



Effect of osmoprotectants on performance of wheat (*Triticum aestivum*) under terminal heat stress condition of North-West India

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ABSTRACT

Field experiment was conducted during *rabi* season of 2013-14 and 2014-15 at Ludhiana to evaluate the effect of various osmoprotectants on productivity of wheat (*Triticum aestivum* L.) under terminal heat stress condition. Results revealed that during 2013-14, foliar application of sodium nitroprusside 400 µg/ml, 2% KNO₃ and thiourea 20 mM recorded higher plant height, leaf area index and dry matter accumulation at harvest over the unsprayed and water sprayed control. Grain yield also increased to the extent of 18.8% by foliar application of sodium nitroprusside 400 µg/ml, 17.9% by 2% KNO₃ and 14.9% by thiourea 20 mM during 2013-14. Injury to wheat plant was observed due to higher doses of osmoprotectants. SNP 1 200 µg/ml and thiourea 60 mM decreased yield by 3.03% and 2.30% over the unsprayed control. During excessive wet season of 2014-15, SNP 800 µg/ml, 3% KNO₃ and thiourea 40 mM performed better as compared to lower doses.

Key words: Grain yield, Growth, Osmoprotectants, Potassium nitrate, Sodium nitroprusside, Thiourea, Wheat

Wheat (*Triticum aestivum* L.) accounts nearly 30% of global cereal production covering an area of 218.5 million ha with an average productivity of 3.26 tonnes/ha (FAO 2014). In India, wheat production was recorded as 95.91 million tonnes from an area of 30 million ha in 2013-14 (DES 2014). With the advent of terminal heat stress (sudden rise in temperature during grain filling period) adversely affecting wheat grown on 36 million ha area, comprising 40% of the temperate environment (Hays *et al.* 2007), there is sheer urgency to find the mitigation strategies. Studies suggested that optimal temperature for grain set and grain filling is between 19°C and 22°C in wheat (Porter and Gawith 1999). Lobell *et al.* (2005) reported that wheat yield in Mexico decreased by 10% for every 1°C increase in night time temperature and grain yield showed a strong negative correlation with increasing minimum temperature. The current trends in northern India also indicate that the 'cool period' for wheat crop is shrinking, while the threat of terminal heat stress is increasing (Joshi *et al.* 2007). These necessitates the mitigation of terminal heat stress in wheat by adopting various strategies like selection of appropriate variety, optimum planting time, and foliar spray of osmoprotectants. Many nitrogenous compounds, inorganic salts, natural and synthetic plant growth regulators and

stress signaling molecules were used based on their specific properties and roles to improve growth of grain, forage and horticultural crops (Farooq *et al.* 2009).

Foliar applications of sodium nitroprusside (Bavita *et al.* 2012), thiourea (Sahu and Singh 1995) and potassium nitrate (Bardhan *et al.* 2007) was reported to be effective for enhancing the productivity of wheat under adverse environmental conditions. Thiols are stress alleviating agents crucial for improving the metabolic imbalances produced in cell during stress. They are also known for maintaining the redox state of the cell and its proper functioning under heat stress (Nathawat *et al.* 2007). Application of thiourea 0.5 kg/ha or 500 ppm as foliar spray twice at tillering and flowering significantly increased the number of spikes, grains/spike, grain weight/spike, biological and grain yield and harvest index of wheat (Sahu and Singh 1995). As potassium is essential for enzyme activation, protein synthesis and photosynthesis, it may act as osmoregulator during stress for increased active uptake of K⁺ by the guard cells and stomatal regulation. Its application extensively improved the cellular as well as metabolic function of plants. It has proved to be very effective in improvement of relative water contents, photosynthesis, oxidant and antioxidants status, gas exchange characteristics and many other processes needed for osmotic stress mitigation in wheat seedlings (Tian and Lei 2006). It is involved in two respiratory electron transport pathways in mitochondria (Zottini *et al.* 2002) where it medicates the modulation of ROS and enhances

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antioxidants defense systems in plants exposed to high temperature stress. Therefore, the efficacy of these chemicals under changing climatic scenario assumes a paramount importance, and needs to be studied.

