INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 11, No. 6, p. 32-45, 2017

RESEARCH PAPER OPEN ACCESS

Effects of sulphur and chlorine on photosynthetic parameters, antioxidant enzyme activities and yield in fresh corn grown under field conditions

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Article published on December 21, 2017

Key words: Photosynthetic rate, Antioxidant enzyme, Yield, Fresh corn, Sulphur

Abstract

Sulphur (S) and chlorine (Cl) roles in plant physiology, abiotic stress tolerance, yield and quality improvements in different crop species have been widely acknowledged. Despite all this however, their influences in fresh corn still remain largely unreported. In the current study, therefore, an integrated field-and-lab experiments approach was used to investigate S (38 kg ha^{-1}) and Cl (84 kg ha^{-1}) effects on photosynthetic parameters [photosynthetic rate (Pn), transpiration rate (Tr), leaf stomatal conductance (gs), leaf chlorophyll (lc) and protein (lp) contents], antioxidant enzyme (SOD and POD) activities and level of lipid peroxidation (MDA content) at different (56, 70, and 85 DAS) growth stages; as well as the total fresh ear yield (kg ha^{-1}) in three fresh corn cultivars (TDN21, JKN2000 and JKN928) grown under field conditions. Results showed that S significantly ($P \leq 0.05$) increased Pn, lc and lp in all cultivars, particularly at 56 DAS. Additionally, S significantly enhanced SOD and POD activities, simultaneously decreasing MDA content, prominently from 70 DAS to 85 DAS period in all three cultivars. On the other hand, Clwas mainly prominent in increasing chlorophyll content, particularly in TDN21 and JKN928 at 70 DAS and 85 DAS, whilst showing a tendency to increase MDA content. Further, S significantly increased total fresh ear yield in fresh corn cultivars by 6.58% to 18.12%, whereas Cl influence was not significant. We conclude that sulphur confers antioxidative and physiological functions against general abiotic stress, contributing to yield improvement in fresh corn grown under field conditions.

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Introduction

Tremendous urban population growth and continued lifestyle changes in China, and the world-over, have resulted in fresh corn (*Zea mays* L. *saccharata* or *rugosa*; *Zea mays* L. *ceratina* and baby corn) becoming an increasingly important major food crop, as a major source of vitamins, tocopherols and carotenoids (Najeeb *et al*., 2011; Zhu *et al*., 2014). It is grown for consumption as fresh vegetable, cobs or other confectionary purposes, including canned kernels, frozen kernels, and frozen cobetts (Ortiz *et al*., 2007; Stall *et al*., 2008). The resultant huge demand has seen fresh corn production surging in arid and semi-arid areas where the crop is even cultivated under partial or non-supplementary irrigation (Zhu *et al*., 2014). Fresh corn plants grown under field conditions, however, like other sessile organisms, are often exposed to combinations of environmental stresses including heat, moisture, light, cold, drought, salinity or toxic metals.

The most drastic effect of these environmental stresses in plants is that they trigger reactive oxygen species (ROS), including superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (·OH) and hydroxygen peroxide (H_2O_2). These ROS cause oxidative damage to the biomolecules such as lipid, protein and nucleic acids, leading to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage (Guo *et al*., 2007). ROS or oxidative damage cause disturbances in the metabolism causing a reduction of chlorophyll content, inhibiting plant growth and respiration, changing the ultra-structure of the cell organelles, and altering the activity and quantity of the key enzymes of various metabolic pathways (Jamal *et al*., 2010).

This consequently reduces the growth, development and productivity of the crop plants.

In order, therefore, to maintain a sustained food production process for the world population under the given scenario, strategies that increase crop plants tolerance to ROS and improve photosynthetic efficiency become imperative.

Sulphur macronutrient plays important protective physiological functions in some crop species on top of being a key constituent of the amino acids cysteine and methionine (Marschner, 2012; Giordano and Raven, 2014). It is a key ingredient in chlorophyll and is vital in physiology and protection of plants against environmental stresses and pests through its antioxidative protective function (Gill and Tuteja, 2010; Anjum *et al.,* 2012; Marschner, 2012; HARSCO, 2015). Sulphur role in ameliorating oxidative stress resulting from the reactive oxygen species (ROS) in plant cells has been well documented in alleviation of metal induced oxidative stress, salinity stress and multiple abiotic stresses in different crop species (Nazar *et al.,* 2011; Mazid *et al.,* 2011a,b; Khan *et al.,* 2014; Giordano and Raven, 2014; HARSCO, 2015).

