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Responses of wheat (*Triticum aestivum* L.) genotypes to sulfur aerosols

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Abstract

An investigation into the effects of S- aerosols on five wheat genotypes (viz., GW-322, GW-366, GW-273, GW-173, JW-336), was carried out in field (October, 2016-March, 2017) at Assam Agricultural University (ICR Farm). Simulated S-aerosols viz., $(\text{NH}_4)_2\text{SO}_4$, CaSO_4 , and K_2SO_4 : @ 300 ppm each ($\approx 30 \text{ kg N ha}^{-1}$) along with a control were misted on the plants, on sunny days in the afternoon (after 2–3 P.M.) at three different growth stages i.e. seedling, maximum tillering and spike initiation stages. Therefore, the total concentration of each of the S-aerosols was $900 \text{ ppm} \approx 0.9\%$. The S-aerosols affected the wheat varieties variably which was proved by the significant changes of net photosynthesis rate (P_n), chlorophyll accumulation, enzyme nitrate reductase activity, cell membrane stability (CMS) and cellular $[\text{Ca}^{2+}]$ and $[\text{K}^+]$ concentrations at maximum tillering and spike initiation stages of the crop. The S and N contents in grains, nitrogen use efficiency (NUE) and sulfur use efficiency (SUE) were also analysed for the crop at harvest. The S-aerosols could augment the SUE and NUE in the wheat crop. The genotype GW-366 was the most responsive under the influence of foliar fertilization with S-aerosols. Among the S-aerosols, $(\text{NH}_4)_2\text{SO}_4$ was the most effective in the work.

Keywords: wheat, S-aerosols, calcium, potassium, photosynthesis and NUE, SUE, CMS, lipid peroxidation

Introduction

Wheat is the second most important cereal crop next to rice in India. Wheat occupies an area of 24.23 million hectares with a production of 70.26 million tonnes in the country (Anonymous, 2011-12) [1]. Sulfur plays important role in wheat productivity as it is the constituent of several amino acids viz., cysteine, methionine, thioredoxins, sulfolipids and co-enzymes such as biotin, coenzyme-A, thiamine pyrophosphate and lipoic acid, (Ernst, 1993) [9]. In the past, the responses of crop plants to basal Sulfur have been studied (Singh *et al.*, 2014) [24]. But, little information is available on the physiological responses of wheat crop to Sulfur while applied in the form of aerosols. The responses of wheat crop to Sulfur aerosols, and how (the mechanism) the S-aerosols do affect physiology of wheat crop are explored in the present investigation.

Materials and methods

Experimental site and situation

The investigation was carried out at the ICR farm, Assam Agricultural University, Jorhat during the year 2016-17. Jorhat is situated at $26^\circ 45' \text{ N}$ latitude and $94^\circ 12' \text{ E}$ longitude with an altitude of 87 meters above mean sea level. The climatic conditions of Jorhat as a whole, is subtropical, humid, dry summer and cold winter.

The aerosol treatment condition

The crop was treated with aerosols, and cultivated in the meteorological conditions of cold winter ($9.4\text{-}29.78^\circ\text{C}$) with high humidity (55-99%), low rainfall (0.15-3.78mm) and lower bright sunshine (4.16-6.24) hours (Table 1).

Experimental materials

Five wheat varieties (viz., GW-322, GW-366, GW-273, GW-173, JW-336) were collected from the eastern wheat-growing zone of India (viz., Uttar Pradesh), and used in the experiment.

Crop husbandry

Seeds were put in a container, and Captan @ 2.5 g kg^{-1} seed was added to it. The fungicide was mixed thoroughly with seeds by agitating them for five minutes. The plots were ploughed

thoroughly, mowed and leveled. Recommended doses of N,P,K fertilizers @ 80:46:42 per hectare were applied as basal. The Randomised Block Design (RBD) with two replications was followed in the experiment. The crop was

irrigated regularly during the growth period. The plots were kept weed free always manually. Prophylactic measures were taken to prevent the crop from the attack of insects and pests.

Table 1: Meteorological data during the crop season (December, 2016 to March, 2017)

Months	Temperature (°C)		Average Relative Humidity in the morning (%)	Average Relative Humidity in the evening (%)	Monthly total Rainfall (mm)	Monthly total Bright sunshine (hours)
	Max.	Min.				
December	26.3	11.9	99	62	0.15	6.24
January	25.2	9.4	98	57	2.1	5.77
February	26.8	13.0	95	55	1.38	4.99
March	29.78	15.74	97	58	3.78	4.16
Total					7.41	21.16

Misting of aerosols on plants

The foliage of plants were misted with S-aerosols @ 300 ppm ($\approx 30 \text{ kg S ha}^{-1}$) at three growth stages of the crop viz., at seedling stage, maximum tillering stage and spike initiation stages. Each of the S-aerosol (1000ml for a single stage) was applied in 3 splits on cloud free and clear sunny days in the afternoon when air temperature was low. The cumulative volume of one aerosol solution was 0.9% ($\approx 300 \text{ ppm} \times 3 = 900 \text{ ppm}$) only. While spraying the aerosol solutions, its drifting was checked from one plant to another using hard board as partition between two plots. A digital pH meter with standard pH (4&7) was used to measure the pH of the S-aerosols which were found as distilled water:7.00; $(\text{NH}_4)_2\text{SO}_4$: 5.34; CaSO_4 : 5.49, K_2SO_4 :5.66

Net photosynthesis (P^n) measurement

In the experiment, plants were incubated, and P^n was measured using a transparent, airtight acrylic assimilation chamber (volume $15 \times 15 \times 15 \text{ cm}^3$). Leaves of uniform small size (5-15nos.) were incubated in a condition of ambient (400ppm) CO_2 , temperature (22°C) and light intensity ($22.56 \mu\text{m photons m}^{-2} \text{ sec}^{-1}$) in the laboratory. A volume of 10 cm^3 air samples was collected by clinical syringe injecting through the rubber port of the chamber, and it was pushed into the Environmental Gas Monitor (EGM-4) through its port. Carbon dioxide concentration after incubation was recorded as displayed by the EGM. Then, ppm- CO_2 absorbed per gram plant dry weight per hour was expressed as the rate of net photosynthesis (Larsson and Kershaw, 1975)^[15].