Foliar applied fertilizers (acting as osmoprotectants under environmental stress) often show a better efficacy which may help to reduce the required dose. These osmoprotectants need further evaluation regarding their potential and economic feasibility. Most of the works are conducted on foliar application of chemicals other than wheat crop. Therefore, this study was thought needful to work out a strategy involving the use of osmoprotectants to enhance the growth and productivity of wheat under irrigated conditions of western Indo Gangetic Plain zone that is prone to terminal heat stress.

MATERIALS AND METHODS

A two year experiment on wheat variety HD 2967 was conducted during 2013-15 at research farm of Punjab Agricultural University, Ludhiana, India, situated at 30°56 N latitude, 75°52 E longitude and 247 m above mean sea level. The climate of the area is characterized as sub-tropical and semi-arid with hot and dry spring summer from April to June, hot and humid summer from July to September and cold autumn winter from November to March. The mean annual rainfall is 705 mm, of which 80% is received during southwest monsoon from July to September and the rest is received through 'Western Disturbances' from December to February. Mean daily values (during different weeks) of meteorological parameters were recorded at the PAU meteorological observatory adjoining to the experimental site during the *rabi* of 2013-15 are presented in Fig.1 The total amount of rainfall received during the crop seasons from November to April was 177 and 221 mm during 2013-14 and 2014-15, respectively. The amount of rainfall received during February and March (reproductive phase) was 36.7 and 35.0 mm during 2013-14 and 38.6 and 84.6 mm in 2014-15. Mean monthly minimum air temperatures recorded in February were 8.2°C and 12.5°C during the year 2013-14 and 2014-15, respectively. The corresponding values for the month of March were 12.5°C and 13.3°C, respectively. The values for the 30 year long term average monthly minimum air temperatures and rainfall recorded were 7.2°C, and 29.9 mm during February and 11.3°C and 26.1 mm during March. These values clearly indicated that there was an increase of 1°C and 1.2°C in average monthly minimum air temperatures during February and March over 30 year long term average in 2013-14. The corresponding increase during these two months were 3.2 and 2°C in 2014-15. Hence, it is easily understood that due to this increase in monthly minimum air temperatures, the experimental crop faced the problem of terminal heat stress severely during both the years, but more prominently during 2014-15.

The experiment was laid in Completely Randomized Block design with 11 treatments and three replications. The treatments of osmoprotectants included thiourea @ 20, 40 and 60 mM, potassium nitrate @1.0, 2.0 and 2.0% and

sodium nitroprusside @ 400, 800 and 1 200 µg/ml. Two additional treatments of water spray and untreated control was also included in the study. Individual plot size was 6.0 × 3 m. Wheat variety HD 2967 was sown by first fortnight of November using a seed rate of 100 kg/ha and row space of 20 cm by seed-cum-fertilizer drill. The fertilizers were applied based on soil test value of experimental plots. The soil of experimental field was loamy sand in texture with pH of 7.8 and low in both available N (189.4 kg/ha), Walkley and Black organic carbon (0.16–0.30%) in 0–15 cm soil layer, medium in 0.5 mol/L NaHCO₃-extractable Olsen-P (9.7 mg/kg) and sufficient (81–83 mg/kg) in 1 mol/l NH₄OAC-extractable K. A half dose of N and full dose of P and K were drilled at sowing through urea, diammonium phosphate (DAP) and muriate of potash and the remaining half dose of N was top dressed in two equal splits at the first and second irrigations. Wheat variety HD-2967 was sown in rows, 23 cm apart, using a seed rate of 100 kg/ha on 8 November during both the years. Four irrigations (each of 7.5 cm, measured by Parshall flume installed in field) were applied uniformly at critical growth stages and weeds were controlled through spraying 2,4-Dichlorophenoxyacetic acid (2,4-D) at 0.50 kg ha applied 35 days after sowing (DAS). The foliar sprays of each osmoprotectants were made two times after anthesis at weekly interval using spray volume of 600 l/ha. In order to make sprays more effective, teepol was mixed at 0.50 ml/l with spray solution as a sticking agent.