Further, sulphur has been revealed to improve yield in various crop species (Xie *et al.,* 2003; Jamal *et al*., 2005, 2006; Tiwari and Gupta, 2006; Jarvan *et al.,* 2008; Fahad *et al.,* 2010; Rasool *et al.,* 2013; Ali *et al.,* 2013; Saha *et al*., 2015; Dash *et al*., 2015).

In addition, chlorine (Cl) micronutrient has been revealed to play a direct role in photosynthesis, stomatal regulation and other protective physiological functions (Fixen, 1993; Lovett *et al*., 2005; Marschner, 2012; Biocyclopedia, 2012) as well as yield and quality improvements (Engel *et al.,* 1997; Chapagain *et al*., 2003). Despite all this, however, the influence of Sand Cl on photosynthesis, antioxidant defense and yield, particularly in fresh corn, has been sparingly explored and little information is available.

The current study, therefore, investigated the effects of Sand Cl on fresh corn plant physiology, primarily focusing on photosynthetic parameters (photosynthetic rate, transpiration rate, leaf stomatal conductance, leaf chlorophyll content and leaf protein content), antioxidant enzyme (SOD, POD)activities and level of lipid peroxidation (MDA content) at different crop growth stages; as well as total fresh ear yield. The results of this study will be of paramount importance in fresh corn plant nutrition improvement, abiotic stress breeding and production.

Materials and methods

Plant materials

Three fresh corn hybrid cultivars were used, namely, *Tiandan 21* (TDN21), a relatively high-sugar-content sweet cultivar (National Maize Improvement Centre of China Agricultural University, Beijing); *Jingkenuo2000* (JKN2000), a low-sugar-content waxy cultivar and *Jingkenuo 928* (JKN928), a sweet-and-waxy cultivar (Maize Research Centre of Beijing Academy of Agriculture and Forestry Sciences).

Field experimental site

Field experiment was carried out at San Fen Chang Station of Hebei Agricultural University, Baoding, China, during 2016-2017 summer seasons. The station (38°44′ N latitude, 115°29′ E longitude and 23m altitude)is in the middle of the Hebei Agricultural Plain and is a typical temperate continental arid climate, with a mean annual temperature of 14°C and an average annual precipitation of 500mm, most (80%) of which falls between July and September. The experimental field soil is clay-loam, and it had pH 6.9, 10.25 g kg^{-1} organic matter, 0.85 g kg^{-1} total nitrogen (N), 30.35 mg kg^{-1} readily available phosphorus (P) and 100.52 mg kg^{-1} readily available potassium (K) in the upper 0.4m.

Field experimental design

Experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The treatments comprised the control (S_0, Cl_0) (CK), and one level each for sulphur (38 kg ha^{-1} S) and chlorine (84 kg ha^{-1}Cl). The unit plot size measured 4.8m x 3.6 m and the plant spacings used were 0.6m inter-row and 0.3m in-row, giving 96 plants per plot. The calculated quantities of N; P; K; S and Cl in the form of urea ($CO(NH_2)_2$), diammonium phosphate ($(NH_4)_2H_2PO_4$), monopotassium phosphate ($K_2H_2PO_4$), potassium sulphate (K_2SO_4) and potassium chloride (KCl), respectively were applied as per the treatments to give 195 kg ha^{-1} N, 150 kg ha^{-1} P, 105 kg ha^{-1} K, 38 kg ha^{-1} S and 84 kg ha^{-1} Cl. All of P, K, S and N were applied at the time of sowing. All agronomic practices were kept normal as per the study area recommendations and uniformly applied to all treatments; and phenological observations and yield components data recorded.

Measurement of parameters

Photosynthetic parameters measurement

Photosynthesis parameters, namely, net photosynthetic rate (Pn), transpiration rate (Tr), leaf stomatal conductance (gs) and leaf chlorophyll content (lc) were measured on the second uppermost fully expanded leaves with five replicates per plot and at three different crop growth stages; namely, jointing (56DAS), flowering (70DAS) and milk (85DAS).

Total leaf chlorophyll content, expressed as SPAD value, was measured using a chlorophyll meter (Konica Minolta SPAD-502, Osaka, Japan), whereas a CI-340 Handheld Photosynthesis System (CID Bio-Science, USA) was used to measure Pn, Tr and gs of the second top most fully expanded leaves.

