The Pharma Innovation Journal 2023; 12(11): 1193-1200

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(11): 1193-1200

www.thepharmajournal.com
Received: 08-09-2023
Accepted: 18-10-2023

Narender Mohan
Department of Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Ajay Pal
Department of Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Vinod Saharan
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Corresponding Author:
Narender Mohan
Department of Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Exploring the use of Zn-SA-Chitosan bio-nano-conjugates for improving terminal heat stress in wheat

Narender Mohan, Ajay Pal and Vinod Saharan

Abstract
A serious environmental barrier to crop productivity in a warming climate is terminal heat stress. In this study, a wheat variety WH 1124, was used to test the potential benefits of Zn-SA-chitosan bio-nano-conjugates (BNCs) in reversing or buffering the harmful effects of terminal heat stress (THS) on antioxidant system. Together, BNCs supplied Zn and SA, which improved the crop's nutritional availability. By improving cellular redox equilibrium and up regulating the antioxidant status involved in stress tolerance and plant growth, BNCs contributed to increased wheat yield. By soothing hydrogen peroxide (H_2O_2), and malondialdehyde (MDA), BNCs (0.01-0.16% w/v) added at the anthesis stage further manipulated cellular homeostasis. The activities of superoxide dismutase, catalase, peroxidase, in flag leaves enduring terminal heat stress on late planting were improved by foliar application of BNCs. The wheat plant was able to mitigate THS thanks to a synergistic combination of improved antioxidative capacity and metabolite accumulation.

Keywords: Bio-nano-conjugates, terminal heat stress, antioxidative potential, metabolites

1. Introduction
Abiotic stress, which includes radiation, drought, salinity, nutrients, and heat stress, is primarily caused by climate change (Sarkar *et al*., 2021) [34]. Heat stress, specifically terminal heat stress (THS), is the primary cause of the yield plateau among all abiotic challenges (Sattar *et al*., 2020) [35]. THS has a negative impact on plant metabolism and growth, prevents cultivated wheat varieties/genotypes from reaching their full potential, and reduces agricultural output by up to 50% per year (Lamaoui *et al*., 2018) [24]. Wheat (*Triticum aestivum* L.) is a one-of-a-kind gift from nature to humanity because of its high nutritional value, which includes 12.1% protein, 1.8% fat, 1.8% ash, and 70% total carbohydrates (Ram & Govindan, 2020) [31]. It is the world's second most widely grown food crop after maize, with 778 million metric tonnes produced in 2021-2022 (FAOSTAT, 2022) [12], and has thus become the staple food crop for millions of people worldwide. It can be produced in a variety of climates, but there are numerous biotic and abiotic variables that limit its output (Akter & Rafiqul Islam, 2017) [1].
Sessile plants try to maintain cellular equilibrium through avoidance and tolerance mechanisms like early maturation, changes in membrane lipid composition, and other enzymatic and non-enzymatic mechanisms (Esfandiari *et al*., 2008) [11]. Temperature increases of 1 °C have been shown to reduce wheat yield by 3-4% (Wardlaw & Wrigley, 1994) [42]. Excess reactive oxygen species (ROS) release such as hydroxyl radicals (OH), and hydrogen peroxide (H_2O_2) by various metabolic pathways is one of the most serious consequences of THS (Sadiq *et al*., 2020) [33]. The antioxidative system, involving anti-oxidants and antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), and Peroxidase (POX), regulates and scavenges the rapidly evolving ROS in plants (Khan *et al*., 2021) [21].

