

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/338909403>

Foliar Spray of Synthetic Osmolytes Alleviates Terminal Heat Stress in Late-Sown Wheat

Article in *International Journal of Plant Production* · January 2020

DOI: 10.1007/s42106-020-00087-4

CITATIONS

3

READS

119

8 authors, including:



Arnab Roy Chowdhury

Bihar Agriculture University

18 PUBLICATIONS 26 CITATIONS

[SEE PROFILE](#)



Mainak Ghosh

Bihar Agriculture University

49 PUBLICATIONS 326 CITATIONS

[SEE PROFILE](#)



Awadhesh Kumar Pal

Bihar Agriculture University

46 PUBLICATIONS 506 CITATIONS

[SEE PROFILE](#)



K. K. Hazra

ICAR-Indian Institute of Pulses Research

139 PUBLICATIONS 1,578 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Weed Control works [View project](#)



Vth International symposium on Lychee, Longan & Other Sapindaceae Fruits [View project](#)

*Foliar Spray of Synthetic Osmolytes
Alleviates Terminal Heat Stress in Late-
Sown Wheat*

**A. Roy Chowdhury, M. Ghosh, M. Lal,
A. Pal, K. K. Hazra, S. Acharya,
A. Chaurasiya & S. K. Pathak**

**International Journal of Plant
Production**

ISSN 1735-8043

Int. J. Plant Prod.

DOI 10.1007/s42106-020-00087-4



Your article is protected by copyright and all rights are held exclusively by Springer Nature Switzerland AG. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".



Foliar Spray of Synthetic Osmolytes Alleviates Terminal Heat Stress in Late-Sown Wheat

A. Roy Chowdhury¹ · M. Ghosh¹ · M. Lal² · A. Pal³ · K. K. Hazra⁴ · S. Acharya¹ · A. Chaurasiya¹ · S. K. Pathak¹Received: 28 April 2019 / Revised: 5 January 2020 / Accepted: 16 January 2020
© Springer Nature Switzerland AG 2020

Abstract

Wheat sowing often gets delayed and the crop faces terminal heat stress, which is severely impacting the crop productivity. Synthetic osmolytes maintain structural integrity of cell membrane and protect oxidative damage of cells under stress conditions. Hence, foliar application of these compounds is likely to alleviate the negative effects of high temperature on crop plants. However, field response of these chemicals on late-sown wheat in the subtropical environment (eastern Indo–Gangetic Plain) is still uncertain. A field experiment was conducted for three years (2013–2016) to envisage the field efficacy of two synthetic compounds *viz.*, potassium nitrate (KNO₃) (1.0 and 0.5%) and glycine betaine (GB) (100 and 50 mM), each sprayed either at respective higher dose at booting or anthesis, or at half the dose at both booting and anthesis for improving grain yield of two contrasting wheat cultivars (DBW14 and K307) facing terminal heat stress. Foliar application of 0.5% KNO₃ at both booting and anthesis stages resulted in the maximum increase (13–16%) in grain yield over no-spray treatment closely followed by 1% KNO₃ at anthesis (10–14%), and 50 mM GB at both booting and anthesis stages (6–9%). However, foliar spray of KNO₃ and GB at only booting stage had a non-significant effect on grain yield. The increase in grain yield with the osmolytes application was attributed to an increase in the number of grains ear⁻¹ (9–16%) and thousand-grain weight (3–10%), demonstrating an increased sensitivity of sink capacity and grain development of wheat to terminal heat stress. The degradation of flag leaf chlorophyll content (34–36%) and increase in the relative membrane permeability (29–52%) during anthesis to grain filling period was observed as the critical yield-limiting factors in late-sown wheat. Foliar spray of osmolytes had a regulatory effect on these physiological anomalies being highest with the treatment 0.5% KNO₃ foliar spray at both booting and anthesis stages followed by 1% KNO₃ foliar spray at anthesis. At the grain filling stage, significant relationships between grain yield and flag leaf chlorophyll ($r = 0.43–0.68$) and relative membrane permeability ($r = -0.47$ to -0.53) were observed. Thus, foliar application of 0.5% KNO₃ at both booting and anthesis stages could be recommended to alleviate the adverse effect of terminal heat stress and improve productivity of late-sown wheat in subtropical environments.

Keywords Potassium nitrate · Glycine betaine · Terminal heat stress · Relative membrane permeability · Chlorophyll degradation · Heat use efficiency

✉ A. Roy Chowdhury
arnabuas@gmail.com

✉ K. K. Hazra
kalikrishna123@gmail.com

¹ Department of Agronomy, Bihar Agricultural University, Bhagalpur, Sabour, Bihar 813210, India

² Department of Soil Science and Agricultural Chemistry, Bihar Agricultural University, Bhagalpur, Sabour, Bihar 813210, India

³ Department of Biochemistry and Crop Physiology, Bihar Agricultural University, Bhagalpur, Sabour, Bihar 813210, India

⁴ Crop Production Division, ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208024, India

Introduction

In the rice-wheat cropping system of eastern Indo–Gangetic Plain (IGP), wheat sowing is often delayed, mostly during the third week of December or later, due to late harvesting of long duration rice (Bhatt et al. 2016). Late-sown wheat crop faces terminal heat stress during the anthesis and grain-filling period (Rane et al. 2007; Dwivedi et al. 2017). Mehla et al. (2000) reported a reduction of wheat yield of about 18% when sowing is delayed from mid-November to end of December. This is primarily attributed to an abrupt rise in air temperature beyond 20–22 °C which coincides with anthesis and grain filling period of wheat (Tewolde et al.

2006). In the eastern IGP region, the daily mean temperature progressively increases from 19.5 °C during last week of February to 27 °C during 3rd week of March, which overlaps with anthesis and grain filling period of wheat, while optimal temperature during this stage should be around 21 °C (Dwivedi et al. 2019). Therefore, the wheat crop in this region faces a supra-optimal temperature of ~6 to 8 °C during anthesis and grain filling period and wheat yield is decreased by 4% for every 1 °C rise in temperature above the optimum temperature of about 20 °C during anthesis to grain filling stage (Dwivedi et al. 2015). Reduction in yield of late-sown wheat, ranging from 21 to 30%, has been reported by different workers in the IGP region (Verma et al. 2000; Pandey et al. 2010; Hazra et al. 2018). This situation urges to find out the suitable strategy that could alleviate the terminal heat stress in late-sown wheat, traditionally grown in eastern IGP.

Previous reports on synthetic osmolytes like potassium nitrate (KNO₃) (Suryavanshi and Buttar 2016), and glycine betaine (GB) (Ashraf and Foolad 2007) unveil their beneficial effects in protecting the crop from high-temperature stress. The principal role involved is to protect the photosynthetic apparatus of leaves under stressful conditions. High-temperature stress adversely affects the membrane integrity. Potassium (K⁺) is known to have an important role in imparting stability and polarization of membrane, activating enzymes, and stomatal movement. Stress-induced accumulation of reactive oxygen species (ROS) induces K⁺ leakage by activating K⁺ efflux channels (Cuin and Shabala 2007; Fayez and Bazaid 2014). Hence, exogenous application of potassium salts may protect cells from oxidative damage by balancing K⁺ in the plant system, and thus may be effective to protect the crop against terminal heat stress. Organic osmolytes maintain osmoregulation and protect the structure of different biomolecules and membranes and scavenge free-radical (Ashraf and Foolad 2007); and thus likely to improve plant functions under stress emanating from abiotic factors. However, most of the studies on these synthetic compounds were conducted under controlled environments. Field efficacy of these compounds has not been tested (Wakchaure et al. 2016) and hence requires field level validation. Moreover, there is a need to standardize the dose of these compounds and time of application to get optimum results.

Therefore, an experiment was conducted to test the efficacy of KNO₃ and GB in improving the grain yield of late-sown wheat crop facing terminal heat stress under field condition of eastern India. The hypotheses of the study were (i) foliar application of osmolyte alleviates the adverse effect of terminal heat stress and improves crop productivity at field condition; (ii) split application of these chemicals on late-sown wheat increases the efficacy at field level over the single high dose application; and (iii) foliar spray of synthetic osmolyte prevents degradation of leaf chlorophyll and

restores membrane integrity in late-sown wheat. Thus, the strategy, if found to be effective, can be easily adopted by the farmers who mostly grow late-sown wheat under rice-wheat cropping system, to ward off the harmful effects of terminal heat stress and enhance the productivity of wheat.