The soluble leaf protein (lp) content was determined according to Bradford (1976), using Coomassie Brilliant Blue G250 as dye and albumin as a standard. 1000ul of the leaf tissue extract was mixed with 5000ul of $C_{47}H_{48}N_3O_7S_2N_a$ (Coomassie Brilliant Blue G250). The mixture cooled at room temperature for 30 minutes, and then, the protein content measured at 595nm using the spectrophotometer. For the control, 1000ul of 7.8 PH potassium phosphate buffer was used instead of the supernatant. The leaf protein content of the samples was then calculated in relation to a prepared standard protein curve.

Assay for antioxidant enzyme activities and level of lipid peroxidation

Assay for analysing the level of antioxidant enzymes (POD and SOD) and content of MDA were measured in leaf tissues obtained from sampled plants from the sulphur and chlorine treated and control plots, at three different growth stages; viz, jointing (56DAS), flowering (70DAS) and milky dough (85 DAS). A sample of 5 plants per plot was selected, all the collected leaf samples immediately frozen in liquid nitrogen and then stored at -80°C for further study. For antioxidant enzymes determination, approximately 0.5g of plant tissue was grounded in 5ml potassium phosphate buffer (pH7.8). The homogenate was then centrifuged for 20 min at 8500 rpm and the supernatant obtained used for enzyme assay. All the procedures were performed at 4°C according to Ge *et al.* (2006).

POD determination

Guaiacol peroxidase (POD) activity was determined at 34°C with guaiacol according to modified protocol of Guo *et al.* (2007). The reaction mixture contained 2910ul potassium phosphate buffer (25mM, pH7.0), 50ul of 20mM guaiacol, 20ul of 40mM H_2O_2 and 20ul of enzyme extract. The mixture was thoroughly mixed and incubated at 34 ℃ for 3 min as the reaction solution. 20ul of 20% TCA was added in the reaction solution to terminate the enzyme activity and then, light absorbance of the reaction solution was measured at 470nm. As a control, 7.0 PH phosphate buffer was used instead of H_2O_2 in the reaction solution. The POD content was then calculated as follows:

POD (μmol mg^{-1} $protein^{-1}$) = (Sample OD ×5ml)/ (FW × 0.02);

where, Sample OD refers to observed sample value and 0.02 is the total volume of enzyme extract used.

SOD determination

Superoxide dismutase (SOD) activity was determined by the nitrobluetetrazolium (NBT) method, by measuring the photoreduction of NBT at ultraviolet wavelength 560nm according to a modified protocol of Prochazkova *et al.* (2001).

The reaction mixture contained 104mM methionine (Met) 500ul, 300uM nitro-blue tetrazolium (NBT) 1000ul, 0.8mM EDTA 500ul, 320uM riboflavin 50ul and 50ul of supernatant (enzyme assay). PH 7.8 phosphate buffer was used instead of supernatant in the reaction solution as a control. After incubation at 30℃ for 15 min less than 4000 LX of light intensity, the absorbance of the reaction solution was measured at 560 nm using a Beckman Coulter DU800 UV/Visible spectrophotometer. The SOD content was then calculated as:

SOD (unit mg^{-1} protein) = (Sample OD – Control OD) × Volume of total enzyme (5ml)/(FW × 0.05)

where Sample OD and Control OD refers to observed sample value and control value respectively, FW refers to the weight of fresh leaf sample used and 0.05 is the volume of enzyme extract used.

Estimation of the level of lipid peroxidation (MDA content)

The level of lipid peroxidation was quantified as melondialdehyde (MDA) content according to the protocols of Guo *et al.* (2007) and Zhang (1992) with slight modifications. An aliquot of supernatant (2000ul) was mixed with 20ml of 0.6% thiobarbituric acid (TBA), prepared from 10% trichloacetic acid (TCA). For the control, PH 7.8 phosphate buffer was used instead of supernatant in the reaction solution. The mixture was thoroughly mixed and incubated at 100°C for 15 min and then quickly cooled in ice. After centrifugation at 4000 rpm for 10min, the light absorbance of the reaction solution was measured at 450nm and 532nm. Correction of non-specific turbidity was made by subtracting the absorbance value taken at 600nm. The level of lipid peroxidation was calculated using extinction coefficient of 155mM cm^{-1}.MDA concentration was estimated as: **c** = 6.45 × (OD_{532} – OD_{600}) – (0.56×OD_{450}). Then, MDA content (μg g^{-1}FW) was calculated as: MDA = (**c** × 5ml)/FW; where OD_{532}, OD_{600} and OD_{450} refers to recorded adsorbed values at 532nm, 600nm and 450nm respectively and FW refers to the weight of fresh leaf sample used.