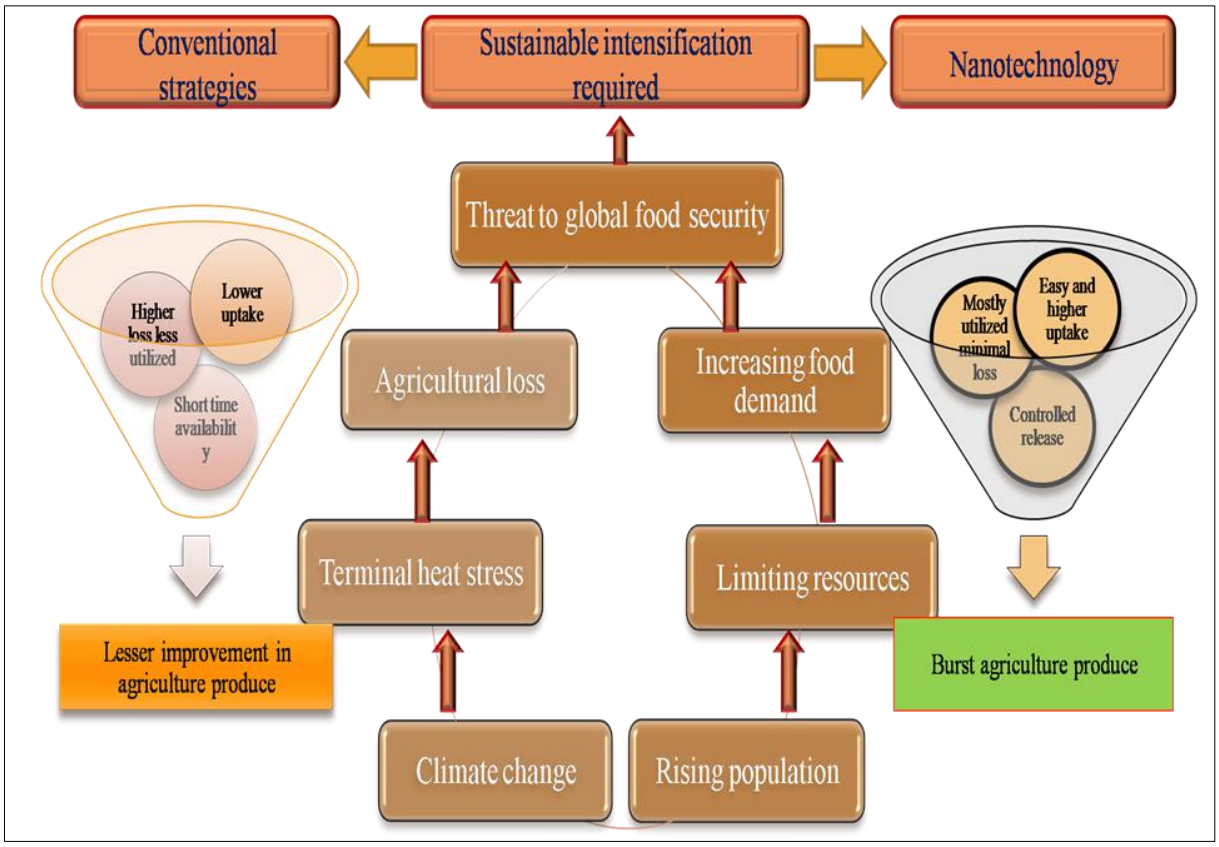

Fig 1: Diagrammatic representations of climate change and resulting food insecurity with some strategies and their impact on agricultural produce

To combat THS, farmers and researchers have discovered a variety of alternative mitigating strategies such as timely sowing, fertigation, exogenous application of nutrients, and phytohormones (Mohan *et al*., 2023) [26]. Even though, more technologies, such as nanotechnology, must be investigated in this direction to address global concerns about biotic and abiotic stresses in crops. Biopolymer and metal-based nano-materials have been widely used in agriculture (Gobi *et al*., 2021; Hossain *et al*., 2021) [15, 19]. Chitosan, a hetero-amino-polysaccharide derived from crustaceans, insects, and fungi cell walls, is the second most abundant polymer in nature after cellulose. Studies have shown that because of its excellent biocompatibility, biodegradability, and bioactivity, it improves plants' ability to reduce the negative effects of unfavourable conditions by affecting multiple physiological responses (Prajapati *et al*., 2022) [30].

Bionanoconjugates (BNCs) are a complex of two or more nano scale molecules with a large surface area and distinct biological activity (Azmana *et al*., 2021) [3]. Zinc (Zn) and salicylic acid (SA) play important roles in plant physiology and biological responses. Ionic gelation, which involves mixing an acidic chitosan solution with a water-soluble negatively charged cross-linker (TPP), is the most common physical cross-linking process for bionanoconjugate synthesis (Choudhary *et al*., 2017) [8]. Their bionanoconjugation with chitosan biopolymers can increase the potential biological activity of nanopolymers (Gobi *et al*., 2021) [15]. Zinc is required for plant development and is involved in the expression of antioxidant enzyme genes such as SOD, POX, and GR. Salicylic acid is a water-soluble phenolic chemical that functions as a signalling molecule in plants to regulate physiological activities. It promotes growth by interfering with enzyme activity and activating growth-promoting cellular functions. Taking the preceding information into account, the current study was proposed to investigate the effect of bionanoconjugate on biochemical changes in wheat during terminal heat stress.

2. Materials and Methods

In the present research, wheat variety WH 1124, was lately grown to expose them to late sowing stress (THS) and were further raised in pot house. The biochemical investigations were conducted in the laboratories of the Department of Biochemistry, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The analytical grade chemicals were used in the study and were obtained from E-Merck, Sigma Chemical Co. (USA), Sisco Research Laboratories (Mumbai), and Himedia.

2.1 Preparation of Zn-SA-chitosan Bionanoconjugates (BNCs)

The ionotropic gelation method was followed to synthesize Zn-SA-chitosan BNCs wherein, chitosan solution was mixed with drop-wise introduction of TPP (cross-linker) on a magnetic stirrer to allow cross linking. Subsequently, $ZnSO_4$ and SA were carefully added to mixture. The mixture was centrifuged and the pellet obtained was lyophilized and used for foliar application (Kumaraswamy *et al*., 2018) [23]. The procured BNCs at various concentrations (0.01-0.16% w/v) from T1-control, T2-bulk Zn, T3-bulk SA, T4-BNC 0.01%, T5-BNC 0.02%, T6-BNC 0.04%, T7-BNC 0.08%, T8-BNC 0.12%, T9-BNC 0.16%, were supplied wheat during anthesis.

2.2 Antioxidative metabolites

To estimate the metabolite, a 200 mg leaf tissue was homogenised in 2.0ml of ice-cold 0.01 M phosphate buffer (pH-7.0), to estimate H_2O_2 content. The homogenate was

centrifuged for 10 min. at 10,000 rpm, and the supernatant was collected to calculate hydrogen peroxide (Sinha, 1972) [40]. 0.4 ml of extract was mixed with 0.6ml of 0.1 M phosphate buffer (pH 7.0) and 3.0ml of a 5% (w/v) potassium dichromate and glacial acetic acid (1:3, v/v) mixture. The quantity of H_2O_2 was determined using the standard curve of H_2O_2 (10-100 µmole). Further, to calculate the MDA content in a neat and clean pestle mortar, 200 mg of fresh leaf sample was hand homogenized in 2.0ml of 0.1% TCA (Heath & Packer, 1968) [18]. The homogenate was centrifuged for 15 min. at 8000 rpm, and the supernatant was collected to calculate MDA. 2.3ml of 20% TCA containing 0.5% TBA was added to 0.5ml of supernatant. The mixture was heated in a 95 °C water bath for 30 min. before being quickly cooled in an ice bath. The absorbance at 532 nm was measured, and the non-specific absorbance at 600 nm was subtracted. MDA content was calculated using the molar extinction coefficient ($155mM^{-1}$ cm^{-1}).