For dry matter sampling in wheat, an area of 0.25 m² (0.5 m × 0.5 m) h was taken from the side rows at 40, 70 and 100 days after sowing (DAS) in both the years. Dry matter accumulation (DMA) was expressed in g/m². The sampled plants were dried in hot air oven at 65°C till constant weight. The leaves of wheat plants were detached and grouped into 5 grades based on their size. The area of 5 leaves grades was measured with leaf area meter (Model LI-COR-3 100). Then the leaf area index (LAI) was obtained dividing leaf area by ground area. Observations were recorded on yield performance of crops. Yield attributing parameters, i.e. ear bearing tillers/m² in wheat was recorded using 1/m² quadrat from three places in each plot at before harvesting of crop. Weight of 1 000- grains after harvesting was sampled randomly from the harvested bulk grains of every plot, and 1000 grains were counted through a seed counter, and their weight was expressed in g.

The harvesting of wheat was done manually on 18 April, 2014 and 21 April, 2015 from the net plot area. The harvested crop was tied in labeled bundles and kept for sun drying. The harvested crop was tied in labeled bundles and kept for sun drying and then threshing was carried out. Plants were harvested at maturity from the net plot area (6 × 2.6m), leaving two rows at each side and 0.5 m towards the row from the gross area. The grains were threshed from these spikes and weight of grains/spike was noted. A representative sample of grains was taken from the produce of each net plot and thousand grain weight was counted. Grain and straw yield were recorded from the net plot area. After sun drying for about 5 days, it was threshed manually

to separate out seeds from the straw, cleaned and weighed. A sub-sample of straw and grain was collected and oven dried at 60 °C for 48 hr to determine moisture content. Grain yields of wheat was reported at 12% moisture content. Seed and straw yields were computed on oven-dry weight basis. The harvest index was computed by dividing the grain yield by biological yield (grain + straw yield).

The data were analyzed as for the RCBD, using the analysis of variance (ANOVA) by windows based SPSS programme (ver. 16.0, SPSS Inc. 1996) to determine the statistical significance of treatment effects. Wherever, F values were found to be significant at a 5% level of probability, least significant difference (LSD) values were computed for making comparisons among the treatment means.

RESULTS AND DISCUSSION

Growth parameters

Plant height at harvest was recorded higher by foliar application of SNP 400 µg/ml followed by 2% KNO₃, 1% KNO₃ and thiourea 20 mM as compared to water spray and unsprayed control. During 2014-15, moderate doses of SNP 800 µg/ml and thiourea 40 mM and 3% KNO₃ were more effective due to washing off and reduced activity of these osmoprotectants due to excessive rainfall during reproductive growth. The increase in DMA was to the extent of 16.2, 15.9 and 12.9% over untreated control, respectively, by foliar spray of these osmoprotectants. The data recorded (Table 1) on dry matter production during 2014-15 revealed that there was non-significant difference between treatments with control, but among different foliar spray treatments, SNP 800 µg/ml exhibited maximum dry matter followed by 3% KNO₃ and thiourea 40 mM over water sprayed and unsprayed control. During 2013-14, foliar application of 2% KNO₃ followed by SNP 400 µg/ml and thiourea 20 mM had resulted in significantly higher LAI, compared to water spray and control (Table 1). The increase over untreated control was 24.8, 17.6 and 16.8%, respectively. The beneficial effect of SNP might be because NO induced the

improvement in photosynthesis by increasing chlorophyll content, counteracted oxidative damage by decreasing the generation of ROS and increased the uptake of nutrient elements. Similar results are corroborated with the findings of Besma *et al.* (2011)

Increase in growth may be attributed to the beneficial effect of these osmoregulators on photosynthesis, increase in sink strength and later on, assimilate translocation to grains. Similar increase in dry matter was also observed by Sahu and Singh (1995). With respect to LAI, higher LAI was recorded in wheat plants treated with various osmoprotectants, probably due to their positive effect on chlorophyll enhancement. All these chemicals have either N or S that might have enhanced LAI of wheat due to the role of nitrogen in increasing division and elongation of cells as well as activation of metabolic and photosynthesis process.

Yield attributes of wheat

Application of different osmoprotectants resulted in non-significant differences between the mean spike density of all the treatments over water sprayed and untreated control (Table 2). However, maximum number of spike/m² were recorded with 2% KNO₃ followed by SNP 400 µg/ml and thiourea 20 mM, compared to untreated control to the extent of 11.87, 10.9 and 9.4%, respectively. This might be due to reduced flower and immature grain drop, prevention of formation of abscission layer which resulted in formation of more spike and their retention on plants. Similar findings were also reported by Bundy and Andraski (2004). The reason could be that potassium at optimum level enhanced production of spikes by maintaining cell turgor, reducing water loss and providing favorable environment to the plants.

