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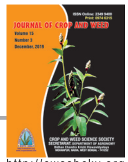

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Canopy temperature depression as influenced by various osmoprotectants in late sown irrigated wheat (*Triticum aestivum* L.)

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ABSTRACT

A field experiment was conducted during rabi season of 2019-20 and 2020-21in late sown irrigated wheat under eastern sub-Himalayan plains of West Bengal to assess the effect of various osmoprotectants on grain yield and canopy temperature depression (CTD)as a means of alleviating terminal heat stress. The experiment was laid out in randomized block design (RBD) with seven treatments, each replicated thrice. The treatments were consisted of five osmoprotectants viz., thiourea @ 750ppm(T_1); salicylic acid @ 1.0mM (T_2); potassium nitrate @ 2.0% (T_3); trehalose@ 1.5mM (T_4) and sodium nitroprusside(SNP) @ 800 µg ml^{-1} (T_5) along with water @ 450 l ha^{-1} (T_6)and no spray (control) (T_7).All the osmoprotectants (except trehalose) including water were sprayed twice, at active tillering and anthesis stages, while trehalose was sprayed at weekly interval starting from 48 hours before heat stress and continued during entire stress period. It was revealed that foliar spray of SNP recorded the highest aerial biomass production (9.38 and 6.76 t ha^{-1}during 2019-20 and 2020-21, respectively) vis-à-vis grain yield (3.54 and 2.58 t ha^{-1}during 2019-20 and 2020-21, respectively), being at par with trehalose and potassium nitrate during both the years. Canopy temperature depression (CTD) did not vary significantly during pre-anthesis and anthesis stages in both the years under various osmoprotectant treatments. However, during grain filling stage, maximum CTD was recorded with SNP treatment (8.3 and 5.2°C in 2019-20 and 2020-21, respectively) followed by potassium nitrate (7.4°C) and trehalose (7.1°C) in 2019-20, being at par with each other. However, SNP treatment was statistically superior to other osmoprotectants in terms of CTD during 2020-21 which is evident through significant positive correlation (r=0.596)between grain yield and CTD at grain-filling. Amongst the osmoprotectants, SNP showed its superiority in terms of CTD and grain yield signifying its potentiality in alleviating terminal heat stress.

***Keywords*:** Yield, canopy temperature depression, osmoprotectants, wheat

Wheat is the most important staple food crop of India during the post-rainy (*rabi*) season (November-April).Presently, Indo-Gangetic Plains (IGP) of South-Asia including India contributes almost 15% of the global wheat production and is increasingly being vulnerable to heat stress (Jha *et al*., 2014).Growing long duration rice varieties during *kharif* is the most popular practice in the eastern part of the country including sub-Himalayan plains of West Bengal.Consequently, subsequent sowing of wheat is very often gets delayed due to wet soil conditions resulting from late receipt of monsoon rain in high intensity and withdrawal of monsoon is also latein this region (Mitra *et al*., 2014; Mitra and Das, 2015).Under these circumstances, delayed sowing in wheat is a very common phenomenon in this tract. It has also been reported that even under restricted irrigation and straw mulching, we can go for this crop under late sowing with selection of appropriate varieties (Das and Mitra, 2013; Singha *et al*., 2018).

Sub-Himalayan plains of West Bengal experiences maximum and minimum temperatures ranging between 27-32 and 11-19°C, respectively during last week of February to mid-April (Anonymous, 2020), coinciding with anthesis to maturity stage of late sown wheat.The optimum temperature required during the entire post-anthesis stage of wheat is around 12-22°C. Exposure of temperature above 30°C during this period of wheat, commonly termed as terminal heat stress,causes irreversible morphological, anatomical, physiological and phenological changes in the crop (Sharma and Sharma, 2017) and ultimately affects yield. Late sowing mediated terminal heat stress may cause 18-34% loss in grain yield of wheat in eastern India (Dwivedi *et al*., 2017). Therefore, for sustaining and improving yield of late sown wheat facing terminal heat stress, suitable stress indicator trait and proper management strategy should be framed out with due priority.