2.3 Antioxidant enzymes

After foliar application, the flag leaf was removed and homogenized according to standard extraction conditions for extraction buffer temperature, molarity, and pH. A leaf tissue (1g) was hand homogenized in 4ml of 0.1 M phosphate buffer (pH 7.0) containing 1.0% PVP in a pre-chilled mortar and pestle. The homogenate thus obtained was centrifuged at 10,000 x g for 20 min. in a refrigerated centrifuge at 4 °C. The supernatant as enzyme extract (EE) was collected and used to assay antioxidant enzymes *viz.* superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX). Superoxide dismutase (SOD) (EC 1.15.1.1) was tested for its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). 2.5ml of 60mM Tris-HCl (pH 7.8), 0.1ml of 420mM L-methionine, 0.1ml of 1.8mM NBT, 0.1ml of 90mM riboflavin, 0.1ml of 3mM EDTA, and 0.1ml of EE were added to a 3.0ml reaction mixture (Beauchamp & Fridovich, 1971). After addition of riboflavin the tubes were shaken vigorously before being placed 30 cm below the light source of two fluorescent 20 W lamps (40 min.) to stop the reaction. To protect the tubes from further light exposure, they were wrapped in a black cloth. As a control, no colour development was observed in the un-irradiated tube. The reaction mixture without the enzyme extract produced the most colour, while the EE reduced OD. The OD was recorded at 560 nm and percent inhibition was calculated using the following formula:

$$\text{Percent inhibition} = \frac{(V - v)}{v} * 100$$

Where,

V = Rate of reaction in the absence of enzyme extract.

v = Rate of reaction in the presence of enzyme extract.

One enzyme unit was defined as the amount of enzyme required to inhibit the photochemical reduction of NBT by 50%.

For catalase (CAT) (EC 1.11.1.6), a reaction mixture containing 0.5ml 0.1 M phosphate buffer (pH 7.5), 0.4 ml 0.2 M H_2O_2, and 0.1ml EE was incubated at 37 °C (Sinha, 1972) [40]. To stop the reaction, 3ml potassium dichromate reagent (PDR) was added. After heating the reaction mixture in a boiling water bath for 10 min., the OD at 570 nm was measured using the PDR as a blank. To estimate the residual amount of H_2O_2 in the mixture, the absorbance of the sample was subtracted from that of the control (assay mixture devoid of EE). One enzyme unit was defined as the amount of enzyme required to catalyze the oxidation of 1.0 µmole of H_2O_2 in one minute under given assay conditions. For, peroxidase (POX) (EC 1.11.1.7), a reaction mixture contained 2.75ml of 50mM phosphate buffer (pH 6.5), 0.1ml of 0.5% hydrogen peroxide, 0.1ml of 0.2% Ortho-dianisidine, and 50 µl of EE (Shannon *et al*., 1966) [38]. The reaction was started by adding 0.1ml of H_2O_2. The assay mixture without H_2O_2 served as the control. The absorbance change was measured at 430 nm every 15 seconds for 3 min. One enzyme unit was defined as the amount of enzyme required to produce a one-unit-per-minute change in OD.

2.4 Statistical analysis

The data were analyzed by analysis of variance for the completely randomized design (CRD) using OPSTAT software available on www. http//hau.ernet.in home page where each observation was replicated thrice and CD at 5% was calculated (Sheoran *et al*., 1998) [39].

3. Results

3.1 Effect of BNCs on metabolites under THS at post-anthesis stages at regular intervals

The H_2O_2 content is shown in Table 1 under late-sown conditions. H_2O_2 concentration increased until the 20th DAT, in WH 1124 from 102.4 to 144.0 µmole g^{-1} fwt in control, 102.7 to 143.4 µmole g^{-1} fwt in bulk Zn, and 100.5 to 141.5 µmol g^{-1} fwt in bulk SA. Additionally, application of BNCs (1200 ppm) showed a significant reduction in increase in H_2O_2 concentration, from 83.7 to 119.5 µmole g^{-1} fwt. Similarly, the MDA content increased as the DAT increased, reaching its peak on the 20th DAT for both varieties (Table 1). The MDA content significantly increased from their mean basal level 3.7 µmole g^{-1} fwt on 0th DAT to their higher level 6.0 µmole g^{-1} fwt on the 20th DAT. However, the increase was far higher in controls than in BNCs. BNCs treated plants exhibited decreased MDA rise, particularly in 800 ppm treated plants (Table 1).

3.2 Effect of BNCs on enzymes of the antioxidant system of flag leaf in wheat under THS

SOD activity gradually raised from the 0th to the 20th DAT. However, compared to bulk and control plants, the greatest increase was seen in plants treated with BNCs. The outcome in Table 2 demonstrates the activity in WH 1124 (19.3-48.9 unit g^{-1} fwt), the mean activity increased gradually and more significantly for subsequent samplings from the 0th to the 20th DAT. SOD activity increased the least in control plants and the most in those treated with 800 ppm BNCs. However, in 800 ppm treated plants, the rise was 20.3 to 55.8 unit g^{-1} fwt. On the 20th DAT, 48.9 unit g^{-1} fwt was the highest mean SOD activity, in plants treated with 800 ppm BNCs.

Similarly, for catalase, Table 2 demonstrates the response of plants to BNCs treatments in terms of catalase activity. According to ANOVA analysis, the control plants had the lowest activity levels than those treated with BNCs. Both varieties significantly increased their catalase activity from the 0th to the 20th DAT after exposure to stress. The highest mean activity was reported 25.2 unit g^{-1} fwt, in the T10 treatment. The study also observed that the maximum increase in activity from 0th to 20th DAT was seen in T11

(17.8-36.7 unit g^{-1} fwt).