Foliar application of 400 µg/ml SNP and 2% KNO₃ produced significantly higher number of grains/spike than untreated control. SNP can protect cell membranes and maintain their structure, and function against the toxic and destructive effects of reactive oxygen species during the stress. This, in turn, can lead to more absorption and translocation of minerals from the soil to the plants and, thereby, formation of more grains/spike. Improvement of

Table 1 Effect of foliar spray of osmoprotectants on growth parameters of wheat

Treatment	Plant height (cm)		Leaf area index		Dry matter accumulation(g/m ²)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
No spray	81.86	82.53	2.50	2.44	1058.39	1052.16
Water spray	82.23	82.93	2.56	2.61	1066.47	1072.47
Thiourea 20 mM	85.66	84.88	2.92	2.92	1194.82	1181.60
Thiourea 40 mM	82.86	87.49	2.83	2.84	1149.29	1206.81
Thiourea 60 mM	78.98	82.43	2.56	2.54	1057.39	1081.54
KNO ₃ 1%	86.62	85.22	2.94	2.86	1183.96	1148.48
KNO ₃ 2%	87.65	86.98	3.01	3.04	1226.73	1213.41
KNO ₃ 3%	84.95	88.71	2.75	2.77	1120.07	1223.89
SNP 400 µg/ml	87.96	85.48	3.12	3.13	1230.14	1167.63
SNP 800 µg/ml	83.82	89.24	2.97	3.03	1180.19	1215.49
SNP 1 200 µg/ml	80.93	83.46	2.68	2.70	1083.36	1081.80
LSD (P=0.05)	NS	NS	0.36	NS	114.89	NS

Table 2 Effect of foliar spray of osmoprotectants on yield attributes of wheat

Treatment	No of spikes/m ²		Spike length (cm)		Spikelet/spike		Grains/spike		Grain weight/spike		1 000 grain weight	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
No spray	287.51	283.03	10.05	11.06	18.08	16.27	51.92	46.29	2.03	1.71	38.76	38.10
Water spray	292.41	285.42	10.10	11.21	18.39	16.72	52.85	47.36	2.08	1.75	39.18	38.48
Thiourea 20 mM	317.33	306.90	11.30	11.59	19.95	17.98	61.54	54.32	2.59	2.10	42.17	39.09
Thiourea 40 mM	305.24	311.48	10.77	11.76	19.19	18.57	57.74	56.62	2.34	2.32	40.60	40.88
Thiourea 60 mM	278.41	310.31	10.11	11.72	17.51	17.62	50.30	56.17	1.84	2.12	36.63	37.77
KNO ₃ 1%	316.83	307.25	11.44	11.60	19.92	18.06	60.16	52.41	2.58	2.05	42.98	39.17
KNO ₃ 2%	325.80	314.35	11.92	11.87	20.49	18.66	62.99	56.38	2.83	2.29	43.80	40.59
KNO ₃ 3%	297.47	320.24	10.61	12.34	18.70	19.03	53.06	57.84	2.11	2.35	39.52	40.66
SNP 400 µg/ml	325.27	293.69	11.69	11.09	20.45	18.04	64.61	55.77	2.89	2.23	44.33	39.93
SNP 800 µg/ml	300.40	328.03	10.65	11.47	20.08	19.31	62.10	58.62	2.63	2.42	42.27	41.62
SNP 1 200 µg/ml	290.14	288.84	10.35	10.91	18.24	17.75	51.12	46.07	1.90	1.68	37.20	37.80
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	8.73	7.19	0.53	0.24	NS	NS

chlorophyll content, enzyme activity, maintenance of water balance and sufficient build up of food reserves for formation of more grains might be reason for increase in grains/spike with foliar application of SNP. Also, availability of K from KNO₃ protects the cells from oxidative damage by the increased activity of antioxidants and turgor maintenance in guard cells, which might have resulted in higher grains/spike in our study. Application of thiourea 20 mM also led to 17.98 % increase in number of grains spike than untreated control (Table 1). The increased mitotic activity with foliar application of thiourea in wheat might have resulted in formation of more number of grains/ spike. Anjum *et al.* (2011) also reported that enhanced number of grains and test weight with foliar spray of thiourea in wheat emerged as a driving factors in improving grain yield/plant.