Total fresh ear yield estimation.

Total fresh ear yield (kg ha^{-1}) was estimated from the yield components data recorded as described in our previous paper (Zenda *et al.*, 2017). The crop was harvested on attaining the appropriate maturity levels as per the harvest indices for each cultivar. Because of the intrinsic yield and quality differences of varieties, the ears were not harvested at the same days after sowing (DAS), but rather, the ear water content was adopted as the harvesting index. The fresh corn ears were harvested at 70-78% for TDN21 and 65-72 % ear water contents for JKN2000 and JKN928 cultivars.

Statistical analyses of data

Mean values were calculated from measurements of five replicates and the SE of means were determined. The statistical analyses of data were performed with SPSS statistical software package (Version 17.0) using One-Way ANOVA, followed by Duncan`s multiple range tests (DMRT) to evaluate the significant treatment effects at $p \leq 0.05$ level.

Results and discussion

Treatment effects on photosynthetic parameters

Results on photosynthetic parameters are shown in Table 1. Both sulphur (S) and chlorine (Cl) significantly ($p \leq 0.05$) increased net photosynthetic rate (Pn) in TDN21 and JKN 928 cultivars at jointing (56 DAS) stage. Compared to control, Significantly increased Pnby 18.25%, 6.68% and 21.94% in TDN21, JKN2000 and JKN928 cultivars, respectively at flowering (70DAS) stage. However, Cl effect was not significantly apparent at this stage. The highest Pn (56.29 μm/m²/s) was recorded in JKN928 S-treatment and the lowest (41.76 μm/m²/s) in TDN21 Cl-treatment at milky dough (85 DAS) stage. Our findings confirm a previous report by Rais *et al.* (2013) that sulphur application improves photosynthetic efficiency and growth in plants under environmental stress. This is probably because S is a key ingredient in chlorophyll (Marschner, 2012).

Table 1. Treatment effects on photosynthetic parameters at different growth stages.

Cultivar	Treatment	Photosynthetic parameters at different growth stages											
		Pn (μm/m²/s)			Tr (mmol/m²/s)			Gs (mmol/m²/s)			Leaf chlorophyll content (SPAD values)		
		56DAS	70DAS	85DAS	56DAS	70DAS	85DAS	56DAS	70DAS	85DAS	56DAS	70DAS	85DAS
TDN21	CK	15.96 a	28.38 a	46.05 a	6.75 a	3.84a	2.47	157.25	151.28 a	130.84 a	46.57 a	48.23 a	41.53 a
	S	24.80 b	33.56 b	49.96 a	4.42 b	2.49 b	2.17	128.14	121.98 b	109.77 b	49.17 b	52.50 b	40.90 a
	Cl	22.54 b	30.17 a	41.76 b	5.99 a	4.09 a	2.44	164.12	148.65 a	117.30 a	48.76 b	51.61 b	44.11 b
	Mean	21.10	30.70	45.92	5.72	3.47	2.36	149.84	140.64	119.30	48.16	50.78	42.18
	Std. dev.	4.24	2.54	4.15	1.26	0.84	0.33	27.40	15.69	13.63	1.47	2.42	1.92
	CV (%)	20.09	8.27	9.04	22.03	24.21	13.98	18.29	11.16	11.42	3.05	4.77	4.55
JKN2000	CK	17.31 a	31.74 a	48.71 a	5.86	4.54 a	2.32	138.82	171.03 a	108.57	47.23 a	44.47 a	44.31
	S	18.83 a	33.86 b	53.32 b	4.43	3.56 b	2.07	141.60	123.33 b	106.43	49.62 b	48.10 b	45.67
	Cl	21.66 b	31.58 a	50.38 a	5.19	4.50 a	2.46	132.40	169.46 a	112.97	47.90 a	45.01 a	44.94
	Mean	19.27	32.39	50.80	5.16	4.20	2.28	137.60	154.61	109.32	48.25	45.86	44.97
	Std. dev.	2.44	1.25	2.20	1.3	0.58	0.3	22.61	27.33	9.37	1.28	2.11	2.19
	CV (%)	12.66	3.86	4.33	25.19	13.81	13.16	16.43	17.68	8.57	2.65	4.60	4.87
JKN928	CK	16.31 a	27.08 a	47.09 a	5.82 a	3.68 a	2.82 a	135.38	174.02 a	133.36	47.73 a	49.33 a	47.18 a
	S	18.52 b	33.02 b	56.29 b	4.28 b	2.70 b	2.11 b	128.14	128.30 b	125.89	50.47 b	52.91 b	50.96 a
	Cl	17.71 b	31.34 a	51.14 a	5.25 a	3.55 a	2.61 a	135.24	168.24 a	125.31	48.01 a	50.77 a	43.37 b
	Mean	17.51	30.48	51.50	5.12	3.31	2.51	132.92	156.85	128.18	48.74	51.00	47.17
	Std. dev.	1.07	2.9	4.55	0.8	0.70	0.44	8.72	22.54	8.38	1.60	1.78	4.04
	CV (%)	6.11	9.51	8.83	15.63	21.15	17.53	6.56	14.37	6.54	3.28	3.49	8.56

