-0.0040	-0.05
Numbers of grains pers pike	-0.0043	0.0000	0.0001	-0.0003	0.0053	0.0010	-0.5462	-0.3196	0.0731	**0.0051**	0.02
Straw yield	-0.0015	-0.0002	0.0002	-0.0006	-0.0080	-0.0036	0.1527	0.7688	-0.0848	-0.0029	**-0.03**
Harvest Index	-0.0011	0.0001	-0.0002	0.0005	-0.0108	-0.0055	0.5558	0.5664	-0.0770	-0.0031	-0.08

Effects of ZnO-NPs on physio-biochemical characterization of wheat

Physiological analysis

Chlorophyll content is vital for plant health, driving photosynthesis and crop production. Heat stress reduces chlorophyll, but treatments can enhance it. Biogenic ZnO-NPs treatment resulted in higher chlorophyll compared to synthetic ZnO-NPs and controls (Table 18). Late sowing conditions decreased chlorophyll in both treated and control plants. HD3226 genotype treated with biogenic ZnO-NPs showed the highest chlorophyll content at 43.81 µg/cm2. Average chlorophyll content was 33.45 µg/cm2 under late sown conditions. Lowest content was in DBW187 treated with synthetic ZnO-NPs. Consistent findings were reported by Lehari, 2021; Wang et al., 2018; Pandey et al., 2015 and Megha et al., 2020. Higher chlorophyll content contributes to increased biomass and grain yield, crucial for sustaining carbohydrates during grain development (Farquhar et al., 1989; Abdullah et al., 2011). Relative water content is crucial for evaluating plant water status under heat stress. In this study, RWC was highest in timely sowing conditions, with biogenic ZnO-NPs treatment showing maximum values compared to control and synthetic ZnO-NPs. The average RWC was 84.75% in timely sown conditions, with the highest value of 90.12% in DBW187 genotype treated with biogenic ZnO-NPs. Under late sown conditions, RWC ranged from 78.65% to 86.42%. These findings align with studies by, Lugojan and Ciulca, 2011; Ramani, 2018 and Lehari, 2021.Cell membrane stability serves as a key indicator for screening heat-tolerant wheat genotypes, as described by Sairam et al., (1997). In this study, CMS increased in heat-tolerant genotypes under timely sown conditions, while heat-sensitive genotypes exhibited lower CMS in late sown conditions. The highest CMS (90.62%) was observed in genotype PBW226 under timely sown conditions, while the lowest CMS (65.72%) was found in genotype HD3226 treated with biogenic ZnO NPs. Similar findings were reported by Premachandra et al. (1991), Dhindsa et al. (1991) and Khan et al. (2013) suggesting that a higher membrane stability index indicates reduced membrane damage due to lipid peroxidation caused by active oxygen species, enabling normal physiological cell functions.

Biochemical analysis

Proline, an amino acid crucial for coping with heat stress, accumulates in plants as a compatible solute under various stress conditions. In this study, proline content increased in late sown conditions (Table 18). Under timely sown conditions, proline ranged from 0.43 to 0.95 µg/gfw across all genotypes, whereas late sown conditions showed a range of 1.18 to 2.57 µg/gfw. The highest proline content (2.57 µg/gfw) was observed in genotype PBW226 treated with biogenic ZnO-NPs under late sown conditions. Similar findings were reported by, Ahmed and Hasan (2011), Khan et al. (2013), Amirjani and Mahdiyeh (2013) and Lehari (2021). Gosavi et al. (2014) noted that proline plays a protective role in plants by acting as a cellular osmotic regulator between the cytoplasm and vacuole.Under heat stress, plants accumulate catalase, providing an antioxidant defense. In this study, catalase activity increased in late sown conditions. Catalase activity ranged from 6.34 to 14.5 µmole/gfw in timely sown conditions, and late sown conditions showed an increase from 7.41 to 16.58