Materials and Methods

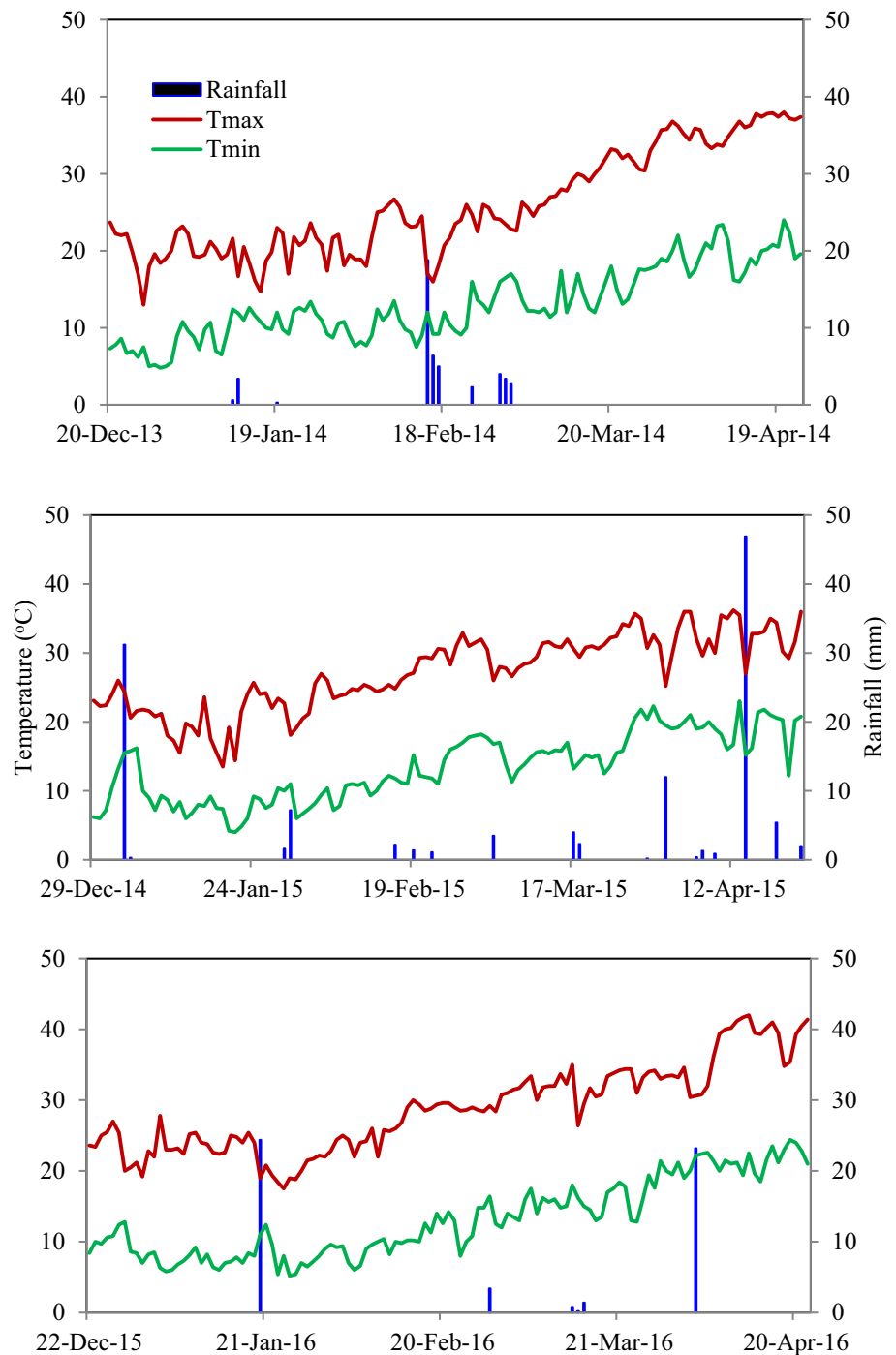
Site Characteristics

Three-year field experiment (2013–2014 to 2015–2016) was conducted at the research farm of Bihar Agricultural University (eastern IGP), Sabour, Bihar, India (25° 50' N and 87° 19' E). The soil was deep and well-drained, silty-loam in texture, mixed *hyperthermic* and belongs to Fluvisol (World Reference Base soil classification), pH 6.7, electrical conductivity 0.26 ds m⁻¹ and soil organic carbon 4.2 g kg⁻¹. The soil available nitrogen (N), phosphorus (P) and potassium (K) in the surface soil (0–15 cm) were 53.9, 7.8 and 51.2 mg kg⁻¹, respectively. The climate of the region is sub-humid subtropical. Weather conditions during the crop season are presented in Fig. 1.

Treatment Description and Experimental Design

The experiment was laid out in a split-plot design with three replications. The dimension of each sub-plot was 2.5 m × 4.0 m. The wheat crop was sown on 20th, 29th, and 22nd December during 2013–2014, 2014–2015 and 2015–2016, respectively, which are considered as late-sown wheat in eastern IGP region (Singh et al. 2015). In this study, two wheat cultivars, *viz.*, DBW 14 and K 307 were taken as main-plot treatment and foliar application of synthetic compounds *viz.*, potassium nitrate (KNO₃) and glycine betaine (GB) were randomized in the subplots. Treatments comprised of foliar spray of 1% KNO₃ at booting, 1% KNO₃ at anthesis, 0.5% KNO₃ at both booting and anthesis, 100 mM GB at booting, 100 mM GB at anthesis, 50 mM GB at both booting and anthesis, along with no-spray or spray control treatment and water spray at both booting and anthesis stages. The treatment abbreviation along with the definition of treatments is given in Table 1. DBW 14 is an early maturing (110–115 days); whereas, K 307 is a late maturing (125–130 days) cultivar. DBW 14 is a bold seeded, semi-erect wheat cultivar having drooping flag leaf and adaptable to irrigated late-sown condition; whereas cultivar K 307 is an erect and bold seeded cultivar mainly grown under timely-sown irrigated condition. The fertilizer grade KNO₃ (13-0-45) and technical grade GB (C₅H₁₁NO₂, molecular weight 117.15 g mol⁻¹) were used in this study. The foliar spray was performed using the manually-operated knapsack sprayer fitted with a flat-fan nozzle and the spray was

Fig. 1 The daily mean minimum and maximum temperatures ($^{\circ}\text{C}$) and rainfall (mm) during the crop growing seasons (2013–2014 to 2015–2016)



directed towards flag leaf of the crop during the windless condition at evening (16:00 to 18:00 h). The total amount of water used for spray was 200 l ha^{-1} .

Crop Management

Pre-sowing irrigation ($\sim 4 \text{ ha-cm}$) was applied for land preparation. At optimum soil moisture condition, the field was prepared by four passes of cultivator followed by planking.

The wheat crop was sown manually by hand-plough with a row-spacing of 22.5 cm using a seed rate of 125 kg ha^{-1} . The recommended fertilizers rate (120 kg N , $40 \text{ kg P}_2\text{O}_5$ and $20 \text{ kg K}_2\text{O ha}^{-1}$) were applied to the crop. The full dose of fertilizer P and K was applied basal. Half of the fertilizer N was applied basal and remaining half dose was top-dressed in two equal splits at first irrigation [21 days after sowing (DAS) and other at 60 DAS]. The crop was irrigated twice during crown root initiation (21 DAS) and late-jointing (50

Table 1 Treatment description and abbreviation used for different treatments

Treatment	Abbreviation
No spray	NS _{CT}
1.0% potassium nitrate (KNO ₃) foliar spray at booting stage	PN _B
1% KNO ₃ foliar spray at anthesis stage	PN _A
0.5% KNO ₃ foliar spray at booting and anthesis stages	PN _{B+A}
100 mM glycine betaine foliar spray at booting stage	GB _B
100 mM glycine betaine foliar spray at anthesis stage	GB _A
50 mM glycine betaine foliar spray at booting and anthesis stages	GB _{B+A}
Water spray at booting and anthesis stages	WS _{B+A}

DAS) stages, and the volume of irrigation was ~4 ha-cm. Two hand weeding was done to keep the plots weed-free. The crop was harvested during 8th in 2013–2014, 17th in 2014–2015, and 9th April in 2015–2016.