Moreover, peroxidase shown in table 3 shows a progressive rise in POX activity when DAT increased from 0^{th} to 20^{th}, with mean basal activities. It was observed that under late sowing, POX activity increased in all treatments, but in BNCs-treated plants of both wheat varieties, the activity increase was more significant. The POX activity under stress increased to its highest levels in 800 ppm BNCs from 35.3 to 59.7 unit g^{-1} fwt. On the 20^{th} DAT, WH 1124 in 1600 ppm BNCs treated plants had the highest POX activity (60.1units g^{-1} fwt).

Table 1: Change in H_2O_2 content in flag leaf of two wheat varieties at regular intervals after foliar application of BNCs

	H_2O_2 (µmole g^{-1} fwt)						MDA (µmole g^{-1} fwt)					
Treatment	**0^{th}**	**5^{th}**	**10^{th}**	**15^{th}**	**20^{th}**	**Mean**	**0^{th}**	**5^{th}**	**10^{th}**	**15^{th}**	**20^{th}**	**Mean**
T1	102.4	110.8	117.2	123.2	144.0	119.5	4.1	4.9	6.9	7.4	7.6	6.2
T3	102.7	116.1	123.8	121.2	143.4	121.5	3.9	5.0	5.9	6.8	7.2	5.8
T4	100.5	118.8	132.5	138.7	141.5	126.4	3.8	4.2	5.3	6.1	6.5	5.2
T6	88.1	105.1	124.8	131.1	136.0	117.0	3.7	4.2	4.8	5.6	5.8	4.8
T7	85.4	98.7	114.5	121.3	134.6	110.9	3.6	4.0	4.8	5.1	5.6	4.6
T8	84.9	95.6	112.8	119.2	128.7	108.2	3.5	3.5	4.4	4.7	5.4	4.3
T9	83.8	97.6	113.0	117.0	123.4	107.0	3.5	3.6	3.9	4.5	5.0	4.1
T10	83.7	97.6	114.0	117.2	119.9	106.5	3.4	3.4	4.0	4.4	5.1	4.1
T11	82.4	94.5	113.0	122.2	126.8	107.8	3.4	3.3	3.7	4.2	5.0	3.9
Mean	92.0	105.7	119.8	124.5	133.8	115.2	3.7	4.1	5.0	5.7	6.0	4.9

Table 2: Change in SOD activity in flag leaf of two wheat varieties at regular intervals after foliar application of BNCs

	SOD (units g^{-1} fwt)						CAT (units g^{-1} fwt)					
Treatment	**0^{th}**	**5^{th}**	**10^{th}**	**15^{th}**	**20^{th}**	**Mean**	**0^{th}**	**5^{th}**	**10^{th}**	**15^{th}**	**20^{th}**	**Mean**
T1	15.2	24.7	29.5	33.4	38.9	28.3	15.0	16.6	18.4	23.7	26.4	20.0
T3	19.1	24.9	32.9	37.7	43.9	31.7	15.8	16.7	19.2	26.8	30.5	21.8
T4	18.5	25.7	32.5	37.3	43.1	31.4	15.8	16.6	20.5	27.8	30.7	22.3
T6	19.2	26.2	38.9	45.4	54.6	36.8	16.1	16.7	20.0	26.5	29.3	21.7
T7	19.3	26.5	39.2	45.7	50.1	36.1	16.9	18.1	22.0	26.7	30.1	22.8
T8	20.9	28.1	42.8	45.4	53.9	38.2	17.2	18.5	22.3	27.8	31.6	23.5
T9	20.3	29.5	42.4	45.7	55.8	38.9	17.7	18.7	22.4	29.7	35.7	24.8
T10	21.2	28.9	39.7	48.4	55.0	38.7	17.5	18.6	22.8	31.4	35.8	25.2
T11	21.0	28.6	39.3	47.9	54.2	38.8	17.8	18.9	23.0	28.4	36.7	25.0
Mean	19.3	26.7	36.7	42.1	48.9	34.7	16.4	17.5	20.6	27.0	31.2	22.5

Similarly, in ascorbate peoxidase (Table 3) activity indicated a maximum rise in mean value across treatments in 1200 ppm BNCs, with the highest value 57.2 unit g^{-1} fwt. Compared to bulk and control plants, stressed plants treated with BNCs had the greatest change in activity. Under stress, BNC-treated plants showed a rapid, progressive rise in activity that was significantly higher than that of the control plants, with an increase of 24.6 unit g^{-1} fwt (from 25.8 to 50.4 unit g^{-1} fwt). The 20^{th} DAT had the highest mean activity levels with values of 66.0 unit g^{-1} fwt (Table 3).