Determination of Chlorophyll content in leaves

A standard protocol as suggested by Arnon (1949)^[2] was used to estimate the total chlorophyll content of the leaves. Ten uniform fresh leaf discs of 5 mm diameter were cut, weighed, and put into 10 ml of 80% acetone in pyrax glass tube. The test tube was sealed by polythene and rubber band to prevent loss of acetone by evaporation. Aluminum foil was used to cover the test tubes for protecting the chlorophyll degradation by photo oxidation. Then, the set up was refrigerated at 4°C for 72 hours. The, supernatant was decanted, its final volume was recorded, and its spectrometric readings were recorded using wave band 645 nm and 663 nm.

Revealing of Nitrate Reductase (NR) activity in plants

Fresh green leaves were collected from field in ice polythene bags for estimation of in- vivo NR activity (Keeper *et al.*, 1971)^[14]. Leaf samples of $10\text{-}15 \text{ mm}^2$ size (200mg) were cut and put into 2.5 ml solution of 200 mM phosphate buffer (pH 7.5), 30 mM KNO_3 , 5 % (v/v) propanol in assay tubes. The samples were incubated for 30 minutes at $33 \pm 2^\circ\text{C}$. Then, the tubes were put in boiling water bath for 15 minutes to stop the

reaction of the mixture. After cooling, 0.2 ml of the reaction mixture was taken into a test tube to which 1 ml of 1% sulphanilamide and 1 ml of 0.025 N-(1-naphthyl)-ethylene diamine dihydrochloride solution were added, and kept for 15 minutes to develop the pink colour in the solution. Finally, the absorbance of the solution was read at 540nm in spectrophotometer. A standard curve for nitrite assay was prepared for calculation of NR activity which was expressed as $\mu\text{moles NO}_2 \text{ formed g}^{-1} \text{ fresh tissue wt. hr}^{-1}$.

Assessment of Cell membrane stability (CMS) and detection of cellular $[\text{Ca}^{2+}]$ & $[\text{K}^+]$

Fresh leaf samples were collected in polythene ice bags. Twenty pieces of leaves (1 cm^2 size) were immersed into 20 cm^3 distilled water in plastic bottles of 60 cm^3 capacity. The bottles were made air tight to avoid leaking of the solution. The samples were checked using magnetic stirrer using electrically operated Vortex Mixture. Thus, leaf samples were washed thrice with distilled water (each 10 min, 20 cm^3) to collect the intercellular ions. Then, the exchangeable ions of the same leaf tissues were extracted by eluting twice in 25 mM Sr_2Cl_2 (each 1 h 20 cm^3) solution. The solutions were collected in plastic bottles, and the plant samples were oven dried at 60°C to a constant weigh. The CMS was calculated using the electrical conductivity readings of these solutions as per the protocol suggested by Bharali *et al.* (2015)^[4]. The Flame photometric method (Jackson, 1973)^[13] was used to measure the $[\text{K}^+]$, whereas, colorimetric titration method (Richards, 1954)^[21] was employed for estimation of intercellular and exchangeable $[\text{Ca}^{2+}]$. The leaf tissues were oven dried, and ion contents were expressed as mM per gram dry weight of the leaves.

Nitrogen use efficiency (NUE)

The modified Kjeldahl method (Jackson, 1973)^[13] was used to estimate total Nitrogen (%) in grains based on the catalytic conversion of organic nitrogen into ammonia, and its acid base titration. The grain NUE was calculated as the multiplication of the per cent Nitrogen in grain by total grain yield per unit area.

Sulfur use Efficiency (SUE) in grains

The SUE of the wheat crop was calculated by multiplying the per cent S content of grain with the grain yield (tha^{-1}).

Statistical analysis

Data for each plant parameter was analysed by Fisher's method of analysis of variance (Panse and Sukhatme, 1978)^[19]. Significance or non-significance of variance due to the treatments was determined by the respective 'F' values. The

standard error of the means (S.Ed. \pm) was calculated by using the following expression.

$$S.Ed(\pm) = \frac{\sqrt{2 \times \text{error mean square}}}{\text{Pooled number of replication}}$$

The critical difference between a pair of treatment means was judged by comparing the values obtained from the product of S.Ed (\pm) and Probability at (0.05).

Results

The results obtained in the investigation into the 'Responses of wheat (*Triticum aestivum* L.) genotypes to Sulfur aerosols' under field conditions are presented in tabular form. The main aim of the work was to study the responses of the wheat genotype in terms of higher SUE, and NUE, to foliar application of Sulfur aerosols. The study, too, delved into the mechanism of altering the physiology of wheat crop by S-aerosol.