Plant water balance is a direct measure of drought response or heat stress of crops. As transpiration is the main cause of changes in leaf temperature, there is a direct relation between canopy temperature, transpiration rate and stomatal conductance (Sofi *et al*., 2019). Deviation of canopy temperature from air temperature is known as canopy temperature depression (CTD) and serves as a reliable stress indicator trait in wheat for detecting abiotic stresses, especially drought and heat (Gowda *et al*., 2011). Higher positive values of CTD, when measured as difference between air and

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canopy temperature (Tair-Tcanopy), can fairly detect the degree of stress faced by the crop. It is a single value parameter which takes care of atmosphere-soil-plant continuum and can be measured easily with hand held infra-red thermometer (Kumari *et al*., 2013). It has been reported from various studies that use of osmoprotectants may be beneficial in mitigating the heat stress through their osmotic adjustments. Osmoprotectant refers to a wide variety of compounds like amino acids (glutamate), low molecular weight compounds (sugars and sugar alcohols), inorganic salts having osmotic properties, methylated tertiary N compounds along with several low molecular weight metabolites. Osmotic adjustment of plants by endogenous accumulation of these compounds in response to several abiotic stresses is now a well-established fact. The principal mechanism involved is the protection of photosystems and cellular membranes from irradiance dependent photo-oxidative damage and scavenging of reactive oxygen species (ROS) (Nawaz *et al*., 2019). Positive impact of some of these compounds with exogenous foliar application in proper dose and time has also been found by some workers in alleviating terminal heat stress in wheat (Suryavanshi and Buttar, 2018). However, most of the studies were conducted under controlled environments. Efficacies of these chemicals under real field conditions have not been tested extensively to a greater extent, particularly under sub-Himalayan plains of West Bengal where late sowing in wheat is very common and terminal heat incur significant losses due to increase in temperature since end-Februray. Our objective of this study to assess the relative effectiveness of some of the osmoprotectants in maintaining lower canopy temperature during terminal growth stages of late sown wheat and minimizing yield penalty due to heat stress. We hypothesized that the osmoprotectant maintaining higher CTD during a particular growth stage of wheat would alleviate terminal heat stress and will have favourable effect on grain yield.

MATERIALS AND METHODS

Field experiments were conducted during two consecutive rabi season of 2019-20 and 2020-21 at the Instructional farm of Uttar Banga Krishi Viswavidyalaya (UBKV), Pundibari, Coochbehar, located under eastern Sub-Himalayan plains of West Bengal (26°24’02.1"N latitude,89°23’21.5"E longitude and at 43 m above mean sea level). The surface soil (0-15 cm) of the experimental site was sandy loam in texture, with slightly acidic pH of 5.6, 0.76% organic carbon, and 173.5, 22.3 and 88.0 kg ha^{-1} of minearalizable N, Olsen’s P and NH4OAC extractable K, respectively.The weather during the two years of the study was cool with maximum and minimum temperatures hovered around 23-32 and 8.4-18.5°C, respectively. But, the rainfall received during post-anthesis period of wheat (February last week to April 1^{st} week) was relatively higher in 2019-20 (54.4 mm) distributed through eight rainfall events, out of which 7 events received only in March,while it was 52.5 mm in 2020-21, distributed evenly through six rainfall events.

The experiment was laid out in randomized block design (RBD) with three replications. Five osmoprotectants viz., thiourea @ 750ppm (T_1); salicylic acid @ 1.0mM (T_2); potassium nitrate @ 2.0% (T_3); trehalose@ 1.5mM (T_4) and sodium nitroprusside (SNP)@ 800 µg ml^{-1} (T_5) were sprayed randomly along with water @ 450 l ha^{-1} (T_6) and no spray (control) (T_7) under each replication. All the osmoprotectants (except trehalose) including water were sprayed twice, at active tillering and anthesis stages. Trehalose was only sprayed at weekly interval starting from 48 hours before heat stress and continued during entire stress period. For all the osmoprotectants laboratory grade chemicals were used. Molecular weight of the osmoprotectants were as follows: Thiourea – 76.12; salicylic acid -138.12; potassium nitrate- 101.10; trehalose -342.29 and sodium nitroprusside (SNP)- 297.95. These molecular weights were used for preparing millimolar (mM)solutions wherever required.