Productivity

A careful study of the meteorological data reveals that the weather parameters played a crucial role in influencing growth, yield attributes and yield of wheat. The weather data for two years revealed distinct variation in minimum and maximum temperature, rainfall and sunshine hours in two cropping seasons. Wheat requires cool weather from seedling stage to flowering stage and warm and sunny days during flowering to maturity, which was a reason for higher yield in 2013-14. Low yield in 2014-15 could be due to high humidity, rains during February and March accompanied with low sunshine hours at the time of flowering (February and March) which resulted in poor seed set. During first year the total rainfall received during this *rabi* season was 85 mm and during second year the rainfall was 150 mm. Foliar spray of KNO₃, SNP and thiourea salts exerted conspicuous effects on grain yield of wheat. During 2013-14, foliar spray of SNP at 400 µg/ml recorded significantly higher grain yield (5.49 tonnes/ha) than water sprayed (4.73 tonnes/ha) unsprayed control (4.62 tonnes/ha) and the increase was 18.8% higher than untreated control. The increased grain yield with foliar application of SNP 400 µg/ml was certainly related to higher number of spike/m², grains/spike, and grain weight. It might have lead to higher water retention and less transpiration rate, accelerated protein synthesis, enhanced photosynthesis rate that maintained higher relative water content under terminal heat stress. SNP may also act as a chain breaker for scavenging ROS produced during a constant rise of minimum temperature which occurred during both 2013-14 and 2014-15 in the month of February and March (reproductive stage). Increasing the concentration of SNP to 1 200 µg/ml reduced the grain yield likely due to detrimental effect on the photosynthetic activity of plant. During same year, 2% KNO₃ recorded significantly and appreciably higher grain yield (5.45 tonnes/ha) than water spray (4.73 tonnes/ha) and unsprayed controls (4.62 tonnes/ha) and the increase was 17.9% over untreated control. KNO₃ spray might have resulted in securing higher photosynthetic activity in effective leaves and supplied developing grains with current photosynthates for proper filling, resulting in higher yield.

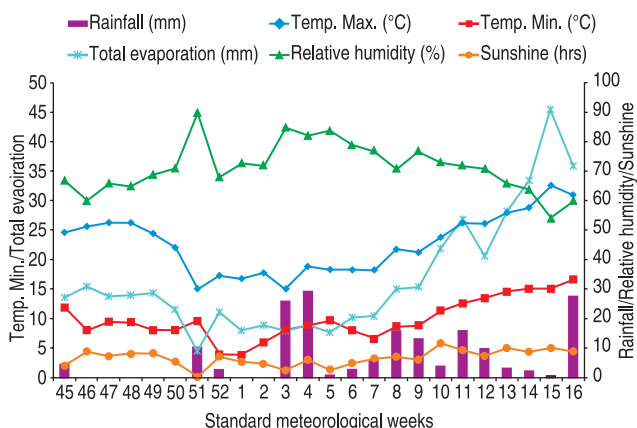


Fig 1 Weekly mean meteorological data recorded during the crop season (2013-14) at Meteorological Observatory, PAU Ludhiana

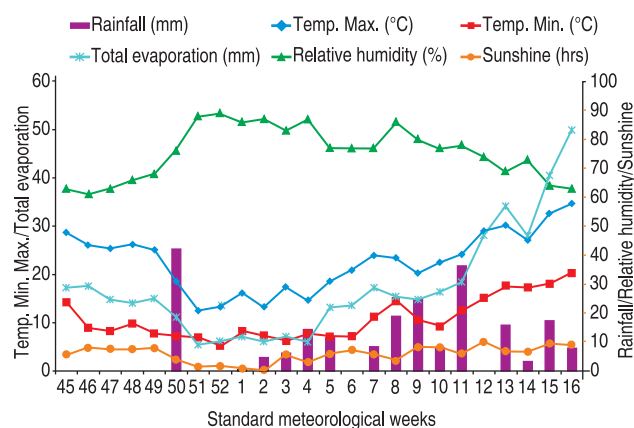


Fig 2 Weekly mean meteorological data recorded during the crop season (2014-15) at Meteorological Observatory, PAU Ludhiana

Besides the beneficial functions of nitrate nitrogen, the prevalence of K^+ in KNO_3 , might have improved grain filling and phytomass production, due to increasing photosynthetic activity and effective translocation of assimilates to reproductive parts resulting in higher yield. Earlier, Tiwari (2002) also suggested that the increase in grain yield might be due to the collective role of N and K as N improves vegetative growth of wheat and K increases root growth and facilitates the translocation of nutrients and photo assimilates.