Data presented in Table 2 showed that there were significant differences in Pⁿ among the varieties, and among treatments at maximum tillering but for treatments only at spike initiation stage. Among the varieties, the GW-322 (6601.4ppm CO₂ absorbed g⁻¹d.w.hr⁻¹) had the highest Pⁿ value followed by GW-273 (6521.1 ppm CO₂ absorbed g⁻¹d.w.hr⁻¹) > GW-366(6459.7 ppm CO₂ absorbed g⁻¹d.w.hr⁻¹)>GW-173(6306.26 ppm CO₂ absorbed g⁻¹d.w.hr⁻¹), and the lowest Pⁿ was found in JW-336(5932.5ppm CO₂ absorbed g⁻¹d.w.hr⁻¹). On an average, among the S aerosols, (NH₄)₂SO₄(6827.9ppm CO₂ absorbed g⁻¹d.w.hr⁻¹) had the highest Pⁿ as compared to control (5446.56ppm CO₂ absorbed g⁻¹d.w.hr⁻¹) at maximum tillering stage.

At spike initiation stage, the variety GW-173 (6648.1ppm CO₂ absorbed g⁻¹d.w.hr⁻¹) showed the maximum Pⁿ followed by GW-322 (6535.9 ppm CO₂ absorbed g⁻¹d.w.hr⁻¹)>GW- 273 (6448.9 ppm CO₂ absorbed g⁻¹d.w.hr⁻¹)>GW-366 (6116.7ppm CO₂ absorbed g⁻¹d.w.hr⁻¹), and the lowest Pⁿ was recorded in JW-336 (5905.7ppm CO₂ absorbed g⁻¹d.w.hr⁻¹) at the spike initiation stage. On an average, (NH₄)₂SO₄ registered the highest Pⁿ (6501.28 ppm CO₂ absorbed g⁻¹d.w.hr⁻¹) as compared to the control (5788.4 ppm CO₂ absorbed g⁻¹d.w.hr⁻¹).

Data presented in Table 3 reveal that the total chlorophyll content in leaf tissue varied significantly among the treatments, and among the varieties at the maximum tillering and spike initiation stages. The highest total chlorophyll content was found in the variety GW-273(2.093mg g⁻¹fw of leaf) >GW- 336 (2.034mg g⁻¹fw of leaf) >GW-322 (1.977mg g⁻¹fw of leaf) >GW-173 (1.976), and the lowest value was in JW-336 (1.847mg g⁻¹fw of leaf). Among the S-aerosol treatments, (NH₄)₂SO₄ recorded the highest total chlorophyll content in leaf tissue (2.262 mg g⁻¹fw of leaf) as compared to the control plants (1.704mg g⁻¹fw of leaf).

At spike initiation stage, over all, the variety GW-273 recorded the highest total chlorophyll content (2.002mg g⁻¹fw of leaf) >GW-366 (1.962mg g⁻¹fw of leaf) >GW-322 (1.906 mg g⁻¹fw of leaf) >GW- 173 (1.879 mg g⁻¹fw of leaf), and the lowest one was found in JW-366 (1.777mg g⁻¹fw of leaf). On an average, among the S-aerosol treatments, (NH₄)₂SO₄ produced the maximum total chlorophyll content in leaf tissues (2.191 mg g⁻¹fw of leaf) as compared to the controlled plants (1.607 mg g⁻¹fw of leaf).

Data presented in Table 4 indicate that there were significant variations in nitrate reductase (NR) activity in leaf tissues among the treatments, and among the varieties at the

maximum tillering stage. The highest NR activity was observed in GW-273 (0.698 nmol NO₂⁻ g⁻¹fw of leaf hr⁻¹) >GW- 366 (0.684nmol NO₂⁻ g⁻¹fw of leaf hr⁻¹) >GW-322 (0.644nmol NO₂⁻ g⁻¹fw of leaf hr⁻¹) >GW-173 (0.643nmol NO₂⁻ g⁻¹fw of leaf hr⁻¹), and the lowest was in JW-336 (0.573 nmol NO₂⁻ g⁻¹fw of leaf hr⁻¹). Among the S-aerosol treatments, (NH₄)₂SO₄ exhibited the highest NR activity (0.705nmol NO₂⁻ g⁻¹ fw of leaf hr⁻¹) as compared to the Controlled plants (0.532nmol NO₂⁻ g⁻¹ f.w. of leaf hr⁻¹) at the maximum tillering stage. The data presented in Table-4 also reveal non-significant differences of NR activity among the treatments, and a significant variation of NR activity among the varieties at spike initiation stage. The highest NR activity was in GW-366 (0.719nmol NO₂⁻ g⁻¹fw leaf hr⁻¹) followed by GW- 273 (0.707 nmol NO₂⁻ g⁻¹ fw of leaf hr⁻¹) >GW-322 (0.685nmol NO₂⁻ g⁻¹ fw of leaf hr⁻¹) >GW-173 (0.676 nmol NO₂⁻ g⁻¹ fw of leaf hr⁻¹), and the lowest NR activity was in JW-336 (0.618nmol NO₂⁻ g⁻¹ fw of leaf hr⁻¹). On an average, among the S-aerosol treatments, (NH₄)₂SO₄ activated the highest NR (0.7nmol NO₂⁻ g⁻¹ fw of leaf hr⁻¹) as compared to the controlled one (0.65nmol NO₂⁻ g⁻¹ fw of leaf hr⁻¹) at spike initiation stage.