Measurement of Crop Growth and Yield Attributes

At physiological maturity stage, six inner rows (leaving 0.5 m border at each end) from each plot were harvested separately for grain yield estimation. The total number of ears in these six rows were counted and expressed as the number of ears m⁻². A random sample of twenty ears were collected at the same stage to count the total number of kernels and expressed as the number of grains ear⁻¹. Meanwhile, thousand-grain weight (test weight) was measured by taking a sample of two hundred kernels, oven-dried, and weighed. A 100 g sample from the harvested grain was oven-dried at 65 °C for 48 h and grain moisture content (%) at harvest was estimated; finally, the grain yield of each plot was adjusted to 14% moisture content and expressed as t ha⁻¹.

Measurement of Physiological Parameters

Given the notable phenological differences in the two selected cultivars, DBW 14 and K 307, two growth stages ('anthesis' and 'grain filling') were designated for measurement of physiological parameters. According to Zadoks et al. (1974), in wheat crop, the growth stage 'anthesis' appears between Zadoks stages 55 and 70; and 'grain filling' between Zadoks stage 75 and 85. In both these growth stages, flag leaves were collected from each plot at noon time for analysis of leaf chlorophyll and relative membrane permeability. Flag leaf chlorophyll content and relative membrane permeability in each of these stages were estimated using the standard procedures described by Arnon, and Yang et al., respectively (Arnon 1949; Yang et al. 1996).

Chlorophyll Content

The collected flag leaves were kept at 5 °C on moist filter paper, wrapped in aluminium foil and taken to the laboratory in an icebox. Then, the leaves were cut into a disc shape (11 mm diameter); three of these disc shape leaf part were oven-dried (65 °C for 48 h) for estimation of dry weight. Other leaf discs were crushed for extraction of chlorophyll using a mortar with 80% acetone-water solution (v/v) and a pinch of calcium carbonate. The supernatant was made up to 5 ml with distilled water and absorbency was measured at 663 and 645 nm in a UV-VIS spectrophotometer (model Shimadzu, Japan). Total chlorophyll in leaf was estimated using Harborne's equations (Harborn 1973).

$$\text{Total chlorophyll (mg kg}^{-1}\text{)} = 20.2 A_{645} + 8.02 A_{663} \quad (1)$$

Relative Membrane Permeability

From the middle section of flag leaves ten pieces of round-shaped (4 cm diameter) leaf sections were cut and then placed in test tubes containing 10 ml of de-ionized water. Then the samples were shaken vigorously for 10 s; and the initial electrical conductivity (EC₀) of each sample was measured using an electrical conductivity meter (Company, Make Place). The samples were left for 24 h at room temperature, and electrical conductivity was measured again (EC₁). Then, the samples were autoclaved and the final electrical conductivity (EC₂) was estimated after cooling to 25 °C. The EC₀ of the de-ionized water (blank) was subtracted from both EC₁ and EC₂. Heat injury of leaf tissues was measured in terms of relative membrane permeability was calculated using the formula of Yang et al. as follows (Yang et al. 1996):

$$\text{Relative membrane permeability (\%)} = \frac{(EC_1 - EC_0)}{(EC_2 - EC_0)} \times 100 \quad (2)$$

Calculation of Heat Use Efficiency (HUE)

Firstly, the growing degree-days (GDD) of each treatment was calculated by adding the cumulative of mean ambient temperatures from the date of sowing to harvesting of crop taking the base-temperature of 0 °C. Heat-use efficiency (HUE) was computed for each treatment using the following formula (Sastry et al. 1985):

$$\text{HUE (kg ha}^{-1}\text{ degree centegrade days}^{-1}\text{)} = \frac{\text{Seed or total dry matter yield kg ha}^{-1}}{\text{Growing degree days (degree centegrade days)}} \quad (3)$$

Statistical Analysis

Statistical analysis of the data was performed applying analysis of variance (ANOVA) technique of split-plot design (Cochran and Cox 1963) using online statistical program OPSTAT. The significance of different sources of variations was tested by Fisher Snedecor's 'F' test at $\alpha=0.05$. Standard error of mean and least significant difference (LSD) at 5% level of significance were worked out for each parameter to compare the difference between the treatment means. The principal component analysis (PCA) was performed using the window-based software PAST (version 3.14).

Results

Weather Condition

Anthesis stage of crop appeared during the last week of February to second week of March, and crop reached its physiological maturity during last week of March to second week of April, depending upon the time of sowing in the respective year and cultivars (Fig. 1). The crop had faced 16, 27 and 29 high-temperature days [i.e. maximum temperature (T_{max}) > 30 °C] in the first, second, and third year, respectively. The average maximum temperature during the last week of February to end of March (anthesis to grain filling) was 28.7, 30.8, and 31.7 °C during the year 2013–2014, 2014–2015 and 2015–2016, respectively. In all 3 years, very less amount of rainfall was received during anthesis to grain filling period.

Flag Leaf Relative Membrane Permeability

Figure 2 shows that increase in the relative membrane permeability from anthesis to grain filling period was prominent (24–41%, $p < 0.01$), being higher in the year 2014–2015 (41%, $p < 0.01$). Foliar spray of 1% KNO_3 at booting maintained relative membrane permeability at anthesis. The effect of spray treatment on relative membrane permeability was prominent at the grain filling stage. All the KNO_3 treatments (PN_B , PN_A , PN_{B+A}) were found to be statistically at par with others but significantly lower than that of no-spray and water spray treatments. The effect GB foliar spray was marginal on membrane permeability. The increase in relative membrane permeability during anthesis to grain filling period was lowest in PN_A (Fig. 2). Pooled ANOVA results revealed that the effect of cultivar, osmolyte treatment and experimental year were significant ($p < 0.05$) for relative membrane permeability at both anthesis and grain filling stages but none of the interaction effect was found significant (Table 2).

Flag Leaf Chlorophyll Content

In both the cultivars, flag leaf chlorophyll content was reduced during the anthesis to grain filling stage (Fig. 3). Chlorophyll content was comparatively higher in cultivar DBW 14 over K

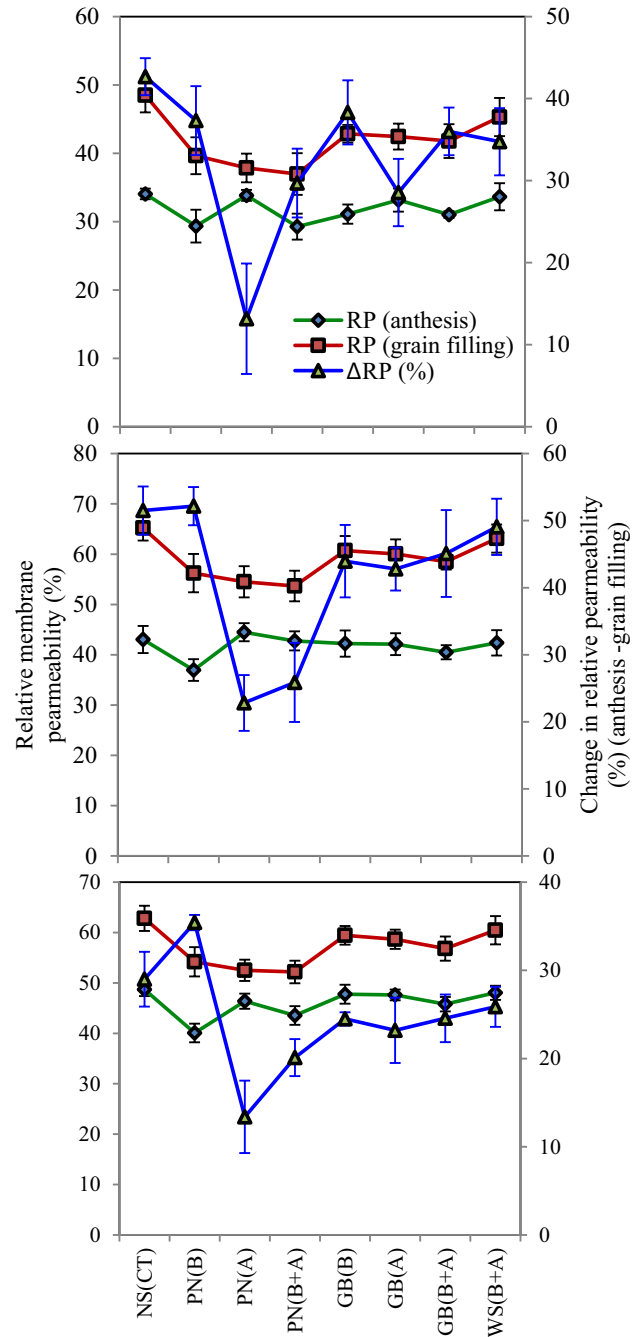


Fig. 2 Flag leaf relative membrane permeability (RP) at anthesis and grain filling stages and changes in flag leaf relative membrane permeability during anthesis to grain filling stages (ΔRP) of late-sown wheat as influenced by different osmolyte treatments (2013–2014 to 2015–2016). Error bars represent the standard error on mean. See Table 1 for definitions of the abbreviations