µmole/gfw. The highest catalase content (16.58 µmole/gfw) was observed in genotype PBW226 treated with biogenic ZnO-NPs. Similar findings were reported by Chakraborty and Pradhan (2012), Mansoor and Naqvi (2013), and Gosavi et al. (2014), suggesting that increased catalase activity under heat stress may be linked to elevated H_2O_2 production, contributing to heat injury. Djanaguiraman et al. (2018) explained that enhanced H_2O_2 scavenging mechanisms, including catalase activities, are crucial anti-drought mechanisms to cope with oxidative stress during water scarcity conditions. Peroxidase enhances plant stress tolerance by neutralizing hydrogen peroxide. In this study, peroxidase activity increased with rising temperatures. Under timely sown conditions, peroxidase activity ranged from 1.23 to 5.83 µmole/gfw across all genotypes, while late sown conditions showed a range of 2.61 to 6.64 µmole/gfw. The highest peroxidase activity (6.64 µmole/gfw) was observed in the PBW226 genotype under timely sown conditions. Similar observations were reported by Chakraborty and Pradhan (2012), Khan et al. (2013), Gosavi et al. (2014), Siddiqui et al. (2015), and Lehari (2021). Superoxide Dismutase activity increased in late sown conditions. The average SOD activity across all genotypes ranged from 8.27 to 12.02 mMol/mg under timely sown conditions, while under late sown conditions, it increased to a range of 10.13 mMol/mg to 14.27 mMol/mg. The highest SOD activity (14.27 Mmol/mg) was found in genotype DBW187 treated with biogenic ZnO-NPs. Similar observations were reported by Chakraborty and Pradhan (2012), Khan et al. (2013), Gosavi et al. (2014) and Siddiqui et al. (2015).

Table 18. Effect of chemical and biogenic ZnO-NPs on physiological and biochemical parameters of four wheat genotypes.

Genotype	Treatment	Chlorophyll content(µg/cm^2)		Relative water Content		CMS %		Proline content		Catalase activity		Peroxidase activity		SOD activity	
		Timely	Late	Timely	Late	Timely	Late	Timely	Late	Timely	Late	Timely	Late	Timely	Late
DBW173	Control	34.15	29.27	81.62	77.48	75.62	66.43	0.43	1.24	6.52	8.26	1.97	2.88	8.27	10.13
HD3226		32.54	28.51	80.71	76.51	77.38	65.72	0.52	1.18	6.34	7.41	1.23	2.61	8.83	10.34
PBW226		40.26	35.39	84.23	81.42	88.24	76.83	0.75	2.38	12.27	14.71	3.84	4.62	9.23	11.12
DBW187		41.53	36.54	88.71	84.31	86.58	74.64	0.56	2.16	10.53	11.54	2.48	3.17	9.18	11.08
DBW173	Synthetic ZnO-NPs	35.48	30.18	82.79	78.94	76.58	67.93	0.54	1.35	7.62	9.64	2.54	3.52	8.76	10.43
HD3226		33.64	29.43	81.35	77.16	78.43	66.26	0.61	1.24	7.14	8.34	2.38	3.11	9.17	10.76
PBW226		41.57	36.84	85.36	82.41	89.64	77.43	0.86	2.46	13.56	15.48	4.21	5.64	10.14	12.27
DBW187		42.36	37.26	89.69	85.36	87.73	75.27	0.67	2.29	11.37	12.67	3.17	4.57	10.23	12.42
DBW173	Biogenic ZnO-NPs	36.52	31.82	83.54	79.38	77.36	68.54	0.62	1.49	8.46	10.54	3.24	4.98	9.43	13.21
HD3226		34.65	30.67	82.43	78.65	79.42	67.19	0.74	1.37	8.17	9.86	3.11	4.12	9.89	13.28
PBW226		42.61	37.15	86.53	83.59	90.62	78.94	0.95	2.57	14.52	16.58	5.83	6.64	11.89	14.09
DBW187		43.81	38.46	90.12	86.42	88.47	76.54	0.71	2.34	12.37	13.42	4.42	5.27	12.02	14.27