Data in Table 5 reveal that there were non-significant differences of lipid peroxidase activity among the treatments and among the varieties at the maximum tillering and spike initiation stages. At maximum tillering stage, the highest lipid peroxidation in leaf was observed in GW- 273 (3.376 nmol MDA g⁻¹ f.w) followed by GW-322 (2.955 nmol MDA g⁻¹ f.w) >GW-366 (2.653 nmol MDA g⁻¹ f.w) >GW-173 (2.359 nmol MDA g⁻¹ f.w), and the lowest was observed in JW-336 (2.157 nmol MDA g⁻¹ f.w). On an average, among the S-aerosol treatments, plants treated with CaSO₄ had the highest rate of lipid peroxidation (2.976nmol MDA g⁻¹ f.w) as compared to controlled plants (2.449nmol MDA g⁻¹ f.w). At spike initiation stage, on an average, the highest lipid peroxidation was found in JW-336 (3.843 nmol MDA g⁻¹ f.w) >GW- 173 (3.752 nmol MDA g⁻¹ f.w), > GW-366 (3.737 nmol MDA g⁻¹ f.w) >GW-273 (3.689 nmol MDA g⁻¹ f.w), and the lowest was recorded in GW-322 (3.574 nmol MDA g⁻¹ f.w). The plants treated with CaSO₄ showed the highest lipid peroxidatn (4.108 nmol MDA g⁻¹ f.w) as compared to controlled plants (3.352 nmol MDA g⁻¹ f.w) at spike initiation stage also.

The data displayed in Table-6 indicate non-significant variations in CMS among the genotypes and among the treatments at both the maximum tillering and spike initiation stages. At maximum tillering stage, the highest CMS was exhibited by GW- 322 (0.33)>followed by GW-273 (0.28)>GW-366 (0.269) >JW-336 (0.236), and the lowest CMS was in JW-336 (0.2). On an average, the highest CMS was in CaSO₄ (0.262), and the controlled plants (0.295) had the lowest of it at maximum tillering stage. At spike initiation stage, among the varieties, the highest CMS was found in GW-173 (0.3)>GW-273 (0.251)>JW-336 (0.213)>GW-366 (0.15)>GW-173 (0.02), and the lowest CMS was found in JW-322 (0.131). On an average, the maximum CMS found in CaSO₄ (0.23) and the lowest was in case of controlled plants (0.015) at spike initiation stage.

Data portrayed in Table 7 (A&B) reveal that sulfur and nitrogen contents in wheat seed at harvest varied non significantly among the treatments and among the genotypes (except for N). On an average, the maximum sulfur content in seed was found in GW-322(0.347)>GW-273 (0.341%) >GW-173(0.323%)> JW-336(0.303%), and the lowest sulfur content was found in GW-366 (0.0.283%). Plants treated with

(NH₄)₂SO₄ (0.328%) had the highest value as compared to the controlled plants (0.316%). On an average, the maximum Nitrogen content in seed was found in JW-336 (0.35%)>GW-173(0.293%)>GW-322(0.267%) >GW- 273 (0.238%), and the lowest was found in GW-366 (0.193%). Plants treated with (NH₄)₂SO₄ (0.34%) had the highest N contents as compared to control (0.243%).

Data in Table 8 (A) reveal SUE in seed varied significantly among the treatments but it was non-significant among the genotypes. On an average, the maximum Sulfur content in seed was found in GW-366 (0.199%)>GW-322(0.189%) >GW-273 (0.178%)>GW-173(0.172%), and the lowest was in JW-336(0.157%). Among the aerosol treatments, (NH₄)₂SO₄ (0.23%) showed the highest value in plants as compared to the control (0.131%). Data presented in Table 8(B) reveal that NUE varied significantly among the treatments and among the genotypes. On an average, the maximum NUE in seed was found GW-322(0.217%)>GW-366(0.216%) >GW-273 (0.211%)>GW-173(0.148%), and the lowest was found in JW-336 (0.125%). Plants treated with (NH₄)₂SO₄ (0.253%) had the highest NUE as compared to control (0.141%).

Data presented in Table 9 reveal that there were non-significant variations of intercellular and exchangeable [Ca²⁺] among the varieties and among the treatments (except intercellular [Ca²⁺]). On an average, the variety GW-366 (1.428 mgg⁻¹d.w.) registered the highest intercellular [Ca²⁺]

followed by JW-336 (1.28 mgg⁻¹d.w.)>GW-273,173 (1.224mgg⁻¹d.w.) while the lowest [Ca²⁺] was in GW-322 (1.204 mgg⁻¹d.w.). Overall, CaSO₄ showed the maximum [Ca²⁺] (1.511mgg⁻¹d.w.) as compared to control (0.945mgg⁻¹d.w.). On an average, the variety JW-336(1.316mgg⁻¹d.w.) registered the highest exchangeable [Ca²⁺] followed by GW-322 (1.305mgg⁻¹d.w.)> GW-366(1.286mgg⁻¹d.w.)>GW-173 (1.246mgg⁻¹d.w.), while the lowest was in GW-273 (1.21mgg⁻¹d.w.). Overall, CaSO₄ showed the maximum [Ca²⁺] (1.396mgg⁻¹d.w) as compared to Control (1.26mgg⁻¹d.w.).

Data presented in Table 10 reveal that there were non-significant variations of intercellular and exchangeable [K⁺] among the varieties and among the treatments. On an average, the variety GW-366 (1.909mgg⁻¹d.w.) registered the highest intercellular [K⁺] followed by GW-273 (1.644)> JW-336(1.613) >GW-173(1.499mgg⁻¹d.w.), while the lowest intercellular [K⁺] was in GW-322 (1.306mgg⁻¹d.w.). Overall, K₂SO₄ (1.741mgg⁻¹d.w) produced the maximum intercellular [K⁺] as compared to the control (1.441mgg⁻¹d.w.). The variety GW-366 (2.079mgg⁻¹d.w) registered the highest exchangeable [K⁺] followed by GW-173 (1.791mgg⁻¹d.w.)> JW-336(1.648)>GW-273(1.638mgg⁻¹d.w.)>GW-322 (1.458mgg⁻¹d.w.), while the lowest [K⁺] was in GW-322 (1.889mgg⁻¹d.w.). Overall, K₂SO₄ showed the maximum [K⁺] (1.955mgg⁻¹d.w.), as compared to the control (1.518mgg⁻¹d.w.).