Note: Data are mean values of 3 replications. Means having similar letter (s) in same column do not differ significantly at $P \leq 0.05$; Pn, photosynthetic rate; Tr, transpiration rate; gs, leaf stomatal conductance; CK, control; S, sulphur; Cl, chlorine; DAS, days after sowing.

Interestingly, leaf chlorophyll content (SPAD values) was significantly enhanced by S treatment at 56 DAS and 70 DAS stages in all the three cultivars (Table 1). Particularly at 70 DAS, S significantly increased SPAD values by 8.92%, 8.09% and 7.30% in cultivars 1, 2 and 3, respectively, compared to CK. Here, we suggest that a positive linear relationship exists between sulphur concentration in leaf tissues and the chlorophyll content which consequently resulted in increased net photosynthetic rate.

Chlorinetreatment showed a tendency to increase Pn, especially in sweet cultivar (TDN21) at jointing stage. In addition, Clresulted in a significant increase in leaf chlorophyll content in TDN21 at all growth stages and at 85 DAS stage in JKN928 cultivar. This observation substantiate the previous findings by Coleman *et al.* (1987) that Cl is an essential cofactor for the activation of the oxygen evolving enzyme associated with photosystem II, the water-splitting step of photosynthesis. It may be correct to suggest that Cl plays important physiological role in increasing leaf chlorophyll content, which have a direct effect on net photosynthetic rate.

Sulphur significantly ($p \leq 0.05$) decreased transpiration rate (Tr) in all the three cultivars at the 56 DAS and 70 DAS growth stages. However, Cl effect was not statistically significant at all in this regard. Treatment effects on leaf stomatal conductance (gs) were not significantly apparent at 56DAS and 85DAS stages. However, at 70DAS, S significantly ($p \leq 0.05$) decreased gs by 19.37%, 27.89% and 26.27% in TDN21, JKN2000 and JKN928 cultivars, respectively. Chlorine effect on gs was not significant at all (Table 1).

Our present findings on Tr and gs are in tandem with Liu *et al.* (2004) who observed that Tr was significantly higher in S_1 (36.3 kg ha^{-1}) than S_2 (41.4 kg ha^{-1}). Further, they observed green leaf area, chlorophyll content and Pn to be enhanced by amount of S applied. We hereby suggest that S plays some osmotic regulatory function by controlling stoma guard cells opening and closing, in addition to enhancing chloroplast protein and chlorophyll synthesis, consequently resulting in improved stomatal conductance and increased net photosynthesis capacity.