307. Foliar spray of 1.0% KNO₃ at booting (PN_B) resulted in higher chlorophyll content at anthesis; whereas at grain filling stage, maximum leaf chlorophyll was recorded in PN_{B+A} treatment, and the result was consistent across the years. The degradation of chlorophyll from anthesis to grain filling was highest in NS_{CT} (34–37%), whereas all KNO₃ treatments reduced chlorophyll degradation, being lowest in PN_{B+A} (21–27%). The degradation of leaf chlorophyll was also reduced with the application of GB; however, its efficacy was relatively less as compared to KNO₃. Table 2 shows that interaction effect of cultivar × chemical spray was significant ($p < 0.05$) for chlorophyll content at grain filling and degradation of chlorophyll.

Yield Attributes and Yield

The significant treatment effect was observed for yield attributes i.e. effective tiller, grains ear⁻¹ and thousand-grain weight (Table 3). The cultivar difference for effective tiller and grains ear⁻¹ was also prominent. The increase in effective tillers with osmolytes spray was maximum up to 9.3%, 8.7%, and 6.5% ($p < 0.05$) in 2013–2014, 2014–2015 and 2015–2016, respectively; and significant interaction effect cultivar × chemical spray was also noticed ($p < 0.05$) (Table 2). Increase in grains ear⁻¹ was observed in KNO₃ treated crop. The positive effect of GB (50 mM) applied at both booting and anthesis (GB_{B+A}) on grains ear⁻¹ was also apparent in first and second years ($p < 0.05$). The treatment PN_{B+A} resulted in 14–19% ($p < 0.05$) increase in grains ear⁻¹ and thousand-grain weight (7–14%) over NS_{CT}. The pooled ANOVA data also confirmed a consistent effect of osmolyte spray on yield attributes of late-sown wheat.

The crop response to osmolytes spray was prominent on crop productivity. The higher grain yield was observed with foliar spray of KNO₃ and GB; however, the effect of KNO₃ was prominent and higher over GB. The maximum yield increment (over NS_{CT}) was registered in PN_{B+A} (13–16%, $p < 0.05$) closely followed by PN_A (10–14%, $p < 0.05$) and GB_{B+A} (6–9%, $p < 0.05$) (Table 4). Figure 4 illustrates that with response to chemical spray treatments, the grain yield is directly associated with the yield attributes (except effective tiller), flag leaf chlorophyll content and relative membrane permeability at grain filling stage.

Heat Use Efficiency (HUE) and Principal Component Analysis

Cultivar DBW 14 had higher HUE over K 307 (Fig. 5). The highest HUE values were associated with the treatment PN_{B+A}, closely followed by PN_A, which were significantly higher over NS_{CT} and WS_{B+A}. Principal component analysis results show that strong association of grain yield with grains ear⁻¹, thousand-grain weight, and leaf chlorophyll at the grain filling stage (Fig. 6). Treatment PN_{B+A} was observed as the best treatment for both the cultivars with a higher weightage of component 1 and component 2.

Discussion

Our results demonstrate that in the sub-tropical environments, the wheat crop is likely to expose a higher mean temperature (> 17 °C) during anthesis which further plunged to

Table 2 Pooled ANOVA for grain yield, yield attributing and physiological parameters (2013–2014 to 2015–2016)

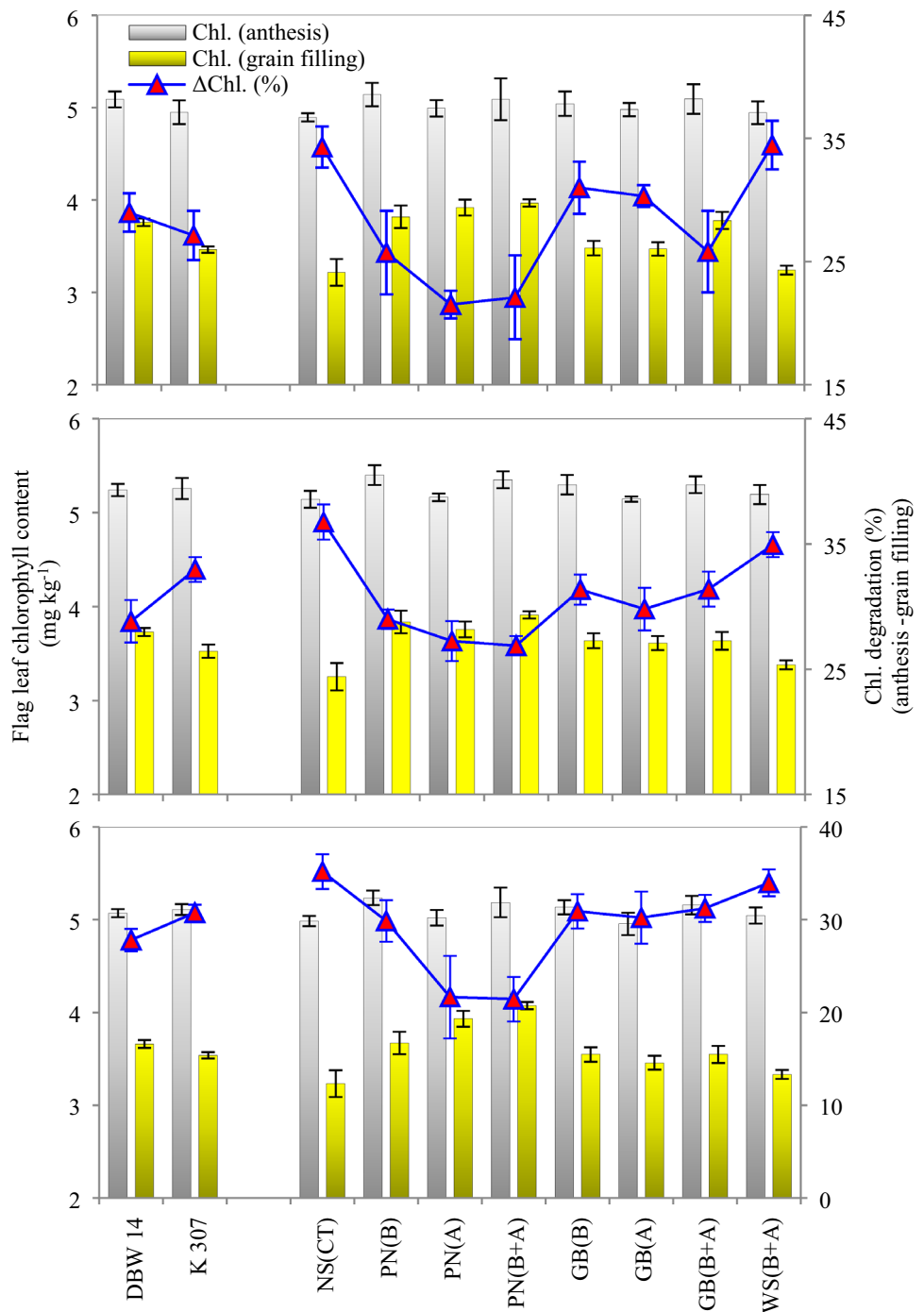
Source of variation	df	GY	ET	GPE	TGW	CHL _A	CHL _{GF}	RP _A	RP _{GF}	CHD _{A-GF}	ΔRP _{A-GF}
Replication	2	20.5	20.9	93.2	9.7	1.00	0.02	3.5	48.3	237.7	744
Year (Y)	2	361.3**	4581.6**	62.3	9.3	1.28	0.02	5004.7**	8428.0**	227.2	7064**
Error (a)	4	4.5	70.1	20.3	7.9	0.47	0.27	16.4	13.5	75.9	518
Cultivar (C)	1	264.8**	7804.1**	2958.7**	7.5*	0.94	1.56**	64.4*	125.0**	109.2	31
Y × C	2	44.9***	822.8*	22.7	0.5	2.58*	0.18	17.0	28.0	251.4	32
Error (b)	6	8.3	376.2	18.8	9.6	1.12	0.36	32.2	28.8	291.5	524
Chemical spray (S)	7	249.8**	3747.8**	1020.0**	138.7**	1.10	8.59**	585.8**	1922.7**	2426.3**	8369**
Y × S	14	25.0	639.1	44.5	28.7	0.05	0.67	144.4	17.2	252.3	1756
C × S	7	60.0**	1138.1*	1388.6**	11.0	0.47	1.55**	44.9	98.1	489.6*	463
Y × C × S	14	30.2	492.1	114.3	34.5	0.50	1.38*	31.5	59.6	490.8	616
Error (c)	84	177.4	5725.1	910.2	187.3	7.10	4.07	849.1	559.2	2623.8	8830
Total	143	1246.7	25417.9	6653.7	444.6	16.62	18.65	6793.9	11328.4	7475.7	28947