Table 2: Effect of sulfur aerosols on rate of net photosynthesis (Pⁿ) of wheat crop at maximum tillering and spike initiation stages

Variety (V)↓ Treatment (T)→	P ⁿ (ppm CO ₂ absorbed g ⁻¹ d.w hr ⁻¹) at the maximum tillering stage					P ⁿ (ppm CO ₂ absorbed g ⁻¹ d.w hr ⁻¹) at spike initiation stage				
	Sulfur aerosols (30 kg ha ⁻¹)					Sulfur aerosols (30 kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	6,582.40	6,862.15	7,077.95	5,883.30	6,601.45	6,584.00	6,555.45	6,865.25	5,600.70	6,401.35
GW-366	6,295.50	7,133.95	6,760.85	5,648.65	6,459.74	6,183.95	6,915.20	5,650.60	5,716.95	6,116.68
GW-273	6,609.35	7,344.55	6,733.35	5,397.30	6,521.14	6,383.85	6,733.35	7,278.65	5,399.65	6,448.88
GW- 173	6,584.85	6,683.35	6,732.40	5,224.43	6,306.26	6,616.70	6,583.60	6,708.30	6,683.90	6,648.13
JW-336	6,492.60	6,115.55	6,042.85	5,079.10	5,932.53	6,545.55	5,718.80	5,817.45	5,541.00	5,905.70
Mean	6,512.94	6,827.91	6,669.48	5,446.56		6,462.81	6,501.28	6,464.05	5,788.44	
Factor	CD (0.05)		S.Ed(±)			CD (0.05)		S.Ed(±)		
Factor (V)	239.932		113.794			n.s.		250.885		
Factor (T)	214.602		101.781			473.136		224.398		
Interaction (TxV)	479.864		227.589			n.s.		501.769		

Table 3: Effect of Sulfur aerosols on total Chlorophyll (Chll) content of wheat crop at maximum tillering and spike initiation stages

Variety (V)↓ Treatment (T)→	Total Chll (mg g ⁻¹ f.w. of leaf) content at maximum tillering stage					Total Chll (mg g ⁻¹ f.w. of leaf) content at spike initiation stage				
	Sulfur aerosols (30kg ha ⁻¹)					Sulfur aerosols (30kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	1.904	2.36	2.048	1.595	1.977	1.839	2.33	1.957	1.499	1.906
GW-366	2.147	2.44	1.91	1.638	2.034	2.085	2.305	1.841	1.617	1.962
GW-273	1.912	2.499	2.198	1.765	2.093	1.823	2.426	2.093	1.667	2.002
GW- 173	1.799	2.197	2.175	1.735	1.976	1.75	2.125	2.087	1.555	1.879
JW-336	1.717	1.815	2.066	1.79	1.847	1.693	1.769	1.953	1.695	1.777
Mean	1.896	2.262	2.079	1.704		1.838	2.191	1.986	1.607	
Factor	CD (0.05)		S.Ed(±)			CD (0.05)		S.Ed(±)		
Factor (V)	0.042		0.02			0.059		0.028		
Factor (T)	0.037		0.018			0.053		0.025		
Interaction (TXV)	0.084		0.04			0.118		0.056		

Table 4: Effect of Sulfur aerosols on Nitrate reductase activity of wheat crop at maximum tillering and spike initiation stages

Variety(V) Treatment(T)→	Nitrate reductase activity (nmol NO ₂ ⁻ g ⁻¹ f.w. of leaf hr ⁻¹) at the maximum tillering stage					Nitrate reductase activity (nmol NO ₂ ⁻ g ⁻¹ f.w. of leaf hr ⁻¹) at spike initiation stages				
	Sulfur aerosols (30kg ha ⁻¹)					Sulfur aerosols (30kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	0.694	0.717	0.706	0.46	0.644	0.717	0.73	0.717	0.579	0.685
GW-366	0.712	0.739	0.7	0.587	0.684	0.726	0.75	0.703	0.699	0.719
GW-273	0.661	0.765	0.72	0.647	0.698	0.667	0.76	0.73	0.67	0.707
GW- 173	0.662	0.696	0.677	0.537	0.643	0.684	0.658	0.682	0.68	0.676
JW-336	0.628	0.61	0.622	0.432	0.573	0.593	0.605	0.648	0.625	0.618
Mean	0.671	0.705	0.685	0.532		0.677	0.7	0.696	0.65	
Factor	CD (0.05)		S.Ed(±)			CD (0.05)	S.Ed(±)			
Factor (V)	0.015		0.007			0.016	0.008			
Factor (T)	0.013		0.006			0.014	0.007			
Interaction (TxV)	0.029		0.014			0.032	0.015			

Table 5: Effect of Sulfur aerosols on lipid peroxidase activity of the wheat crop at maximum tillering and spike initiation stages