During 2013-14, thiourea 20 mM also resulted in higher grain yield (5.16 tonnes/ha) than the water sprayed and untreated control and the increase was 14.9% over untreated control. Thiourea application had a role in enhancing canopy photosynthesis and metabolic transport of photosynthetic assimilates to grains via an effect on phloem loading (Sahu and Singh 1995). The reason for this increase might be that it can provide both sulphur and nitrogen to plants and almost 80% of the N applied was recovered in grain at the final harvest. Yadav (2005) has reported that thiourea significantly improve yield of wheat.

However, SNP 1 200 $\mu\text{g}/\text{ml}$ and 60 mM thiourea acted as growth retardant, being statistically at par with the untreated control. During 2014-15 foliar spray of SNP 800 $\mu\text{g}/\text{ml}$ (5.34 tonnes/ha) recorded the highest grain yield. 3% KNO_3 was the next best treatment but the differences with control were non-significant. However, SNP 1 200 $\mu\text{g}/\text{ml}$ and 60 mM thiourea were found toxic to the plant and acted as growth retardant, decreasing the yield. Earlier, all the physiological and growth parameters also had similar trend.

Table 3 reveals that all the chemicals recorded higher biological yield except in case of thiourea 60 mM and SNP 1 200 $\mu\text{g}/\text{ml}$. The reason could be an increase in number of spikes bearing tillers with foliar applied N and K, which results in accumulation of higher dry matter which enhance biological yield of wheat. Results also reveals that foliar spray of osmoprotectants recorded no effect on straw yield during both the years. This was because spray was not done during active vegetative growth phase of wheat and plant height increases at a very slow rate after anthesis. Foliar sprays of all the osmoprotectants did not show any significant effect on harvest index. These data suggested that the increase

Table 3 Effect of foliar spray of osmoprotectants on wheat yield

Treatment	Biological yield (t/ha)		Grain yield (t/ha)		Straw yield (t/ha)		Harvest index (%)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
No spray	10.83	10.75	4.62	4.50	6.20	6.25	42.73	41.86
Water spray	10.91	10.96	4.73	4.57	6.18	6.39	43.36	41.69
Thiourea 20 mM	12.22	12.08	5.31	5.01	6.91	7.07	43.51	44.37
Thiourea 40 mM	11.76	12.33	4.93	5.18	6.82	7.15	41.93	42.11
Thiourea 60 mM	10.82	11.05	4.51	4.61	6.30	6.44	41.72	41.63
KNO_3 1%	12.11	11.74	5.10	4.82	7.01	6.92	42.12	42.16
KNO_3 2%	12.55	12.40	5.45	5.21	7.10	7.19	43.38	43.83
KNO_3 3%	11.46	12.51	4.81	5.29	6.64	7.22	42.02	42.02
SNP 400 $\mu\text{g}/\text{ml}$	12.58	11.93	5.49	5.19	7.09	6.74	43.58	43.95
SNP 800 $\mu\text{g}/\text{ml}$	12.07	12.42	5.21	5.34	6.86	7.08	43.11	43.48
SNP 1 200 $\mu\text{g}/\text{ml}$	11.08	11.06	4.48	4.45	6.60	6.61	40.44	40.32
LSD (P=0.05)	1.17	NS	0.52	NS	NS	NS	NS	NS

in grain yield of wheat due to the application of osmoprotectants was accompanied by a similar increase in straw yield.

The experimental findings suggest that foliar spray applications of either SNP 400 µg/ml or 2% KNO₃ after anthesis were found most suitable for achieving better growth and increased productivity of wheat. Thiourea @ 20 mM was found to be the next best feasible option. Further investigations are required to evaluate the role of suitable concentrations of these osmoprotectants in mitigating terminal heat stress in wheat.

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