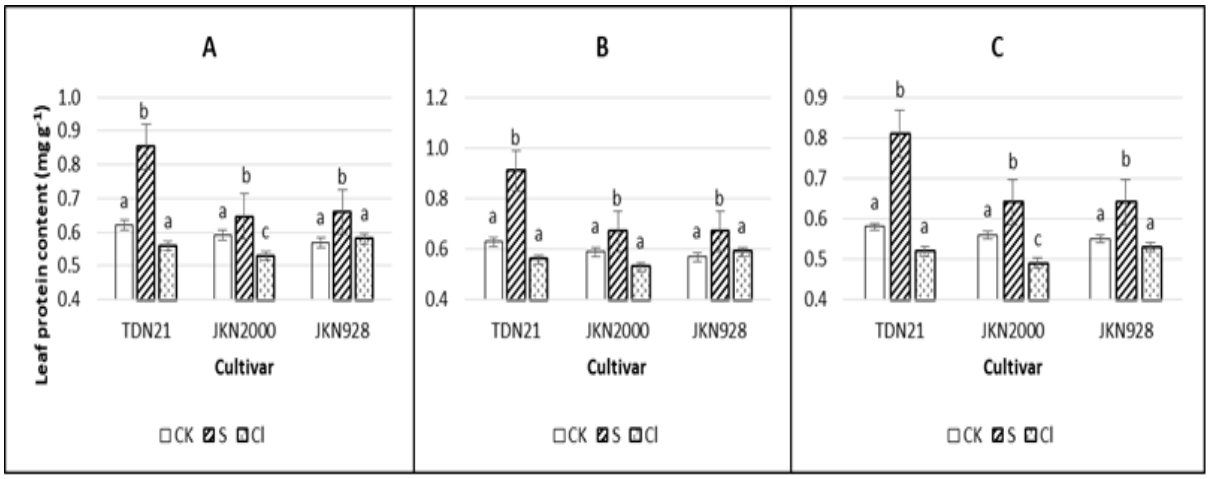

Fig. 1. Treatment effects on leaf protein content (mg g^{-1}) in different fresh corn cultivars at different growth stages; 56 DAS - A, 70 DAS – B, 85 DAS – C.

Previous studies by Fixen (1993), Xu *et al.* (1999), Chapagain *et al.* (2003)and Biocyclopedia (2012) have shown Clto play some biochemical functions in plants including operating as a counter ion for cation transport and as an osmoticum, regulation of stomatal movement. Chlorine, along with potassium, participates in stomatal opening by moving from epidermal cells to act as an osmotic solute that result in water uptake into and a bowing apart of the guard cell pair. Our present investigation, however, could not confirm these previous study findings since Cl could not significantly influence neither Tr nor gs. Sulphur significantly ($p \leq 0.05$) increased leaf protein (lp) content in all the three cultivars at all the growth stages. On the other hand, Cl significantly decreased lp in JKN2000cultivar at 56 DAS and 85 DAS stages. The highest (0.85 mg g^{-1})lp content was recorded in TDN21 CK-treatment at 56 DAS, whilst the least (0.49 mg g^{-1})lp was recorded in JKN2000Cl-treatment at 85DAS stage (Fig. 1).

Plants use S and N jointly to biosynthesize proteins because of S element being a key constituent of the amino acids cysteine and methionine, and hence of proteins (Marschner, 2012; Edis and Norton, 2012; Sahota, 2012). It serves therefore that once the sulphate ions are absorbed through the root cells, they are transported up the plant system into sink organs via a network of sulphate transporters according to the availability of S and plant`s requirements (Nazar *et al.*, 2011; Kopriva *et al.*, 2015).

Our present findings confirm to these previous studies and posit that the leaf protein content has a positive correlation with S addition since S is a critical component of key biomolecules.

In the current study, Cl showed a tendency to decrease lp content in almost all the three cultivars. Generally, Cl is known to have an antagonistic effect to leaf protein content rather than an enhancement effect. It is usually found together with sodium in saline soils, and has been found to have some drastic effects to biomolecules, including proteins, depending with its concentration and the osmotic effects in those saline soils (Lovette, 2005; Biocyclopedia, 2012).