Variety (V) Treatment (T)→	Lipid peroxidation (nmol MDA g ⁻¹ f.w.) at the maximum tillering stage					Lipid peroxidase (nmol MDA g ⁻¹ f.w.) at spike initiation stages				
	Sulfur aerosols (30kg ha ⁻¹)					Sulfur aerosols (30kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	3.967	1.153	3.595	3.105	2.955	3.767	3.96	3.283	3.288	3.574
GW-366	3.54	3.154	1.739	2.18	2.653	3.912	3.992	3.805	3.239	3.737
GW-273	2.895	3.418	3.69	3.501	3.376	4.185	3.594	3.507	3.473	3.689
GW- 173	2.175	2.701	2.262	2.297	2.359	4.725	3.96	2.882	3.443	3.752
JW-336	2.305	2.68	2.485	1.16	2.157	3.949	3.278	4.825	3.319	3.843
Mean	2.976	2.621	2.754	2.449		4.108	3.757	3.66	3.352	
Factor	CD (0.05)		S.Ed(±)			CD (0.05)	S.Ed(±)			
Factor (V)	n.s.		0.442			n.s.	0.285			
Factor (T)	n.s.		0.395			n.s.	0.255			
Interaction (TxV)	n.s.		0.884			n.s.	0.57			
n.s. Non significant										

Table 6: Effect of sulphur aerosols on Cell Membrane Stability (CMS) of the wheat crop at maximum tillering and spike initiation stages

Variety (V) Treatment (T)→	CMS at the maximum tillering stage					CMS at spike initiation stage				
	Sulfur aerosols (30kg ha ⁻¹)					Sulfur aerosols (30kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	0.133	0.019	0.026	0.018	0.049	0.026	0.022	0.022	0.013	0.021
GW-366	0.022	0.021	0.024	0.13	0.049	0.022	0.024	0.022	0.016	0.021
GW-273	0.023	0.02	0.027	0.019	0.022	0.026	0.026	0.02	0.015	0.021
GW- 173	0.125	0.02	0.021	0.021	0.047	0.022	0.024	0.019	0.016	0.02
JW-336	0.118	0.017	0.019	0.018	0.043	0.019	0.018	0.017	0.015	0.017
Mean	0.084	0.019	0.023	0.041		0.023	0.022	0.02	0.015	
Factor	CD (0.05)		S.Ed(±)			S.Ed(±)				
Factor (V)	n.s.		0.033			n.s.	0.072			
Factor (T)	n.s.		0.03			n.s.	0.064			
Interaction (TxV) n.s. Non Significant	n.s.		0.066			n.s.	0.143			

Table 7: Effect of sulfur aerosols on sulphur and Nitrogen contents in grains at harvest of the wheat crop

Variety (V) Treatment (T)	(A) Sulfur content (%) in grain at harvest					(B) Nitrogen content (%) in grain at harvest				
	Sulfur aerosols (30 kg ha ⁻¹)					Sulfur aerosols (30 kg ha ⁻¹)				
	CaSO ₄	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	0.346	0.153	0.332	0.275	0.308	0.267	0.268	0.292	0.287	0.27
GW-366	0.276	0.214	0.357	0.156	0.046	0.193	0.288	0.214	0.271	0.256
GW-273	0.34	0.266	0.332	0.193	0.163	0.238	0.291	0.3	0.195	0.277
GW- 173	0.295	0.282	0.362	0.18	0.347	0.293	0.318	0.272	0.272	0.285
JW-336	0.326	0.315	0.32	0.417	0.35	0.35	0.293	0.255	0.223	0.26
Mean	0.316	0.246	0.34	0.244	0.243		0.291	0.267	0.249	
Factor	CD (0.05)		S.Ed(±)			CD (0.05)	S.Ed(±)			
Factor (V)	n.s.		0.025			0.093	0.044			
Factor (T)	n.s.		0.022			n.s.	0.039			
Interaction (TxV) n.s. Non Significant	n.s.		0.049			n.s.	0.088			

Table 8: Effect of sulfur aerosols on Sulfur Use efficiency (SUE) Nitrogen Use Efficiency (NUE) in grains at harvest of the wheat crop

Variety(V) ↓ Treatment (T) →	(A) SUE (%) in grain at harvest					(B) NUE (%) in grain at harvest				
	Sulfur aerosols (30 kg ha ⁻¹)					Sulfur aerosols (30 kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	0.193	0.227	0.188	0.147	0.189	0.18	0.357	0.205	0.128	0.217
GW-366	0.198	0.306	0.204	0.086	0.199	0.15	0.273	0.264	0.176	0.216
GW-273	0.164	0.202	0.194	0.153	0.178	0.263	0.296	0.19	0.097	0.211
GW- 173	0.199	0.215	0.134	0.141	0.172	0.131	0.203	0.111	0.148	0.148
JW-336	0.172	0.201	0.13	0.126	0.157	0.114	0.135	0.092	0.157	0.125
Mean	0.185	0.23	0.17	0.131		0.168	0.253	0.172	0.141	
Factor		CD (0.05)	S.Ed(±)			CD (0.05)	S.Ed(±)			
Factor (V)		n.s.	0.046			0.068	0.032			
Factor (T)		0.086	0.041			0.061	0.029			
Interaction (TxV) n.s.: Non Significant		n.s.	0.091			n.s.	0.064			

Table 9: Effect of Sulfur aerosols on Calcium content of the wheat crop at spike initiation stage

Variety (V) ↓ Treatment (T) →	Exchangeable [Ca ²⁺] (mgg ⁻¹ d. w.)					Intercellular [Ca ²⁺] (mgg ⁻¹ d.w.)				
	Sulfur aerosols(30 kg ha ⁻¹)					Sulfur aerosols(30 kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	1.895	1.43	1.295	1.825	1.611	1.535	1.335	1.385	0.56	1.204
GW-366	1.74	1.74	1.14	1.19	1.453	1.86	1.33	1.455	1.065	1.428
GW-273	1.125	1.33	1.175	1.22	1.213	1.645	1.345	1.275	0.63	1.224
GW- 173	1.325	1.135	1.21	1.145	1.204	1.225	1.29	1.04	1.34	1.224
JW-336	1.34	1.14	1.265	1.32	1.266	1.29	1.495	1.205	1.13	1.28
Mean	1.485	1.355	1.217	1.34		1.511	1.359	1.272	0.945	
Factor		CD (0.05)	S.Ed(±)			CD (0.05)	S.Ed(±)			
Factor (V)		n.s.	0.16			n.s.	0.153			
Factor (T)		n.s.	0.143			0.288	0.137			
Interaction (TxV) n.s. Non Significant		n.s.	0.32			n.s.	0.306			