Fertilizers were applied as per the recommended dose, i.e., 120:60:40 kg N: P_2O_5:K_2O ha^{-1} (Anonymous 2012).One- third of the total N along with full P and half K fertilizers were applied as basal. The rest amount of N fertilizers was applied in two equal splits, once at 20-22 days after sowing (DAS) and rest other at 40-42 DAS along with remaining half of K fertilizer. The individual gross plot size was 5 x 3m (15 m^2).The variety used in the experiment was HD 3086, a high yielding, timely sown, irrigated variety, matures in 130-135 days, suitable for NEPZ. Despite being a timely sown variety, it was purposively sown delayed on December 20 during both the years to have a clear exposure of the crop towards terminal heat. Seeds were sown manually behind the plough with 20 cm row spacing and seed rate of 120 kg ha^{-1}. Boron was applied @0.2% in the form of Solubor (B 20%), once at 35-40 DAS and the subsequent oneat 55-60 DAS. Zinc was applied in the form of chelated Zn (Zn-EDTA 12%) @ 0.1% during 2^{nd} spraying of B. Wheat was harvested manually from the net plot having an area of 4 X 2.4 m (10.4 m^2) (skipping the border rows), on April 08 and 02, during 2019-20 and 2020-21, respectively from ground level using a sickle for recording total aerial biomass, grain and straw yield separately and harvest index was calculated accordingly using grain and straw yields.

Canopy temperature was recorded with a hand held infrared thermometer during pre-anthesis, anthesis and grain filling stages following standard protocols (Pask *et al*., 2012). Canopy temperature depression (CTD) was estimated as its deviation from air temperature (Tair-Tcanopy) considering specified values of air temperatures obtained from a hand–held ambient temperature sensor just above the canopy during the observation. The specific air temperatures have been tabulated below:

Table 1 : Air temperatures (°C) during different growth stages of wheat

Growth stage of wheat	Tair (°C)	
	2019-20	2020-21
Pre -anthesis	24.5	29.2
Anthesis	28.4	30.3
Grain filling	31.4	31.1

Analysis of variance method was used for statistical analyses and for drawing conclusions using SPSS software version 20.0.3. In addition the relationship between canopy temperature,CTD and grain yields were assessed using bivariate correlation analysis (Pearson correlation coefficients and two-tailed test of significance) and a linear regression model has been used to highlight the relation between CTD and grain yields.

RESULTS AND DISCUSSION

Biomass production and yield performances

Spike m^{-2} did not vary significantly due to different treatments in both the years of study. However, maximum number of spike m^{-2} (250 and 211 during 2019-20 and 2020-21) was recorded under the foliar spray of sodium nitroprusside (SNP) (Table 2). Spikes m^{-2} is the major yield attributing characters which vary markedly due to variation in type of variety, seeding rate, dose of nitrogen fertilization and other major management practices. In our experiment, spike m^{-2} did not vary significantly due to uniformity amongst all the treatments in terms of basic agronomic managements like variety, seed rate, fertilizer dose, water management, etc. However, the total biomass production, grain yield and straw yields varied significantly under different osmoprotectant treatments. During both the years of study, maximum above ground biomass (9.38 and 6.76 t ha^{-1} during 2019-20 and 2020-21, respectively), grain yields (3.54 and 2.58 t ha^{-1}during 2019-20 and 2020-21, respectively) and straw yields (5.84 and 4.18 t ha^{-1}during 2019-20 and 2020-21, respectively) was recorded under SNP, being at par with potassium nitrate (9.25 and 6.60 t ha^{-1} of biomass ; 3.45 and 2.48 t ha^{-1} of grain yield and 5.80 and 4.12 t ha^{-1} of straw yield during 2019-20 and 2020-21, respectively) and trehalose (9.21 and 6.46 t ha^{-1} of biomass; 3.52 and 2.41t ha^{-1} of grain yield & 5.69 and 4.05t ha^{-1} of straw yield during 2019-20 and 2020-21, respectively) (Table 2). It was noted that the above ground biomass production, grain and straw yields recorded from rest of the treatments were statistically inferior to that registered from SNP, potassium nitrate and trehalose treatments. Increment in above ground biomass, grain and straw yield observed during the two years study in SNP treatment were18.3 and 17.8%; 18.4 and 21.1 % ; 18.2 and 15.8 %, respectively over no spray (control). This was followed by the increments in all of the above parameters obtained under potassium nitrate (16.6 and 14.9%; 15.4 and 16.4% ; 17.4 and 14.1%) and trehalose (16.1 and 12.5%; 17.7 and 13.1%; 15.2 and 12.2%) treatments.