GY grain yield, ET effective tillers, GPE grains ear⁻¹, TGW thousand-grain weight, RP_{GF} relative membrane permeability at grain filling, RP_A relative membrane permeability at anthesis, CHL_A chlorophyll content (anthesis), CHL_{GF} chlorophyll content (grain filling), CHD_{A-GF} Chlorophyll degradation, ΔRP_{A-GF} change in relative membrane permeability (anthesis to grain filling), df degrees of freedom.

*Significant ($p \leq 0.05$)

**Significant ($p \leq 0.01$)

Fig. 3 Flag leaf chlorophyll content (mg kg^{-1}) at anthesis and grain filling stages and chlorophyll degradation (%) during anthesis to grain filling stages ($\Delta\text{Chl.}$) as influenced by different cultivars and chemical spray treatments (2013–2014 to 2015–2016). Error bars represent the standard error on mean. See Table 1 for definitions of the abbreviations



about 27.8–31.7 °C during grain filling period till maturity of the crop. According to Farooq et al. (2011), the optimum temperature for anthesis and grain filling of wheat ranges from 12 to 22 °C. So, the wheat crop is mostly exposed to supra-optimal temperature during its terminal growth phases, which is likewise evident in all the three years of study (Fig. 1). Bamboriya et al. (2017) are also in agreement with the fact that higher ambient temperature during March in eastern India, which often coincides with grain formation

period of wheat, causing terminal heat stress. Notably, the average temperature increased to the tune of about 6–8 °C in just around twenty days after anthesis. Therefore, the crop faced heat stress throughout its anthesis and grain filling period which is the typical terminal heat stress and very common for late-sown wheat in eastern IGP.

In agreement with previous reports, our result likewise demonstrates that the grain yield of late-sown wheat, irrespective of cultivars was markedly low when no alleviation

Table 3 Yield attributing characters of wheat crop as influenced by different chemical spray treatment under late-sown condition in Eastern India

Treatment	ET (nos. m ⁻²)			GPE (nos. ear ⁻¹)			TGW (g)		
	2013–2014	2014–2015	2015–2016	2013–2014	2014–2015	2015–2016	2013–2014	2014–2015	2015–2016
Cultivar (C)									
DBW 14	196.2	196.3	214.2	54.1	56.0	53.7	36.2	36.8	36.4
K 307	214.8	215.0	222.2	45.6	45.9	45.1	35.7	36.3	36.1
LSD (<i>p</i> =0.05)	9.9	11.9	4.2	1.4	3.8	0.9	ns	ns	ns
Chemical spray (S)¹									
NS _{CT}	195.0	194.0	208.3	45.7	47.9	46.4	34.3	34.9	35.4
PN _B	210.3	208.8	221.1	51.9	53.0	50.7	36.6	36.6	36.3
PN _A	206.8	205.8	222.2	53.4	52.9	53.2	36.7	36.8	36.2
PN _{B+A}	213.0	206.1	217.0	54.2	55.6	53.0	37.6	39.8	37.9
GB _B	210.8	208.5	221.2	47.6	48.8	47.3	35.4	35.9	35.9
GB _A	214.0	210.8	218.5	48.3	48.4	47.5	36.7	35.9	36.1
GB _{B+A}	209.5	208.8	221.8	51.0	52.4	49.1	35.6	36.2	36.3
WS _{B+A}	196.3	202.3	215.4	46.6	48.6	47.8	34.6	36.5	35.7
LSD (<i>p</i> =0.05)	10.2	ns	8.6	3.2	3.9	4.6	1.9	2.2	1.1
C×S (<i>p</i> =0.05)	ns	ns	ns	s	s	s	ns	s	s

ET effective tillers, GPE grains ear⁻¹, TGW thousand-grain weight, ns non significant, s significant

¹See Table 1 for definitions of the abbreviations

Table 4 Grain yield (t ha⁻¹) of late-sown wheat crop of Eastern India as influenced by foliar spray treatments

Treatment	Grain yield (t ha ⁻¹)			
	2013–2014	2014–2015	2015–2016	Mean
Cultivar (C)				
DBW 14	3.33	3.39	3.02	3.25
K 307	3.19	2.98	2.76	2.97
LSD (<i>p</i> =0.05)	0.14	0.16	0.18	0.07
Chemical spray (S)¹				
NS _{CT}	3.09	2.95	2.71	2.92
PN _B	3.15	3.25	2.97	3.13
PN _A	3.39	3.35	3.01	3.25
PN _(B+A)	3.49	3.43	3.12	3.34
GB _B	3.26	3.05	2.77	3.03
GB _A	3.24	3.19	2.86	3.10
GB _(B+A)	3.27	3.21	2.95	3.14
WS _{B+A}	3.16	3.03	2.73	2.97
LSD (<i>p</i> =0.05)	0.18	0.18	0.17	0.14
C×S (<i>p</i> =0.05)	ns	s	s	s

ns non significant, s significant

¹See Table 1 for definitions of the abbreviations

measure was taken. Among the different chemical spray treatments, foliar spray of 0.5% KNO₃ at both booting and anthesis (PN_{B+A}) or 100 mM GB (GB_{B+A}) can improve grain yield of wheat, irrespective of varieties, over no-spray treatment (NS_{CT}). The extent to which the grain yield can

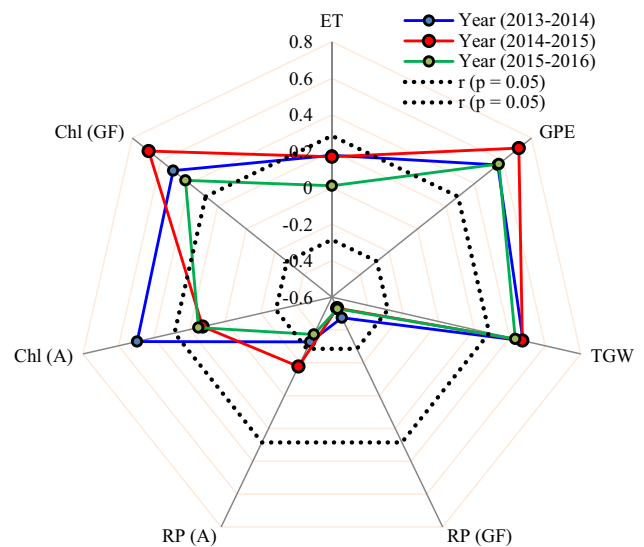


Fig. 4 Pearson correlation coefficient (*r*) between grain yield and plant growth, yield and physiological parameters during the experimental year 2013–2014 to 2015–2016. The dash lines represent the critical ±*r* value (LSD, *p*=0.05, *df*=46). ET effective tillers, GPE grains ear⁻¹, TGW thousand-grain weight, RP(A) relative membrane permeability (anthesis), RP(GF) relative membrane permeability (grain filling), Chl(A) chlorophyll content (anthesis), Chl(GF) chlorophyll content (grain filling)

be increased was about 13–16%, during the three years under the treatment PN_{B+A}. This clearly suggests that split application of low concentration of KNO₃ (0.5%) is more