Table 10: Effect of Sulphur aerosols on Potassium content of the wheat crop at spike initiation stage

Variety (V) ↓ Treatment (T) →	Exchangeable [K ⁺](mgg ⁻¹ d.w.)					Intercellular [K ⁺](mgg ⁻¹ d.w.)				
	Sulfur aerosols(30 kg ha ⁻¹)					Sulfur aerosols(30 kg ha ⁻¹)				
	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean	CaSO ₄	(NH ₄) ₂ SO ₄	K ₂ SO ₄	Control	Mean
GW-322	1.475	1.34	1.625	1.39	1.458	1.465	1.295	1.22	1.245	1.306
GW-366	2.22	2.38	2.425	1.29	2.079	1.795	1.66	2.265	1.915	1.909
GW-273	1.155	1.94	1.635	1.82	1.638	1.96	1.84	1.435	1.34	1.644
GW- 173	1.855	1.194	2.35	1.767	1.791	1.705	1.57	1.445	1.275	1.499
JW-336	1.88	1.79	1.74	1.325	1.684	1.29	1.39	2.34	1.43	1.613
Mean	1.717	1.729	1.955	1.518		1.643	1.551	1.741	1.441	
Factor		CD (0.05)	S.Ed(±)			CD (0.05)	S.Ed(±)			
Factor (V))		n.s.	0.197			n.s.	0.196			
Factor (T)		n.s.	0.176			n.s.	0.175			
Interaction (TxV) n.s. Non Significant		n.s.	0.394			n.s.	0.392			

Discussion

The main objective behind the recent inquisition was to look into the responses of wheat genotypes to misting of S-aerosols at two prominent spike initiations and heading stages of wheat crop. It unfurled a few natural features of importance, and exhilarated reality on the physiological traits of the wheat crop that are influenced by the Sulfur aerosols. It was, too, attempted to delve down the mechanisms of changing and resetting of cell membrane stability of wheat crop exposed to the S-aerosols.

In the investigation, rate of Pⁿ increased significantly due to Sulfur aerosols. Among the aerosols, the highest increment was in (NH₄)₂SO₄ (20.2%) followed by K₂SO₄ (18.3%), and the lowest was in CaSO₄ (16.3%) as compared to control at maximum tillering stage. At spike initiation stage also, rate of Pⁿ increased significantly due to the S-aerosols. Among the aerosols, K₂SO₄ (12.4%) increased maximum rate of Pⁿ followed by CaSO₄ (10.4%) & (NH₄)₂SO₄ (10.4%) as compared to control. The rate of Pⁿ in wheat varieties varied significantly following foliar feeding. The Pⁿ rate in crop varieties also differed due to the differences in nitrogen input

from the aerosols at their growth stages. In the study, the S-aerosols were applied at two different growth stages of wheat crop. The Pⁿ rate in the crop varieties also differed due to the differences in nitrogen input from the aerosols at their growth stages. The Pⁿ depression in higher plants by nitrogenous pollutants was reported previously by Hill and Bennett (1970)^[11]; Bharali and Bates (2015, 2016)^[4, 5]. One of the reasons for decreases in Pⁿ rate was the presence of higher chloroplastic pH due to outnumbering protons in the chloroplast than required (six) for a NO₂ reduction. Such build up in pH is prone to damaging of ribulose-1-5- bisphosphate carboxylase/oxygenase. The secondary influence on the ultra-structure of the chloroplast in plants caused by NO₂ might be linked to Pⁿ depression (Heldt *et al.*, 1986)^[10].

In the present study, NR activity increased significantly due to Sulfur aerosols. Among the aerosols, the highest increment was in (NH₄)₂SO₄ (24.5%) followed by K₂SO₄ (22.3%), and the lowest was in CaSO₄ (20.7%) as compared to control at maximum tillering stage. At spike initiation stage also, NR activity increased significantly due to the aerosols. Among the aerosols, (NH₄)₂SO₄ (7.1%) increased maximum NR activity

followed by K_2SO_4 (6.6%), and the lowest was in $CaSO_4$ (3.9%) as compared to control. It was noted that the NR activity in wheat was lower in shoots at the different stages of the crop. The wheat varieties also differed in respect of NR activity in their leaves. It is a fact that NR enzyme catalyses the reduction of nitrate to nitrite, and its levels of activity are determined by the supply of nitrate. The inhibition of NR activity might be due to the feed-back inhibition of the larger ammonium ions (Hisamatsu *et al.*, 1988) [12].

In the experiment, chlorophyll content in leaf tissue increased significantly due to Sulfur aerosols. Among the aerosols, the highest increment was in $(NH_4)_2SO_4$ (24.6%) followed by K_2SO_4 (18%), and the lowest was in $CaSO_4$ (10.1%) as compared to control at maximum tillering stage. At spike initiation stage also, chlorophyll content in leaf tissue increased significantly due to the aerosols. Among the aerosols, K_2SO_4 (23%) increased the maximum chlorophyll content in leaf tissue followed by $CaSO_4$ (20.4%), and the lowest was in $(NH_4)_2SO_4$ (0.63%) as compared to the controlled plants. Nitrogen impacts on structure of chlorophyll and protein molecules (Bojovič & Markovič, 2009) [6]. It was reported that foliar feeding of plants with amino acids, microelements and Sulfur increased the productivity of spring barley (Staugaitis, Petrauskienė, 2006 [22]; Pranckietienė *et al.*, 2015 [16]) due to chlorophyll enhancement in leaves.