Results indicated the superiority of SNP, potassium nitrate and trehalose over other osmoprotectants used in the study. Positive impact of SNP, potassium nitrate and trehalose in sustaining yield under terminal heat was attributed to the beneficial effects of these osmoprotectants on numbers on grains $spike^{-1}$ and or the individual grain weight (data not shown). Biochemically, these osmoprotectants played a key role to protect flag leaf from oxidative damage due to generation of reactive oxygen species (ROS) (Srivastava *et al*., 2016) through favourable osmoregulations like regulation of endogenous osmolyte concentration in cells; stabilizing cell membranes and scavenging ROS with anti-oxidant enzymes (Nawaz *et al*., 2019). Data also reflected that the grain yield obtained across the treatments of SNP, potassium nitrate and trehalose was almost 40% higher in 2019-20 than in 2020-21. The possible reason of this variation could be the better grain-filling leading to higher number of filled grains $spike^{-1}$ and test weight in 2019-20 than 2020-21. It was due to higher degree of stress imposed in 2020-21 under uninterrupted evapo-transpirative demand and higher mean temperature of 22.7°C prevailed during entire grain filling period in March. But, the mean temperature during the grain-filling period in 2019-20 was relatively low (22.6°C) with frequent rainfall spells which might have lowered the evaporative demand and consequently reduced the intensity of stress. It was clear from the study that these osmoprotectants could perform very well under stress situation. Considering the prevalence of terminal heat stress in eastern India causing 18-34% yield loss in wheat (Dwivedi *et al*., 2017), these osmoprotectants could partially restore yield penalty.

Canopy temperature and canopy temperature depression (CTD)

Canopy temperature as well as CTD did not vary significantly under various treatments at pre-anthesis and anthesis stages during both the years, though in most of the cases,osmoprotectant treatments showed

Table2 : Biomass production and yield performance of wheat under various osmoprotectant treatments

Treatments	Spikes m^{-2}		Above ground biomass (tha^{-1})		Grain yield (tha^{-1})		Straw yield (tha^{-1})		Harvest index (%)	
	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
T1	246	192	8.10	5.87	3.06	2.20	5.04	3.68	37.77	37.41
T2	242	195	8.02	5.94	3.03	2.21	5.00	3.73	37.77	37.15
T3	245	208	9.25	6.60	3.45	2.48	5.80	4.12	37.30	37.55
T4	247	206	9.21	6.46	3.52	2.41	5.69	4.05	38.26	37.30
T5	250	211	9.38	6.76	3.54	2.58	5.84	4.18	37.74	38.20
T6	244	207	8.01	5.95	3.02	2.22	4.98	3.74	37.77	37.25
T7	242	201	7.93	5.74	2.99	2.13	4.94	3.61	37.73	37.07
SEm (±)	**12.14**	**9.99**	**0.24**	**0.22**	**0.13**	**0.10**	**0.21**	**0.12**	**1.33**	**0.42**
LSD (0.05)	**NS**	**NS**	**0.74**	**0.68**	**0.41**	**0.31**	**0.65**	**0.39**	**NS**	**NS**

T1:TUat AT and anthesis @ 750ppm; T2:SA at AT and anthesis @ 1.0 mM., T3:KNO_3spray at AT and anthesis @ 2%; T4:Trehalose at weekly interval starting from 48 hrs. before heat stress (spraying to be continued during the entire stress period)@1.5 mM., T5: SNP twice at AT and anthesis @ 800 µg/ml; T6: Water at AT and anthesis @ 450 litre/ha); T7: No spray (control)

Table 3 : Canopy temperature and canopy temperature depression (CTD) at various stages of wheat under various osmoprotectant treatments