Table 3: Change in POX activity in flag leaf of two wheat varieties at regular intervals after foliar application of BNCs

	POX (units g^{-1} fwt)						APX (units g^{-1} fwt)					
Treatment	**0^{th}**	**5^{th}**	**10^{th}**	**15^{th}**	**20^{th}**	**Mean**	**0^{th}**	**5^{th}**	**10^{th}**	**15^{th}**	**20^{th}**	**Mean**
T1	30.8	37.7	39.9	45.6	47.2	40.2	25.8	30.7	42.2	46.7	50.4	39.8
T3	30.8	36.2	39.8	45.7	50.5	40.6	27.0	36.0	44.3	50.6	59.5	43.5
T4	32.0	37.7	42.5	47.8	48.9	41.8	26.7	35.7	47.4	55.0	61.6	45.3
T6	34.4	38.4	43.4	48.0	51.7	43.2	24.9	36.4	49.1	58.5	67.6	47.3
T7	35.4	38.7	43.9	48.7	53.4	44.0	28.4	40.3	53.6	62.0	68.0	50.5
T8	36.0	39.8	45.1	49.3	54.2	44.9	35.6	46.8	56.4	64.2	69.7	54.5
T9	35.3	39.5	45.7	50.7	59.7	46.3	35.3	48.0	58.5	67.8	74.2	56.8
T10	36.0	40.0	46.8	52.0	59.4	46.8	35.0	48.2	60.0	66.4	76.3	57.2
T11	36.8	41.2	45.9	51.8	60.1	47.0	35.2	49.6	60.8	65.8	74.5	57.2
Mean	34.1	38.4	43.2	48.7	53.2	43.5	29.6	40.0	51.0	58.2	66.0	49.0

4. Discussion

Food insecurity is becoming a concern in many countries as a result of climate change. Crop-sowing and harvesting practises have evolved due to considerations associated with environmental change (Fig 1). The north-western and north-eastern plains of India have seen a lot of late wheat planting as a result of the delayed harvesting of crops such as cotton, paddy, sugarcane, pigeon pea, etc. Due to this, the wheat crop is subjected to greater ambient temperatures following anthesis, or terminal heat stress (THS), which drastically lowers crop production (Dubey *et al*., 2020; Newport *et al*., 2020) [10, 28]. It has not yet been determined what effect mixing different biomaterials will have, especially in bionanoconjugates (BNCs). It is yet unknown how these BNCs affect proline metabolism, the underlying biochemical response system, and the mechanism behind their defence against THS in wheat.

4.1 Response of antioxidant metabolites in wheat under THS and effect of BNCs

Ascorbic acid, a ubiquitous metabolite in plant cells, is a crucial non-enzymatic antioxidant that works through AOS to protect plants from oxidative damage caused by biotic or abiotic stimuli (Shah *et al*., 2019) [36]. Stress tolerance is a result of this metabolite's exceptional redox-buffering capacity (Parveen *et al*., 2021) [29]. Ascorbic acid content rose more in the heat-resistant wheat variety (WH 1124) in the current study than in the heat-sensitive wheat variety (WH 542). Ascorbic acid content was considerably increased by applying BNCs in addition to bulk and control (Tables 1). The findings are consistent with those of Gunes *et al*. (2008) [16], who discovered that ascorbic acid levels were relatively higher in genotypes that were more stress-resistant than in susceptible genotypes (Gunes *et al*., 2008) [16]. Glutathione is another low molecular weight plant metabolite that also functions as a stress indicator in plants. It regulates a number of essential cellular functions (Ranjit *et al*., 2016) [32]. It seems to be a critical signal molecule because it establishes a direct connection between environmental stress and important adaptive responses. According to May *et al*., (1998) [25], it is a non-enzymatic antioxidant that improves stress tolerance to provide protection. Following treatment, the glutathione concentrations in the flag leaves of WH 1124 and WH 542 plants increased at different developmental stages under THS, peaking on the 20th day of observation (Table 2). Additionally, compared to the bulk and control, the delivery of BNCs resulted in a noticeably higher increase in glutathione content. The mean glutathione concentration in WH 1124 and WH 542 was found to increase to 32.2 and 39.0 percent, respectively, upon application of BNCs (800 ppm) (Table 2).

The generation of H_2O_2, a hazardous naturally occurring plant metabolite, primarily by SOD, is one of the outcomes of photorespiration that is known to worsen lipid degradation and increase membrane electrolyte leakage (Awasthi *et al*., 2015) [2]. The results of this study strongly imply that wheat grown under stressed environments greatly increased in H_2O_2. The H_2O_2 concentration of late-sown wheat was further reduced or regulated by applying BNCs to sensitive and resistant wheat varieties from 0th to 20th DAT (Table 1). In our study, displayed in Table 1, demonstrate that under unfavourable conditions, malondialdehyde (MDA) levels might indicate the extent of damage and are a marker of lipid peroxidation. A decrease in MDA levels indicates enhanced antioxidant capacity, heightened resistance to cell damage, and, ultimately, decreased stress because it may also be utilised as a biomarker for lipid peroxidation (Dhanda *et al*., 2004) [9]. Even after treatment, the MDA content in the flag leaf of WH 1124 increased; the highest value was noted on the 20th DAT. The results showed that under stress, flag leaf experienced higher oxidative damage compared to treated plants than control. The current study clearly shows that BNCs (800–1600 ppm) decreased MDA levels in comparison to the control, increasing the plant's ability to withstand stress. Furthermore, the decrease in the negative effects of THS leading to an increase in membrane stability may also be attributed to the foliar spray of BNCs containing SA, which has a signalling function that plays a role in the stimulation of heat tolerance in wheat plants as indicated by the decrease in MDA content. It might also be connected to the gradual release of zinc, sulphur, and nitrogen from the chitosan framework of the BNCs, which was supplied slowly as required during stress. Chakraborty & Pradhan, (2012) [7] discovered that the accumulating metabolite concentration in wheat varieties was three times higher in sensitive varieties. Our results are in line with their findings of Wang *et al*., (2014) [41] in which 0.3mM of SA inhibited lipid peroxidation and increased MSI in wheat leaves when exposed to high temperatures and light stress. Applying chitosan, an immunity-boosting substance that co-encapsulates the stress-relieving components Zn and SA, may be the cause of the reduction in H_2O_2 accumulation (more than 10%). Moreover, these BNCs may have enhanced H_2O_2 activities in signalling cascades. Considering the aforementioned results, it is clear that BNCs containing Zn, SA, and chitosan in their nano-conjugated form protect and benefit wheat.