In the study, NUE increased significantly due to the S-aerosols. Among the aerosols, the highest increment in NUE was shown by $(NH_4)_2SO_4$ (44.2%) followed by K_2SO_4 (18%), and the lowest was recorded by $CaSO_4$ (16%) as compared to controlled one. The S-aerosols increased NUE in the varieties, and the varieties showed remarkable differences in their NUE in grains. However, NUE in grains decreased with the higher concentration of nitrogen aerosols in the experiment. Cations viz., $[Ca^{2+}]$ and $[K^+]$ present in intercellular and exchangeable sites varied non-significantly among the varieties due to the S-aerosol treatments. The S-aerosols viz., $(NH_4)_2SO_4$, $CaSO_4$, K_2SO_4 couldn't deplete cations substantially from the water free spaces and exchangeable sites of the cell as compared to controlled plants, because the aerosols were in the same range of pH (5.34 to 5.66), where it was neither acidic nor very basic in nature. The S-aerosols exerted beneficial effects on most of the physiological parameters including yield and yield attributes. There were no significant changes in CMS of the wheat varieties as no significant impacts of the S-aerosols were found on either the lipid peroxidation or leaching of cations from the cellular locations. Rather, the aerosols increased N and S contents and their use efficiencies (i.e. NUE & SUE) in grains at harvest. The S-aerosols were not involved in damaging the membrane. Therefore, no loosening or breaking of the membrane to leach out the ions which were plausibly adhered to the intercellular and exchangeable sites of the cells. Hence, whatever intercellular and exchangeable ions were present; they could be recovered in the extracting solutions following incubations with distilled water and $SrCl_2$ respectively. Moreover, the S-aerosols could increase the $[Ca^{2+}]$ and $[K^+]$ ions in the cellular locations following the treatments of wheat crop with respective aerosols. Thus, in case of Ca_2SO_4 , intercellular $[Ca^{2+}]$ (by 0.54-9.7%) and exchangeable $[Ca^{2+}]$ (by 4.8-21.1%) increased as compared to the control. Likewise, the intercellular $[k^+]$ (by 34-46.9%) and exchangeable $[k^+]$ (by 21.8-27.7%) enriched in the cellular locations following the K_2SO_4 aerosol treatment. In plant cells, calcium plays integral role on fabricating plasma membrane, and supports in sustaining membrane stability

(Legge *et al.*, 1982) [16]. Calcium ions characteristically combine with modulator proteins e.g. Calmodulin (CaM) and it forms Ca-CaM (Dieter, 1984) [8] to display sub-clinical signaling that brings about physiological differences and productivity of the crop.

In general, ammonia (gaseous NH_3 or particulate NH_4^+) or so called NHy (Bharali *et al.*, 2017) [3] at high concentration (>1 Mm) is toxic to plants (Mehree and Mohr, 1989) [18]. Apart from disconnecting electron transport chain in chloroplast (Lilley *et al.*, 1975) [17], ammonia lessens cations viz., $[Ca^{2+}]$, $[K^+]$ and $[Mg^{2+}]$ (Boxman. *et al.*, 1991) [7]. In contrast, in the present investigation, perhaps, $(NH_4)_2SO_4$ on dissociation produced NH_4^+ and SO_4^{2-} where, SO_4^{2-} neutralized the cellular acidity produced by NH_4^+ , and it was channeled to participate in N-assimilation pathway to increase its use efficiency in the grain. Because, in aqueous solution, SO_2 exists in three ionic forms viz. Sulphite (SO_3^{2-}) leading to SO_4^{2-} at pH >6.0 , Bisulphite (HSO_3^-) at pH 2-4 and un-dissociated sulphurous acid (H_2SO_3) dominates at pH 1 (Sounders and Wood, 1973) [23].

In the present work, GW-366 followed by 273 emerged as the most efficient genotype physiologically, as these possessed higher NUE, SUE, CMS at crop maturity. The genotype GW-366, also contained higher cellular ions viz., $[Ca^{2+}]$ and $[K^+]$ in the intercellular and exchangeable sites. Further, as regard to the field application of sulfur aerosols, $(NH_4)_2SO_4$ was more effective than $CaSO_4$ and $>K_2SO_4$ and controlled distilled water. Thus, among the aerosols, $(NH_4)_2SO_4$ @30kg ha^{-1} could be applied as foliar spray to explore the potential economic yield of the selected wheat varieties. Because, $(NH_4)_2SO_4$ not only supported S and N-nutrition, but also maintained the CMS with higher retention of intercellular and exchangeable ions in wheat.

From the forgoing dialogue, it is evident that S and N derived from the aerosol $(NH_4)_2SO_4$ might have played pivotal roles in physiological process of wheat crop including the quality and quantity of wheat storage protein. N is one of the important constituents of nucleotides, proteins, chlorophyll and enzymes. Similarly, sulfur is involved in plant growth, metabolism and enzymatic reactions. It's known that sulfur is one of the constituents of amino acids such as cystine, cysteine, and methionine. However, identification of molecular markers e.g. QTL for physiological traits for NUE & SUE, amino acids and fatty acids in wheat varieties (e.g. 'GW-273) following incubation in S-aerosols remains as one of the important thrusts in wheat improvement program in near future.

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