Treatments	Canopy temperature (°C)						Canopy temperature depression (°C)					
	Pre -anthesis		Anthesis		Grain filling		Pre -anthesis		Anthesis		Grain filling	
	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
T1	18.9	23.0	22.1	24.1	23.2	27.7	5.6	6.2	6.3	6.2	8.2	3.4
T2	18.4	22.7	22.2	24.4	24.6	27.8	6.1	6.5	6.2	5.9	6.8	3.3
T3	19.3	22.7	20.9	23.7	24.0	27.1	5.2	6.5	7.5	6.6	7.4	4.0
T4	18.2	23.3	21.2	22.9	24.3	27.3	6.3	5.9	7.2	7.4	7.1	3.8
T5	19.1	22.7	20.7	23.9	23.1	25.9	5.4	6.5	7.7	6.4	8.3	5.2
T6	18.8	23.2	22.1	24.5	25.3	28.3	5.7	6.0	6.3	5.8	6.1	2.8
T7	18.6	22.8	22.3	25.3	25.8	28.9	5.9	6.4	6.1	5.0	5.6	2.2
SEm(±)	**0.44**	**0.31**	**0.52**	**0.56**	**0.39**	**0.31**	**0.44**	**0.31**	**0.52**	**0.56**	**0.39**	**0.31**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**1.23**	**0.98**	**NS**	**NS**	**NS**	**NS**	**1.23**	**0.98**

T1:TUat AT and anthesis@ 750ppm; T2:SA at AT and anthesis @ 1.0 mM., T3:KNO_3spray at AT and anthesis @ 2%; T4: Trehalose at weekly interval starting from 48 hrs. before heat stress (spraying to be continued during the entire stress period)@1.5 mM., T5: SNP twice at AT and anthesis @ 800 µg/ml; T6: Water at AT and anthesis @ 450 litre/ha); T7: No spray (control)

Table 4 : Pearson correlation coefficient among grain yield, canopy temperature and canopy temperature depression during 2019-20 and 2020-21

	2019-20			2020-21		
	CT-GF	**CTD-GF**	**GY**	**CT-GF**	**CTD-GF**	**GY**
CT-GF	1			1		
CTD-GF	-1	1		-1	1	
GY	-0.298^{NS}	0.298 ^{NS}	1	-0.596**	0.596**	1

*CT-GF = Canopy temperature at grain filling; CTD-GF = Canopy temperature depression at grain filling; GY= grain yield, NS= non-significant; ** significant at 1% level*

relatively cooler canopy temperature *vis-à-vis* higher CTD than water spray and no spray (control) treatments (Table 3).The crop received its second irrigation and topdressing with N and K fertilizers at around 6-7 weeks and the crop entered into its reproductive stage within next couple of days. Enough moisture prevailed in the soil during pre-anthesis and anthesis stages might had met up the evapo-transpirative demand of the crop leading to transpirative cooling of canopy, irrespective of treatments, and masked the response of osmoprotectants. However, significant effect of osmoprotectant treatments on canopy temperature and CTD were apparent at grain-filling stages during both the years. In 2019-20 significantly lower canopy temperature (23.1°C) and consequently higher CTD (8.3°C) was recorded from SNP treatment. The canopy temperature at this stage under no spray treatment was much higher (25.8°C) with less CTD (5.6°C). However, CTD was statistically at par with thiourea (8.2°C), potassium nitrate (7.4°C) and trehalose (7.1°C) treatments. Rest of the treatments had significantly higher canopy temperature and lower CTD than these treatments. But, in 2020-21 the SNP alone was statistically superior in maintaining lower canopy temperature (25.9°C) and higher CTD (5.2°C) over rest of the treatments.The stress was intensified during 2020-21 and under the stressed condition SNP showed its superiority over rest of the osmoprotectants in maintaining cooler canopy and sustaining higher yield. Farooq *et al*. (2017) revealed that foliar applied SNP in different doses variably and favourably influenced grain yield of wheat under abiotic stress by accumulation of soluble phenolics, proline and protecting the biological membranes from oxidative damage. It was also observed that canopy temperature progressively increased as the crop entered into anthesis from pre-anthesis stage, irrespective of treatments and year of study, with only exception in the case of trehalose during 2020-21, where a marginal 0.4°C decrease in canopy temperature was noticed from pre-anthesis to anthesis stage, but further increased at subsequent grain-filling stage. It was attributed to further increase in air temperature and evaporative drying of soil moisture with the advancement of crop growth. During 2019-20, CTD values either increased or remained almost same with the advancement of crop growth from pre-anthesis to grain filling stage in all the osmoprotectant treatments except water and no spray where the CTD values increased as the crop passed from pre-anthesis to anthesis stage, but further decreased at grain filling stage indicating relatively higher canopy temperature in water and no spray treatment at the terminal stage.There was relatively mild stress during 2019-20 due to frequent rainfall in the month of March coinciding with anthesis to grain-filling stage, leading to transpirative cooling of canopy with concurrent increase in air temperature causing greater deviation of canopy temperature from air temperature, in the osmoprotectant treatments in particular. On the contrary, during 2020-21 the general trend was almost same values of CTD with minute fluctuations from pre-anthesis to anthesis stage, for all the osmoprotectant treatments (except trehalose) followed by drastic decrease in the values of CTD. The gradual intensification of the degree of heat stress and consequent increase in canopy temperature with concurrent increase in air temperature, especially at grain filling stage could be the reason for this. The increase in CTD in trehalose treatment from pre-anthesis to anthesis was attributed to receipt of more number of sprays (six sprays altogether). The water supplied to the canopy along with the trehalose during the spray might be accountable for transpirative cooling of canopy leading to higher CTD. While comparing the relative effectiveness of the osmoprotectants, it was revealed that in the year of more intense stress, i.e., 2020-21 least reduction of CTD from anthesis to grain filling period was found in SNP treatment (18.7%) followed by potassium nitrate (39.3%), salicylic acid (44.06%), thiourea (45.1%) and trehalose (48.6%). The findings suggested the ability of SNP to maintain favourable cool canopy temperature.Though salicylic acid, thiourea and trehalose were having some extent of positive impact in maintaining higher CTD throughout the post-anthesis period, the positive effect of only trehalose reflected in grain yield, whereas other two could not produce significant effect. This might be due to the relative non