4.2 Response of antioxidant enzymes in wheat under THS and effect of BNCs

AOS is developed as a result of THS, which is highly harmful to plants. To prevent or lessen the harm caused by AOS, plants have created an antioxidant defence system that includes the enzymes SOD, CAT, POX, and APX (Caverzan *et al*., 2016) [6]. One antioxidant that is responsible for converting O_2 to H_2O_2 is SOD, which is also considered to be the first line of defence against AOS. Flag leaves showed elevated SOD activity, according to the current study. When compared to bulk and control, the plants treated with BNCs exhibited the greatest increase in activity. Furthermore, a boost in mean activity of 37.8 and 34.6% was seen in flag leaf when treated with 800 ppm BNCs compared to the control (Table 2). The hazardous SOD product is removed by CAT, however less successfully than with the APX-GR system. Found in conjunction with peroxisomes, the enzyme catalase is widely distributed in plants but absent from chloroplasts. It plays a critical role in converting the highly reactive H_2O_2 produced during photorespiration into less dangerous forms (Foyer *et al*., 1994; Ighodaro & Akinloye, 2018) [13, 20]. Heat is one among the several abiotic environments that alter its activity (Mohan *et al*., 2020) [27]. The current study found that THS exposure significantly raised CAT levels in wheat plants. The fact that CAT activity in WH 1124 leaves increased by nearly two times under THS circumstances suggests that CAT is important for THS tolerance. When BNCs (800 ppm) were applied in a stressed environment, the mean CAT activity of WH 1124 in flag leaf rose by 24.1% (Table 2).

Hemoproteins called peroxidases use H_2O_2 to catalyse the oxidation of a variety of substrates (Banci, 1997) [4]. THS enhanced peroxidase activity in flag leaves after anthesis in the current study; exhibited a greater augmentation (Table 3). Furthermore, the data demonstrated that the plants treated with 800 ppm BNCs exhibited increased mean activity in with values of 15.0% under THS. The results suggest that a plant can be protected against oxidative damage by increased peroxidase activity or induction in the flag leaf under THS. Another and more effective H_2O_2 detoxifying mechanism present in plant cells is the ascorbate/glutathione (AsA/GSH) cycle, which operates in the cytosol and chloroplast (Table 3). Ascorbate peroxidase (APX), a major antioxidant enzyme in the AsA/GSH cycle, is essential for detoxifying H_2O_2 because it uses ascorbate as an electron donor (Foyer & Noctor, 2005) [14]. The THS was found to increase APX activity in the present investigation, and this was further corroborated by BNCs, especially at 800 ppm and higher concentrations.

When 1200 ppm BNCs were applied to flag leaves under late planted conditions the mean APX activity increased to 43.8%, in comparison to the control (Tables 3).

According to the results above, applying BNCs topically to wheat increased its antioxidant capacity up until the 20th DAT. Under THS, however, the resistant cultivar (WH 1124) exhibited the highest growth. The results supported earlier research by Shailesh *et al*., (2017) [37], which shown that foliar spray of isolated SA increased the overall antioxidant capability of four wheat types under heat stress, with the tolerant variety exhibiting the most increase. In summary, the foliar application of Zn, SA, and chitosan to wheat plants during their reproductive phases resulted in an increase in antioxidant activity, which in turn boosted the activities of CAT, SOD, POX, and APX under late-sown conditions (Hameed *et al*., 2020; Kumar *et al*., 2021) [17, 22]. It might result from SA's beneficial effects on physiological characteristics and other biochemical features that are maintained during foliar spraying. According to statistical data, foliar application of BNCs (100–1600 ppm) boosted the levels of antioxidants in flag leaves, reduced MDA, and enhanced antioxidant enzyme activities (Sharma *et al*., 2023) [26]. Even though certain enzymes exhibit their peak activity at concentrations as high as 1600 ppm, it is thought that 800 ppm was the concentration that performed the best in the pot conditions used in the current study. This is due to the fact that the outcomes at 800 ppm are statistically equivalent to those at higher BNC concentrations.

5. Conclusion

Prominent results were obtained from an experiment carried out in the department of Biochemistry's screen house. Under THS, the activity of several H_2O_2 detoxifying enzymes, including as catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase, increased. The enzymes also showed peaks at different times after BNC administration. On the twentieth day following foliar spray, the enzyme activity peaked; however, WH 1124 exhibited the highest growth in comparison to WH 542 improved stress-induced activity of these enzymes may have contributed to WH 1124's improved tolerance capacity by scavenging AOS. The crop's innate system was further activated by the foliar application of BNCs, which also markedly increased the activities of antioxidant defence and proline-metabolizing enzymes. Furthermore, BNCs applied topically enhanced the activation of antioxidants and defense-related enzymes. Late-sowing elusively enhanced activities of APX above 50%, followed by SOD, CAT, and POX. Notably, these enzymes rose gradually after BNCs were applied topically. On the twentieth day after treatment, the highest activity was observed and can be stated that usage of BNCs greatly preserved the redox system by reducing the accumulation of H_2O_2 and MDA and minimising damage brought on by oxidative stress. BNC treatments have also somewhat boosted the accumulation of metabolites, all of which are essential for maintaining crop redox equilibrium and quenching AOS. All things considered, it is evident that applying BNCs enhanced the plants' ability to endure by enhancing their capacity for antioxidation and maintaining their redox balance by lowering stress in both types growing under late-sown circumstances. We assert that Zn and SA are supplied to the wheat crop by the co-encapsulated chitosan nanostructures in a gradual and sustained manner over an extended duration. In order to supply a consortium of micronutrients to the crop under stressful conditions, we anticipate that the co-encapsulation strategy used in this study will be developed for the synthesis of nano-composites/fertilizers containing several micronutrients.