Fig. 5 Heat use efficiency ($\text{kg ha}^{-1} \text{ } ^\circ\text{C day}^{-1}$) as influenced by wheat cultivars and chemical spray treatments. Error bars represent standard error of means. See Table 1 for definitions of the abbreviations

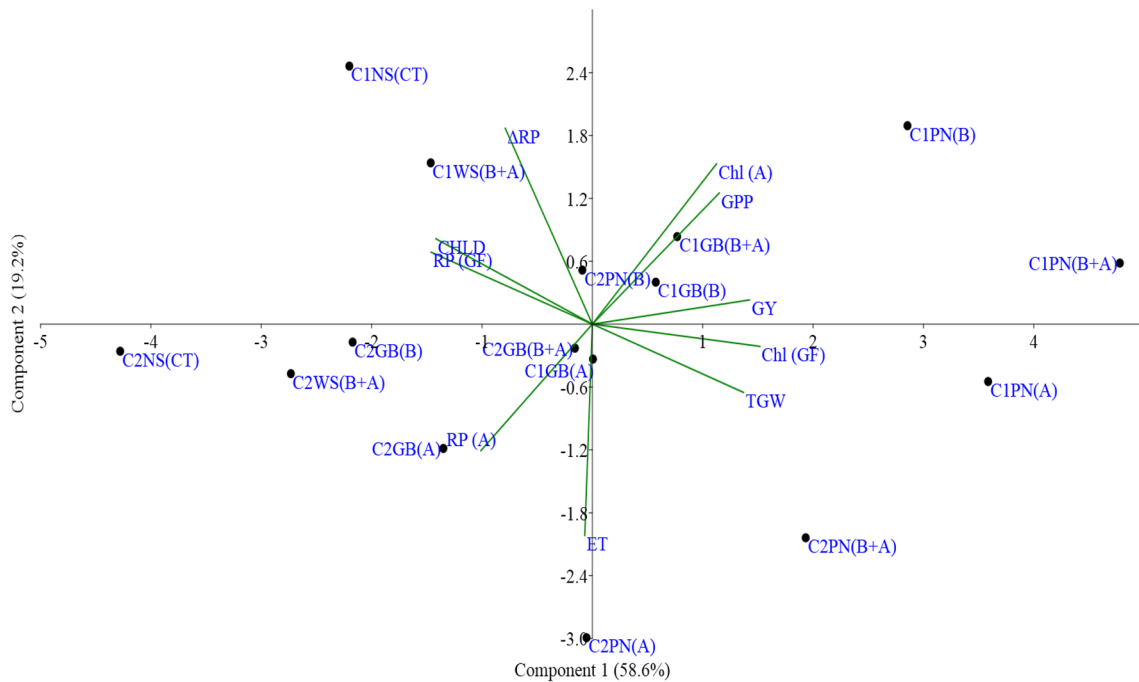
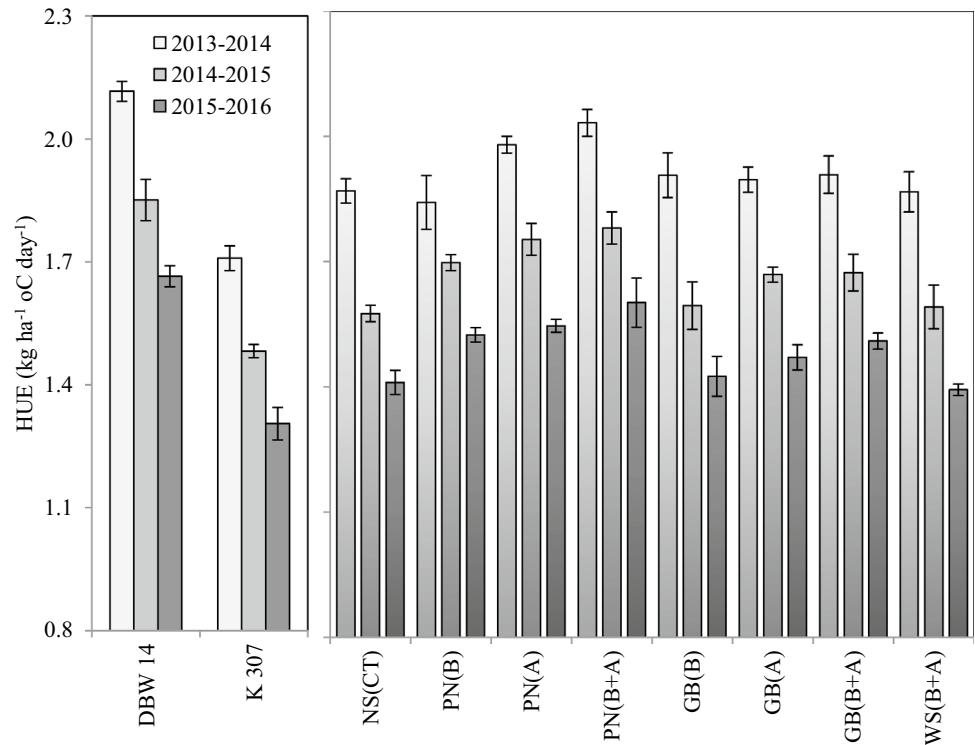


Fig. 6 Scatter plot of treatment combinations on PCA coordinates based on growth, yield and physiological parameters. *C1* cultivar DBW 14, *C2* cultivar K 307, *ET* effective tillers, *GPP* grains panicle⁻¹, *TGW* thousand-grain weight, *RP(A)* relative membrane permeability (anthesis), *RP(GF)* relative membrane permeability

(grain filling), *Chl(A)* chlorophyll content (anthesis), *Chl(GF)* chlorophyll content (grain filling), *CHLD* chlorophyll degradation (anthesis to grain filling), ΔRP change in relative membrane permeability (anthesis to grain filling)

effective in protecting yield loss of late-sown wheat facing terminal heat stress under field condition. For both the chemicals, split application at both booting and anthesis had higher efficacy and resulted in a yield increase in late-sown wheat. Hence, our second hypothesis is accepted. Exogenous application of potassium (K) based compounds known to maintain K^+ balance and enzymes activities in the plant and thereby can alleviate the adverse effect of heat stress on important physiological functions in cereals (Pasala et al. 2016). Likewise, the positive response of the crop to organic osmolyte (GB) under stress conditions possibly associated with improved osmoregulation, membrane integrity, and protecting cells from free radicals under stress conditions (Wahid and Shabbir 2005; Ashraf and Foolad 2007).

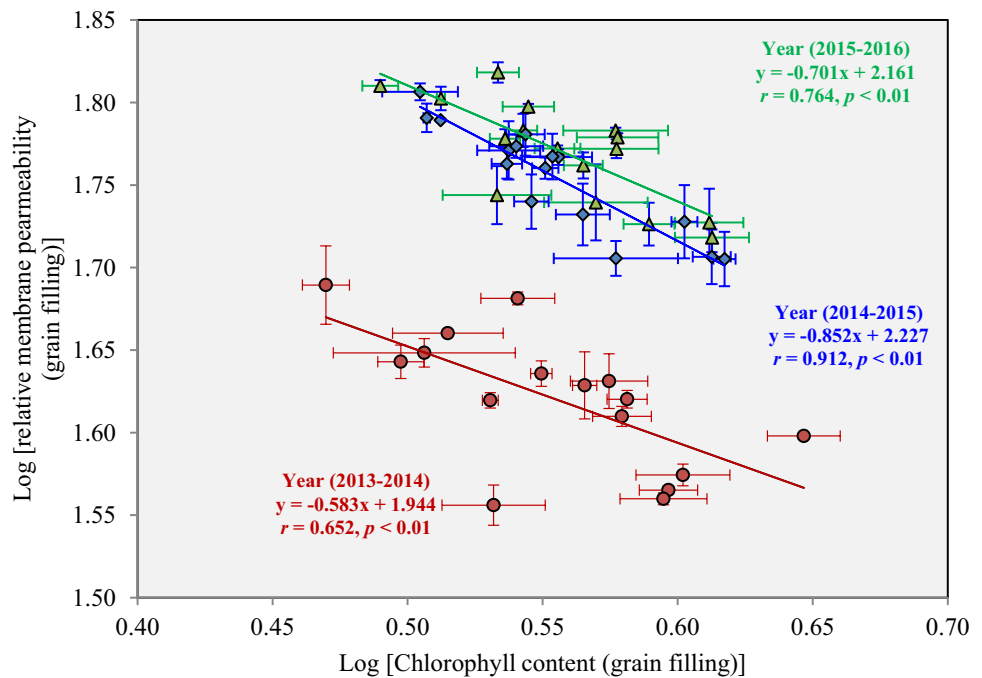
Our results demonstrate that grain yield of late-sown wheat has a close association with yield attributes like grains ear^{-1} and thousand-grain weight. The increase in grain yield with chemical spray treatments was primarily attributed to a combined effect of the increase in these yield attributes. Nouri and Etmnan (2011) also specified that the reduction in grain yield of late-sown wheat facing terminal heat stress is mainly because of reduction in grains ear^{-1} and thousand-grain weight. Correlation study revealed the positive correlation among grain yield, grains ear^{-1} and thousand-grain weight in all the three years of the experiment (Fig. 4) which also substantiate better yield under the treatments PN_A and PN_{B+A} due to the positive impacts of these treatments on grains ear^{-1} and thousand-grain weight. The reason behind this positive effect of KNO_3 is its ability to modulate source-sink homeostasis and increased reserve mobilization leading to enhanced crop yield (Srivastava et al. 2016). Higher correlation (r) between grain yield and grains ear^{-1} , thousand-grain weight also confirmed this. Where it is apparent from the study that ear bearing tillers in wheat is not affected by terminal heat stress; as the ear formation completed before the occurrence of high-temperature periods.