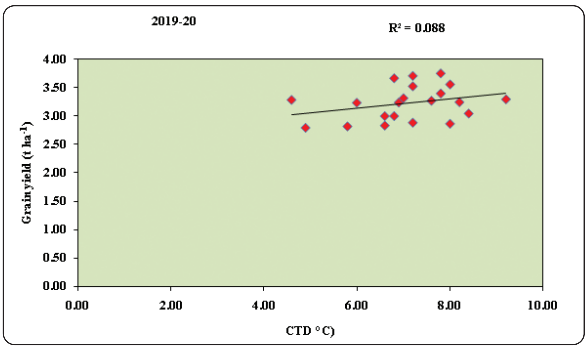

Fig.1: (a) Relationship between canopy temperature depression at grain-filling and grain yield of wheat in 2019-20

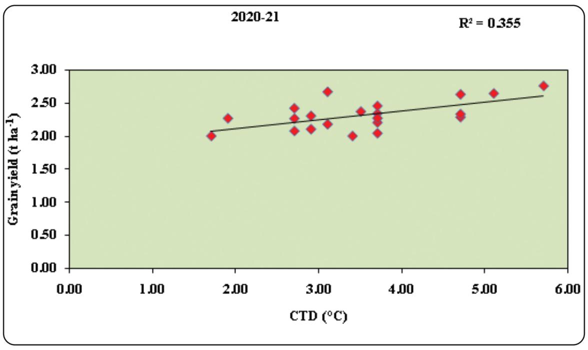

Fig.1: (b) Relationship between canopy temperature depression at grain-filling and grain yield of wheat in 2020-21

effectiveness of those osmoprotectants to influence some stress related physiological or biochemical parameters in a positive direction. Despite a positive correlation with CTD at grain filling and grain yield (r=0.298) during 2019-20, it was not significant due to exposure of the crop to a mild stress as indicated by the canopy temperature. However, correlation analysis indicated a significant negative correlation (r=-0.596 with $p<0.01$) between canopy temperature at grain filling and grain yield during the year 2020-21; while a significant positive correlation (r=0.596 with $p<0.01$) was found between CTD and grain yield in that year experiencing greater intensity of heat stress (Table 4). This can be further supported by linear regression model which showed a stronger relation between CTD and grain yield (R^2=0.355; $p<0.01$) during 2020-21 and a weaker association between these two variables in 2019-20 (Fig. 1a and 1b).

From this two years study,it can be concluded that foliar spray of sodium nitroprusside@ 800 µg ml^{-1} twice at active tillering and anthesis stage served as the most effective osmoprotectant in maintaining cooler canopy and thus sustaining yield. Foliar spray of 2% potassium nitrate twice at active tillering and anthesis stage could also bring about a cooler canopy with higher yield performances. Exogenous applications of these two osmoprotectants could mitigate the ill effects of terminal heat stress in late sown wheat crop grown under eastern sub-Himalayan plains.

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