6. Credit authorship contribution statement

Narender Mohan wrote the manuscript and prepared the figures. Ajay Pal and Vinod Saharan conceived, designed, supervised, reviewed, and edited the writing.

7. Declaration of competing interest

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.

8. Acknowledgements

Chaudhary Charan Singh Haryana Agriculture University, Hisar, Haryana supported this work. We apologize to the authors whose work could not be cited in this review owing to space limitations.

9. References

1. Akter N, Rafiqul Islam M. Heat stress effects and management in wheat. A review. Agronomy for Sustainable Development. 2017, 37(5). https://doi.org/10.1007/s13593-017-0443-9
2. Awasthi R, Bhandari K, Nayyar H. Temperature stress and redox homeostasis in agricultural crops. In Frontiers in Environmental Science; c2015. https://doi.org/10.3389/fenvs.2015.00011
3. Azmana M, Mahmood S, Hilles AR, Rahman A, Arifin M, Bin A, Ahmed S. A review on chitosan and chitosan-based bionanocomposites: Promising material for combatting global issues and its applications. International Journal of Biological Macromolecules. 2021 July;185:832–848. https://doi.org/10.1016/j.ijbiomac.2021.07.023
4. Banci L. Structural properties of peroxidases. Journal of Biotechnology. 1997;53(2-3):253–263. https://doi.org/10.1016/S0168-1656(97)01677-5
5. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 1971;44(1):276–287. https://doi.org/10.1016/0003-2697(71)90370-8
6. Caverzan A, Casassola A, Brammer SP. Antioxidant responses of wheat plants under stress. Genetics and Molecular Biology. 2016;39(1):1-6. https://doi.org/10.1590/1678-4685-GMB-2015-0109
7. Chakraborty U, Pradhan B. Oxidative stress in five wheat varieties (*Triticum aestivum* L.) exposed to water stress and study of their antioxidant enzyme defense system, water stress responsive metabolites and H2O2 accumulation. Brazilian Journal of Plant Physiology. 2012;24:117–130.
8. Choudhary RC, Joshi A, Kumari S, Rv K, Saharan V. Preparation of Cu-chitosan nanoparticle and its effect on growth and enzyme activity during seed germination in maize. Journal of Pharmacognosy and Phytochemistry JPP. 2017;6(64):669–673.
9. Dhanda SS, Sethi GS, Behl RK. Indices of Drought Tolerance in Wheat Genotypes at Early Stages of Plant Growth. Journal of Agronomy and Crop Science. 2004;190(1):6–12.