Under elevated temperature, leaf chlorophyll degrades due to disruption of the structure of chloroplast (Xu et al. 1995) and also thermo-stability of cell membrane gets disturbed (Hasan et al. 2007) resulting in electrolyte leakage from the cell. These physiological events are manifested through lower chlorophyll content and higher membrane permeability of leaf tissues, leading to reduced source activities and transport of photosynthates to sink which ultimately limits the grain yield. Therefore, leaf chlorophyll content and relative membrane permeability have been taken into consideration to assess the improvement in stress tolerance. Relative membrane permeability and chlorophyll content were estimated, at anthesis and grain filling stages (Figs. 2 and 3), from flag leaf of wheat as it is the principal contributory organ for the supply of photosynthates to the developing grain (Borrill et al. 2015).

Results demonstrate that a significant improvement in flag leaf chlorophyll content under all the treatments having the foliar application of KNO_3 at booting and anthesis over no spray and only water spray treatments. It can also be seen that flag leaf chlorophyll content degraded to the lowest extent (about 21–27%) between anthesis to grain filling period under the treatments PN_{B+A} and PN_A , whereas corresponding values for the treatments SP_{CT} and WS_{B+A} hovered around 34–36% and 33–34%, respectively (Fig. 3). It clearly depicts the superiority of the treatments PN_{B+A} and PN_A for retaining higher levels of flag leaf chlorophyll throughout the post-anthesis and grain filling period under high-temperature stress. It helped in maintaining normal photosynthetic activities in the flag leaf of wheat during this period and thus contributed to the higher yield attributes and ultimately grain yield. A significant correlation between flag leaf chlorophyll content at grain filling and grain yield (Fig. 4) also supports the fact. The lower reduction of flag leaf chlorophyll content in PN_{B+A} and PN_A treatments may be attributed to the beneficial roles of K^+ in maintaining stomata conductance, decreasing mesophyll resistance, increasing RUBP carboxylase activity (Zhao et al. 2001), detoxification of reactive oxygen species (Cakmak 2005) and export of photosynthates to the developing sink (Cakmak 1994). It is also evident that flag leaf chlorophyll content during grain filling was always higher in DBW 14 than K 307 (Fig. 3). It might be due to the fact that post-anthesis high-temperatures spell exerted a greater negative impact on K 307 than DBW 14. A probable reason for this is relatively shorter grain filling period of DBW 14 as compared to K 307.

Injury to the cell membrane of flag leaf, as evidenced by the values of relative membrane permeability across the two varieties, was significantly lower under all the treatments with KNO_3 over no-spray and water spray during both anthesis and grain filling period. Lowest values of relative membrane permeability at both the crop growth stages were recorded under PN_{B+A} . However, the lowest percentage change in relative permeability from anthesis to grain filling was observed under the treatment PN_A and is almost comparable with the percent change in relative membrane permeability values recorded under the treatment PN_{B+A} (Fig. 2) which signifies the highest impact of these treatments in protecting the cell membranes of flag leaves. The substantially lower values of relative membrane permeability in PN_{B+A} and PN_A treatments indicates a lower level of membrane injury, which might have prevented degradation of chlorophyll under these treatments and imparted 'stay-green' characteristics in flag leaves leading to uninterrupted supply of photosynthates to the developing grains and ultimately helped in improving grain yield. Irrespective of the treatment, the increased heat intensity during the second and third year of experiment led to the higher increase in relative

Fig. 7 Linear relationship between flag leaf relative membrane permeability and chlorophyll content at grain filling stage of crop. Vertical and horizontal error bars represent standard error of means.



permeability during anthesis to grain filling over the first year (Fig. 2)

Relationship between flag leaf chlorophyll content and relative permeability also supports that about 40–80% variation in flag leaf chlorophyll content at grain filling may be attributed to the variation in relative membrane permeability during the same period (Fig. 7). Detoxification and decreased production of reactive oxygen species lead to less damage in cell membrane under the KNO_3 treatments receiving supply of K^+ in flag leaf as foliar sprays indicate a lower degree of stress in flag leaf which is reflected through lower relative membrane permeability in these treatments. The result is in concordance with the findings of Biehler and Fock (Biehler and Fock 1996). Lower flag leaf relative membrane permeability in DBW 14 than K 307 at both the anthesis and grain filling period may be due to lower ill effects of heat stress in a variety having a shorter grain filling period.

Results demonstrate that with the increase in heat at terminal growth stage (anthesis to grain filling) the HUE drops sharply (Fig. 5). The higher values of HUE in $\text{PN}_{\text{B+A}}$ and PN_{A} was primarily attributed to the increase in grain yield over no spray treatment under late-sown condition. The PCA graph also shows that a parallel association of grain yield with flag leaf chlorophyll content at grain filling, thousand-grain weight, and grains ear^{-1} (Fig. 7).

Relationship between flag leaf chlorophyll content and relative permeability also supports that about 40–80% variation in flag leaf chlorophyll content at grain filling may be attributed to the variation in relative membrane permeability during the same period (Fig. 6). Detoxification and

decreased production of reactive oxygen species lead to less damage in cell membrane under the KNO_3 treatments receiving supply of K^+ in flag leaf as foliar sprays indicate lower degree of stress in flag leaf which is reflected through lower relative membrane permeability in these treatments. The result is in concordance with the findings of Biehler and Fock (Biehler and Fock 1996). Lower flag leaf relative membrane permeability in DBW 14 than K 307 at both the anthesis and grain filling period may be due to lower ill effects of heat stress in a variety having shorter grain filling period.

Results demonstrate that with increase in heat at terminal growth stage (anthesis to grain filling) the HUE drops sharply. The higher values of HUE in $\text{PN}_{\text{B+A}}$ and PN_{A} was primarily attributed to the increase in grain yield over no spray treatment under late-sown condition. The PCA graph also shows that a parallel association of grain yield with flag leaf chlorophyll content at grain filling, thousand grain weight, and grains ear^{-1} (Fig. 7).

Conclusions

The results of the three-year field experiment demonstrate that foliar spray of KNO_3 could alleviate the negative effect of terminal heat stress and improve the grain yield of late-sown wheat in eastern India. Maximum improvement in grain yield (~14%) was recorded with split application of 0.5% KNO_3 both at booting and anthesis stages ($\text{PN}_{\text{B+A}}$) closely followed by single spray of 1.0% KNO_3 only at anthesis stage (PN_{A}). The study suggests that the

split application increased the field efficacy of KNO_3 and GB foliar spray over one-time application with the higher dose. Irrespective of cultivars, foliar spray of osmolytes was found effective in protecting flag leaf chlorophyll, and also maintained a higher level of membrane integrity throughout the post-anthesis period. Multi-location trials with the promising treatments may further validate the results in the IGP region, where terminal heat stress is a rule, rather an exception. Thus, foliar spray of KNO_3 could be a feasible strategy to alleviate terminal heat stress in late-sown wheat, and thus recommended.