Gene expression analysis of heat stress responsible genes in wheat

The quantity of all RNA samples had A260/A230 ratio over 1.1 which indicated that DNAse treatment successfully removed the genomic DNA. Quality of RNA samples was also checked by agarose gel electrophoresis (1%). 4 distinct bands of 28S RNA, tRNA 18S RNA and 5SRNA were obtained indicating good quality of RNA (Figure 2-A). Quantitative Real-time PCR analysis revealed upregulation of various heat shock protein genes (HSP17, HSP23, and HSP70) in heat-tolerant wheat genotypes (DBW187, PBW226) and down regulation in heat-sensitive genotypes (HD3226, DBW173) (Figure 2-B). The study examined different heat shock treatments (25˚C, 34˚C, and 37˚C) in both control and treated conditions (Table 19). HSP17 showed variable expression among genotypes, with consistent upregulation in both conditions. In control conditions, DBW187 exhibited the highest fold expression (2.43 folds) at 37˚C, and in treated conditions, PBW226 showed the highest expression (5.80 folds) at 37˚C. Similar expression patterns were observed for HSP23 and HSP70, with PBW226 consistently showing the highest fold expressions (Figure 3). The study suggests that HSP17 is constitutively expressed but heat stress-inducible in wheat leaves, and its upregulation correlates with increased heat stress in tolerant genotypes. Comparable findings were reported by Tiwari et al. (2014), Sheoran et al. (2015), Singhal (2016), Tounsi et al. (2019), Wang et al. (2020), and Wang et al. (2023).

Table 19. Effect of ZnO-NPs on expression of HSP17, HSP23 and HSP70 genes in four wheat genotypes under various heat shock treatments.

Treatments	Genotype	CT of house-keeping gene	HSP17				HSP23				HSP70		
			CT Target gene	ΔCT	ΔΔCT	Fold change	CT Target gene	ΔCT	ΔΔCT	Fold change	CT Target gene	ΔCT	Δ
25ºC control samples	HD3226	18.240	22.263	4.023	1.008	0.497	22.809	4.023	1.525	0.347	28.764	10.524	1
	DBW173	18.457	22.560	4.103	1.088	0.470	22.206	4.103	0.706	0.613	28.583	10.126	0
	PBW226	18.113	21.909	3.796	0.781	0.582	21.905	3.796	0.750	0.595	28.126	10.014	0
	DBW187	18.206	22.112	3.906	0.891	0.539	21.783	3.906	0.534	0.691	28.366	10.160	0
25ºC treated samples	HD3226	17.996	21.547	3.551	0.536	0.690	22.127	3.551	1.088	0.470	27.330	9.334	0
	DBW173	18.014	21.734	3.720	0.705	0.614	21.558	3.720	0.501	0.707	27.632	9.617	0
	PBW226	17.901	21.010	3.109	0.462	0.726	21.518	3.109	0.316	0.803	27.141	9.240	0
	DBW187	17.953	21.155	3.202	0.555	0.681	21.641	3.202	0.388	0.764	27.263	9.310	0
34ºC control samples	HD3226	17.685	20.299	2.614	-0.033	1.023	20.851	2.614	-0.134	1.097	25.926	8.241	-(
	DBW173	17.747	20.137	2.390	-0.257	1.195	20.947	2.390	-0.100	1.072	26.591	8.844	-(
	PBW226	17.925	20.047	2.122	-0.525	1.439	20.651	2.122	-0.574	1.489	26.116	8.191	-(
	DBW187	17.724	20.000	2.276	-0.371	1.293	20.293	2.276	-0.731	1.660	25.934	8.209	-(
34ºC Treated samples	HD3226	16.813	20.311	3.498	-0.938	1.916	20.857	3.498	-0.819	1.765	27.310	10.497	-(
	DBW173	16.939	20.377	3.438	-0.998	1.997	20.933	3.438	-0.868	1.826	27.424	10.485	-
	PBW226	16.677	20.678	4.001	-1.020	2.027	20.992	4.001	-1.178	2.263	24.721	8.044	-
	DBW187	16.743	20.737	3.993	-1.028	2.039	21.092	3.993	-1.144	2.210	24.799	8.056	-
37ºC control samples	HD3226	17.460	20.640	3.179	-1.257	2.389	21.252	3.179	-1.072	2.102	27.892	10.432	-
	DBW173	17.516	20.743	3.227	-1.209	2.312	20.876	3.227	-1.503	2.834	27.952	10.436	-
	PBW226	17.270	20.465	3.195	-1.241	2.364	20.874	3.195	-1.259	2.393	27.530	10.260	-
	DBW187	17.388	20.541	3.153	-1.283	2.433	21.038	3.153	-1.212	2.317	27.616	10.228	-
37ºC Treated samples	HD3226	16.363	19.650	3.287	-1.734	3.326	19.942	3.287	-1.914	3.768	23.967	7.604	-
	DBW173	16.499	19.789	3.290	-1.731	3.320	20.024	3.290	-1.968	3.912	24.063	7.564	-
	PBW226	15.868	18.350	2.483	-2.538	5.809	18.950	2.483	-2.411	5.317	23.010	7.143	-2
	DBW187	16.037	19.028	2.991	-2.030	4.084	19.252	2.991	-2.277	4.848	23.287	7.250	-