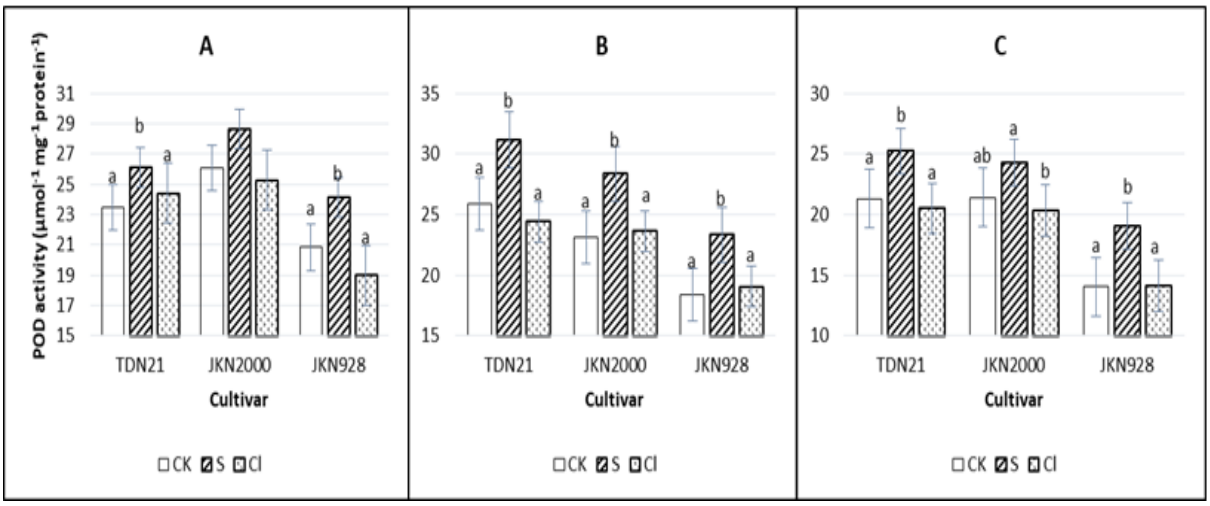

Fig. 2. Treatment effects on POD activity (µmol^{-1} mg^{-1}protein^{-1}) in different fresh corn cultivars at different growth stages; 56 DAS - A, 70 DAS – B, 85 DAS – C.

Treatment effects on antioxidant enzyme (POD and SOD) activities

Sulphur significantly ($p \leq 0.05$) increased POD activity in TDN21 (at 56 DAS), in all the three cultivars (at 70 DAS), and in TDN21 and JKN928 cultivars (at 85 DAS) (Fig. 2).In contrast, compared to control, Cl showed a tendency to decrease POD activity, though non-significantly in the present study.

Leaf SOD activity (unit mg^{1} protein-^{1}) was significantly ($p \leq 0.05$) enhanced by S treatment at all the growth stages, in almost all the cultivars (Fig. 3). Compared to the control, S increased SOD activity at 70 DAS stage by 39.11%, 31.60% and 27.10% in TDN21, JKN2000 and JKN928 cultivars, respectively. Meanwhile, Cl treatment only showed significant ($p \leq 0.05$) influence on SOD at 70 DASin TDN21 where the parameter was increased (Fig. 3c).

Our present research resultsconfirm previous studies reports that noted sulphur as an essential macronutrient for plant growth and development because of its antioxidative protective function (Scherer, 2001; Jamal *et al*., 2010; Mazid *et al*., 2011a,b; Bouranis *et al.,* 2012; Sahota, 2012).

The plant antioxidant machinery components include enzymatic antioxidants (SOD, POD, CAT, APX and GR) and non-enzymatic antioxidants such as carotenoids and tocopherols (Gill and Tuteja, 2010). Sulphur functions in this defence system as a key constituent of gluthathione (GSH), one of the most crucial intracellular defense metabolites against ROS induced oxidative damage in plants (Kopriva and Koprivova, 2005; Ohkama-Ohtsu and Wasaki, 2010).

GHS depresses or scavenges the formation of toxic ROS such as superoxide and lipid hydro peroxides thereby providing a protective role against oxidative and environmental stress (HARSCO, 2015).

It therefore serves as a thiol buffer in the protection of proteins via direct reaction with ROS or by the formation of mixed disulphides. Khan *et al.* (2014) posited that the redox properties of S in proteins, and of S-containing metabolites, are particularly important in the interaction between the reductive assimilation processes of photosynthesis and ROS that arise as by-products of electron transport chains. Increased S metabolism and production of S metabolites are critical in maintaining redox state of the cell as a response against increasing environmental induced oxidative stress.

These metabolites also enhance tolerance by modulating physiological and molecular processes and by up-regulating genes for stress tolerance (Nikiforova *et al.*, 2004; Jamal *et al.*, 2010; Nazar *et al.*, 2011).