https://doi.org/10.1111/j.1439-037X.2004.00592.x
10. Dubey R, Pathak H, Chakrabarti B, Singh S, Gupta DK, Harit RC. Impact of terminal heat stress on wheat yield in India and options for adaptation. Agricultural Systems, 2020, 181. https://doi.org/10.1016/j.agsy.2020.102826
11. Esfandiari E, Shakiba MR, Mahboob SA, Alyari H, Shahabivand S. The effect of water stress on the antioxidant content, protective enzyme activities, proline content and lipid peroxidation in wheat seedling. Pakistan Journal of Biological Sciences. 2008;11(15):1916–1922. https://doi.org/10.3923/pjbs.2008.1916.1922
12. FAOSTAT. World Food and Agriculture - Statistical Yearbook 2020. In World Food and Agriculture - Statistical Yearbook; c2020. https://doi.org/10.4060/cb1329en
13. Foyer CH, Lelandais M, Kunert KJ. Photooxidative stress in plants. Physiologia Plantarum. 1994;92(4):696–717. https://doi.org/10.1111/j.1399-3054.1994.tb03042.x
14. Foyer CH, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. The Plant Cell. 2005;17(7):1866–1875.
15. Gobi R, Ravichandiran P, Shanker Babu R, Yoo DJ. Polymers Biopolymer and Synthetic Polymer-Based Nanocomposites in Wound Dressing Applications: A Review; c2021. https://doi.org/10.3390/polym13121962
16. Gunes A, Inal A, Adak MS, Bagci EG, Cicek N, Eraslan F. Effect of drought stress implemented at pre-or post-anthesis stage on some physiological parameters as screening criteria in chickpea cultivars. Russian Journal of Plant Physiology. 2008;55(1):59–67.
17. Hameed A, Khalid A, Ahmed T, Farooq T. Nano-priming with Zn-chitosan nanoparticles regulates biochemical attributes and boost antioxidant defence in wheat seeds. Nano-Priming with Zn-Chitosan Nanoparticles Regulates Biochemical Attributes and Boost Antioxidant Defence in Wheat Seeds; c2020. p. 207–221.
18. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 1968;125(1):189-198. https://doi.org/10.1016/0003-9861(68)90654-1
19. Hossain A, Skalicky M, Brestic M, Maitra S, Alam MA, Syed MA, *et al*. Consequences and mitigation strategies of abiotic stresses in wheat (*Triticum aestivum* l.) under the changing climate. Agronomy, 2021, 11(2). https://doi.org/10.3390/agronomy11020241
20. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine. 2018;54(4):287–293. https://doi.org/10.1016/J.AJME.2017.09.001
21. Khan A, Ahmad M, Ahmed M, Iftikhar Hussain M. Rising atmospheric temperature impact on wheat and thermotolerance strategies. Plants. 2021;10(1):1–24. https://doi.org/10.3390/plants10010043
22. Kumar A, Prajapati D, Devi KA, Pal A, Choudhary U, Dashora A, *et al*. Slow-release Zn application through Zn-chitosan nanoparticles in wheat to intensify source activity and sink strength. Plant Physiology and Biochemistry. 2021;168:272–281. https://doi.org/10.1016/j.plaphy.2021.10.013
23. Kumaraswamy RV, Kumari S, Choudhary RC, Pal A, Raliya R, Biswas P, *et al*. Engineered chitosan based nanomaterials: Bioactivities, mechanisms and perspectives in plant protection and growth. In International Journal of Biological Macromolecules, 2018, 113. https://doi.org/10.1016/j.ijbiomac.2018.02.130
24. Lamaoui M, Jemo M, Datla R, Bekkaoui F. Heat and drought stresses in crops and approaches for their mitigation. Frontiers in Chemistry; c2018. https://doi.org/10.3389/fchem.2018.00026
25. May MJ, Vernoux T, Leaver C, Montagu M Van, Inzé D. Glutathione homeostasis in plants: implications for environmental sensing and plant development. Journal of Experimental Botany. 1998;49(321):649–667.
26. Mohan N, Jhandai S, Bhadu S, Sharma L, Kaur T, Saharan V, *et al*. Acclimation response and management strategies to combat heat stress in wheat for sustainable agriculture: A state-of-the-art review. Plant Science, 2023, 111834. https://doi.org/https://doi.org/10.1016/j.plantsci.2023.111834
27. Mohan N, Kumari Ni, Jattan M, Avtar R, Rani B, Sushil. Response of antioxidative system of brassica juncea (l.) czern. To terminal heat stress. Bangladesh Journal of Botany. 2020;49(4):1185–1188. https://doi.org/10.3329/bjb.v49i4.52659
28. Newport D, Lobell DB, Balwinder-Singh, Srivastava AK, Rao P, Umashaanker M, *et al*. Factors constraining timely sowing of wheat as an adaptation to climate change in eastern India. Weather, Climate, and Society. 2020;12(3):515-528. https://doi.org/10.1175/WCAS-D-19-0122.1
29. Parveen A, Ashraf MA, Hussain I, Perveen S, Rasheed R, Mahmood Q, *et al*. Promotion of growth and physiological characteristics in water-stressed *Triticum aestivum* in relation to foliar-application of salicylic acid. Water (Switzerland). 2021, 13(9). https://doi.org/10.3390/w13091316
30. Prajapati D, Pal A, Dimkpa C, Harish Singh U, Devi KA, Choudhary JL, *et al*. Chitosan nanomaterials: A prelim of next-generation fertilizers; existing and future prospects. Carbohydrate Polymers. 2022;288:119356. https://doi.org/10.1016/J.CARBPOL.2022.119356
31. Ram S, Govindan V. Improving wheat nutritional quality through biofortification. In Wheat Quality for Improving Processing and Human Health; c2020. https://doi.org/10.1007/978-3-030-34163-3_9
32. Ranjit SL, Manish P, Penna S. Early osmotic, antioxidant, ionic, and redox responses to salinity in leaves and roots of Indian mustard (Brassica juncea L.). Protoplasma. 2016;253(1):101–110. https://doi.org/10.1007/s00709-015-0792-7
33. Sadiq Y, Zaid A, Khan MMA. Adaptive physiological responses of plants under abiotic stresses: Role of phytohormones. In Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives I: General Consequences and Plant Responses; c2020. https://doi.org/10.1007/978-981-15-2156-0_28
34. Sarkar S, Islam AKMA, Barma NCD, Ahmed JU. Tolerance mechanisms for breeding wheat against heat stress: A review. South African Journal of Botany. 2021;138:262–277.

https://doi.org/10.1016/j.sajb.2021.01.003
35. Sattar A, Sher A, Ijaz M, Ul-Allah S, Rizwan MS, Hussain M, *et al*. Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. PLoS ONE, 2020, 15(5). https://doi.org/10.1371/journal.pone.0232974
36. Shah T, Latif S, Khan H, Munsif F, Nie L. Ascorbic acid priming enhances seed germination and seedling growth of winter wheat under low temperature due to late sowing in Pakistan. Agronomy; c2019. https://doi.org/10.3390/agronomy9110757
37. Shailesh K, Singh VP, Sweta M, Ajay A. Exogenous applied salicylic acid alleviates adverse effects of high temperature on photosynthesis in late sown wheat (*Triticum aestivum*). Indian Journal of Ecology. 2017;44(4):100–104.
38. Shannon LM, Kay E, Lew JY. Peroxidase Isozymes from Horseradish Roots. Journal of Biological Chemistry. 1966;241(9):2166–2172. https://doi.org/10.1016/s0021-9258(18)96680-9
39. Sheoran OP, Tonk DS, Kaushik LS, Hasija RC, Pannu RS. Statistical software package for agricultural research workers. Recent Advances in Information Theory, Statistics & Computer Applications by DS Hooda & RC Hasija Department of Mathematics Statistics, CCS HAU, Hisar; c1998. p. 139-143.
40. Sinha AK. Colorimetric assay of catalase. Analytical Biochemistry. 1972;47(2):389–394. https://doi.org/10.1016/0003-2697(72)90132-7
41. Wang Y, Zhang H, Hou P, Su X, Zhao P, Zhao H, *et al*. Foliar-applied salicylic acid alleviates heat and high light stress induced photo inhibition in wheat (*Triticum aestivum*) during the grain filling stage by modulating the psbA gene transcription and antioxidant defense. Plant Growth Regulation. 2014;73(3):289–297. https://doi.org/10.1007/s10725-014-9889-9
42. Wardlaw I, Wrigley C. Heat Tolerance in Temperate Cereals: An Overview. Functional Plant Biology. 1994;21(6):695. https://doi.org/10.1071/pp9940695