Conclusion

The findings from these studies demonstrates the potential of biogenic zinc oxide nanoparticles (ZnO-NPs), synthesized through environmentally friendly methods, to alleviate the detrimental effects of heat stress on wheat crops. These nanoparticles exhibited remarkable properties and positively influenced seed germination and growth parameters, underscoring their capacity to enhance wheat productivity and quality. Genetic variability analyses highlighted opportunities for selective breeding, while path analysis illuminated key traits for targeted improvement. Moreover, our investigation into physiological and biochemical responses revealed that ZnO-NPs mitigated the adverse impacts of heat stress on wheat plants. Gene expression analysis shed light on the differential expression of heat shock proteins in heat-tolerant and heat-sensitive genotypes, offering insights into the molecular responses of heat-tolerant and heat-sensitive genotypes. Thereby, this research highlights the promise of nanotechnology as a modern agricultural tool to enhance wheat resilience in the face of climate change, thereby contributing to global food security.

Declarations

Declarations

Not applicable.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

All authors contributed to the study conception and design. N.K. conceived the idea and supervises the experiments, writing and editing. A.A. material preparation, data collection and analysis. P.K. statistical analysis. R.K., R.S.S. creative experiment design and manuscript editing., A.S., L.K.G., P.K. and R.D. screened the language, values, statements and facts mentioned in the manuscript.

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Figures

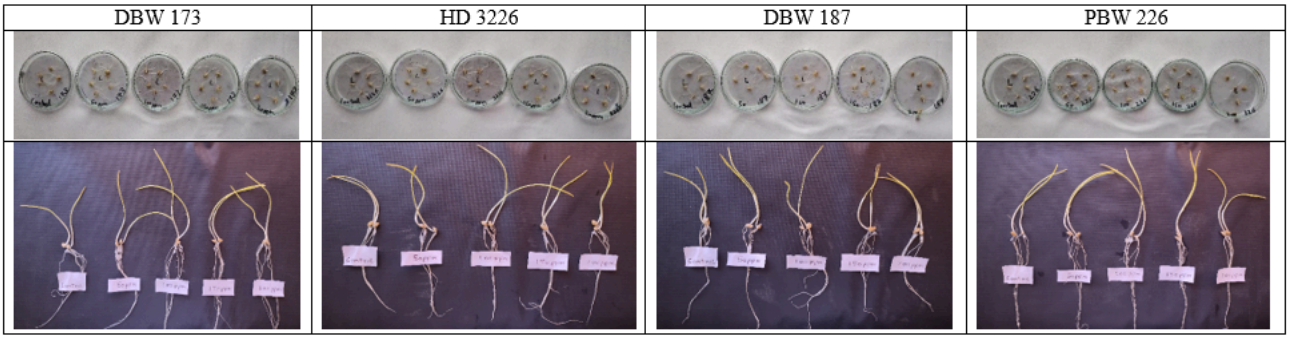


Figure 1

Effects of Biogenic ZnO-NPs on germination percentage and seedling length of different wheat genotypes under control and treatment conditions