Acknowledgements The authors are indebted to the Directorate of Research, Bihar Agricultural University, Sabour, Bihar for giving financial assistance and infrastructural facilities required for this study.

Compliance with Ethical Standards

Conflict of Interest No potential conflict of interest was reported by the authors.

References

- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24(1), 1–15.
- Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, 59, 206–216.
- Bamboriya, S. D., Bamboriya, S. D., & Bana, R. S. (2017). Agronomic management of terminal heat stress in wheat. *Popular Kheti*, 5(1), 2–6.
- Bhatt, R., Kukal, S. S., Busari, M. A., Arora, S., & Yadav, M. (2016). Sustainability issues on rice–wheat cropping system. *International Soil and Water Conservation Research*, 4(1), 64–74.
- Biehler, K., & Fock, H. (1996). Evidence for the contribution of the Mehler–peroxidase reaction in dissipating excess electrons in drought–stressed wheat. *Plant Physiology*, 12, 265–272.
- Borrill, P., Fahy, B., Smith, A. M., & Uauy, C. (2015). Wheat grain filling is limited by grain filling capacity rather than the duration of flag leaf photosynthesis: A case study using NAM RNAi plants. *PLOS One*, 10(8), e0134947.
- Cakmak, I. (1994). Activity of ascorbate–dependent H_2O_2 –scavenging enzymes and leaf chlorosis are enhanced in magnesium and potassium–deficient leaves, but not in phosphorus–deficient leaves. *Journal of Experimental Botany*, 45, 1259–1266.
- Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science*, 168, 521–530.
- Cochran, W. G., & Cox, G. M. (1963). *Experimental design*. New York: Wiley.
- Cuin, T. A., & Shabala, S. (2007). Compatible solutes reduce ROS induced potassium efflux in *Arabidopsis* roots. *Plant Cell and Environment*, 30, 875–885.
- Dwivedi, S. K., Basu, S., Kumar, S., Kumar, G., Prakash, V., Kumar, S., Mishra, J. S., Bhatt, B. P., Malviya, N., Singh, G. P., & Arora, A. (2017). Heat stress induced impairment of starch mobilisation regulates pollen viability and grain yield in wheat: Study in Eastern Indo–Gangetic Plains. *Field Crops Research*, 206, 106–114.
- Dwivedi, S. K., Basu, S., Kumar, S., Kumari, S., Kumar, A., Jha, S., Mishra, J. S., Bhatt, B. P., & Kumar, G. (2019). Enhanced antioxidant enzyme activities in developing anther contributes to heat stress alleviation and sustains grain yield in wheat. *Functional Plant Biology*. <https://doi.org/10.1071/FP19016>.
- Dwivedi, S. K., Kumar, S., & Prakash, V. (2015). Effect of late sowing on yield and yield attributes of wheat genotypes in Eastern Indo Gangetic Plains (EGIP). *Journal of AgriSearch*, 2, 304–306.
- Farooq, M., Bramley, H., & Palta, J. A. (2011). Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Science*, 30, 1–17.
- Fayez, K. A., & Bazaid, S. A. (2014). Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. *Journal of the Saudi Society of Agricultural Sciences*, 13(1), 45–55.
- Harborne, J. B. (1973). *Phytochemical Methods. A guide to modern techniques of plant analysis* (pp. 204–208). Fakenham: Chapman & Hall.
- Hasan, M. A., Ahmed, J. U., Bahadur, M. M., Haque, M. M., & Sikder, S. (2007). Effect of late planting heat stress on membrane thermostability, proline content and heat susceptibility index of different wheat cultivars. *Journal of the National Science Foundation of Sri Lanka*, 35(2), 109–117.
- Hazra, K. K., Ghosh, P. K., Venkatesh, M. S., Nath, C. P., Kumar, N., Singh, M., Singh, J., & Nadarajan, N. (2018). Improving soil organic carbon pools through inclusion of summer mungbean in cereal–cereal cropping systems in Indo–Gangetic plain. *Archives of Agronomy and Soil Science*, 64, 1690–1704.
- Mehla, R. S., Verma, J. K., Gupta, R. K., & Hobbs, P. R. (2000). Stagnation in the productivity of wheat in the Indo–Gangetic plains: Zero–till–seed–cum–fertilizer drill as an integrated solution. Rice–wheat consortium for the Indo–Gangetic plains. Rice–wheat consortium paper series. No. 8, New Delhi, India.
- Nouri, A., Etmiman, A., Teixeira da Silva, J., & Mohammadi, R. (2011). Assessment of yield, yield–related traits and drought tolerance of durum wheat genotypes (*Triticum turgidum* var. Durum Desf.). *Australian Journal of Crop Science*, 5(1), 8–16.
- Pandey, B., Pandey, R. K., Dwivedi, D. K., & Singh, R. S. (2010). Phenology, heat unit requirement and yield of wheat (*Triticum aestivum*) varieties under different crop growing environment. *Indian Journal of Agricultural Sciences*, 80(2), 136–140.
- Pasala, R. K., Khan, M. I. R., Minhas, P. S., Sultana, R., Per, T. S., Deokate, P. P., Nafees, A., Singh, Y., & Rane, J. (2016). Can plant bioregulators minimize the crop productivity losses caused by drought, heat stress and salinity? An integrated review. *Journal of Applied Botany and Food Quality*, 89, 113–125.
- Rane, J., Pannu, R. K., Sohu, V. S., Saini, R. S., Mishra, B., Shoran, J., Crossa, J., Vargas, M., & Joshi, A. K. (2007). Performance of yield and stability of advanced wheat genotypes under heat stressed environments of Indo–Gangetic Plains. *Crop Science*, 47, 1561–1573.
- Sastry, P. S. N., Charkravarty, N. V. K., & Rajput, R. P. (1985). Suggested index for characterization of crop response to thermal environment. *International Journal of Ecology and Environmental Sciences*, 11, 25–30.
- Singh, P. K., Singh, K. K., Baxla, A. K., & Rathore, L. S. (2015). Impact of climatic variability in wheat yield prediction using DSSAT v 4.5 (CERES–Wheat) model for the different agro–climatic zones of India. In A. K. Singh (Ed.), *Climate change modelling, planning and policy for agriculture* (pp. 45–56). India: Springer.
- Srivastava, A. K., Ratnakumar, P., Minhas, P. S., & Suprasanna, P. (2016). Plant bioregulators for sustainable agriculture; integrating redox signalling as a possible unifying mechanism. *Advances in Agronomy*, 137, 1–33.
- Suryavanshi, P., & Buttar, G. S. (2016). Mitigating terminal heat stress in wheat. *International Journal of Bioresource Stress Management*, 7(1), 142–150.

- Tewolde, H., Fernandez, C. J., & Erickson, C. A. (2006). Wheat cultivars adapted to post-heading high temperature stress. *Journal of Agronomy and Crop Science*, *192*, 111–120.
- Verma, U. N., Thakur, R., Pal, S. K., Singh, M. K., & Singh, S. P. (2000). Nutrient management of late planted wheat on Alfisol of Bihar Plateau. *Indian Journal of Agronomy*, *45*(1), 118–123.
- Wahid, A., & Shabbir, A. (2005). Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. *Plant Growth Regulation*, *46*, 133–141.
- Wakchaure, G. C., Minhas, P. S., Ratnakumar, P., & Choudhary, R. L. (2016). Optimizing supplemental irrigation for wheat (*Triticum aestivum* L.) and the impact of plant bio-regulators in a semi-arid region of Deccan Plateau in India. *Agricultural Water Management*, *172*, 9–17.
- Xu, Q., Paulsen, A. Q., Guikema, J. A., & Paulsen, G. M. (1995). Functional and ultra structural injury to photosynthesis in wheat by high temperature during maturation. *Environmental and Experimental Botany*, *35*, 43–54.
- Yang, G., Rhodes, D., & Joly, R. J. (1996). Effects of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficient and glycinebetaine-containing maize lines. *Functional Plant Biology*, *23*(4), 437–443.
- Zadoks, J. C., Chang, T. T., & Konzani, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*, 415–421.
- Zhao, Z., Chen, G., & Zhang, C. (2001). Interaction between reactive oxygen species and nitric oxide in drought-induced abscisic acid synthesis in root tips of wheat seedlings. *Australian Journal of Plant Physiology*, *28*, 1055–1061.