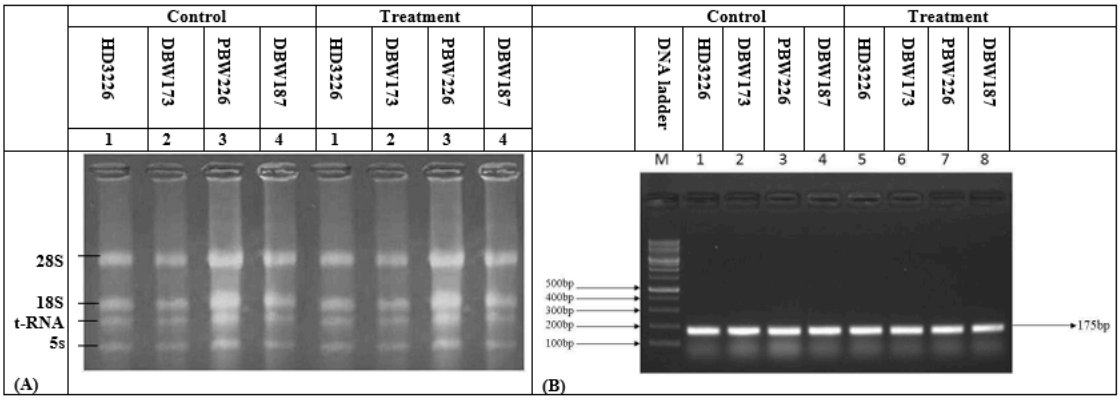


Figure 2

(A) Total RNA isolated from four wheat genotypes under control and treated condition. (B) Amplification of GAPDH gene using Semi quantitative PCR under control and treated condition.

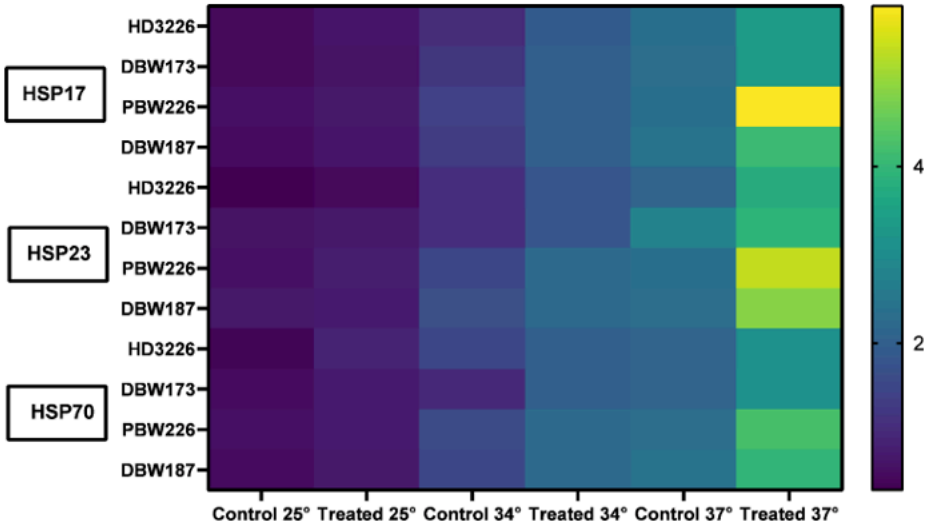


Figure 3

Relative expression of heat stress responsible genes of HSP17, HSP23 and HSP70 in all the varieties in response to different thermal treatments in control and treated conditions.

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