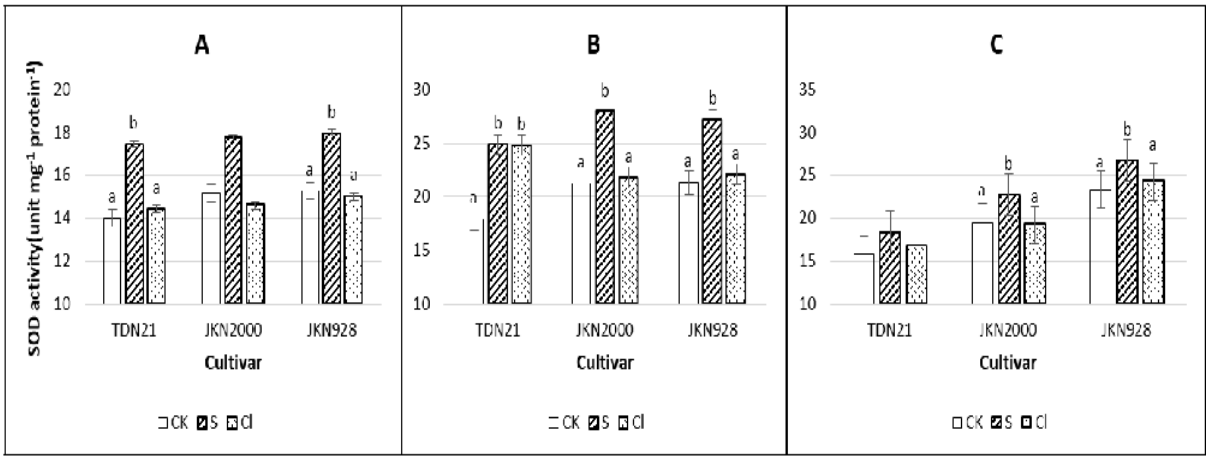

Fig. 3. Treatment effects on SOD activity (unit $mg^{-1}protein^{-1}$) in different fresh corn cultivars at different growth stages; 56 DAS - A, 70 DAS – B, 85 DAS – C.

In addition, S application has been reported to improve photosynthetic efficiency and growth in barley plants under salinity (Astolfi and Zuchi, 2013). Thus, the S-containing metabolites are linked to antioxidant system and are useful in reversing the adverse effects of abiotic stress because of their free radicals scavenging property. Further, these sulphur containing metabolites enhance tolerance by modulating physiological and molecular processes and by up-regulating genes for stress tolerance.

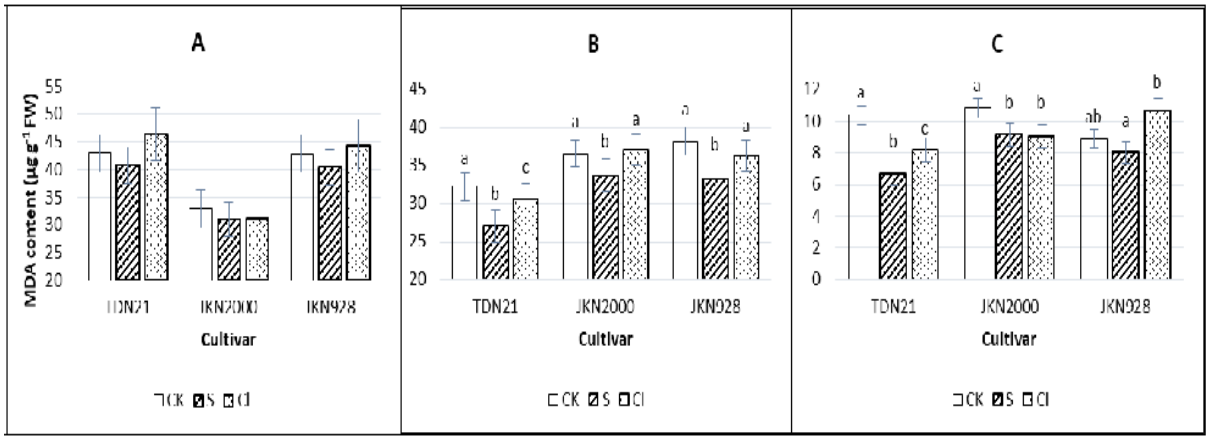

Fig. 4. Treatment effects on MDA content (µg g^{-1} FW) in different fresh corn cultivars at different growth stages; 56 DAS - A, 70 DAS – B, 85 DAS – C.

However, in the current study, Cl could not significantly enhance POD activity at all. It has already been discovered that great differences exist in tolerance to Cl salts among crops and plants of the same species (Fixen, 1993). Generally, Cl is known as a toxic element for plants, although its toxicity is associated with the osmotic effect in saline soils. In other cases, chloride is not toxic even when it is in higher concentrations compared to the other micronutrients. This fact is confirmed in the present study. The chlorine effect on antioxidant enzymes was not significantly apparent suggesting that the available concentration of Cl was just sufficient to allow for normal growth without lethal effects, hence no major influence on antioxidant enzyme activities.

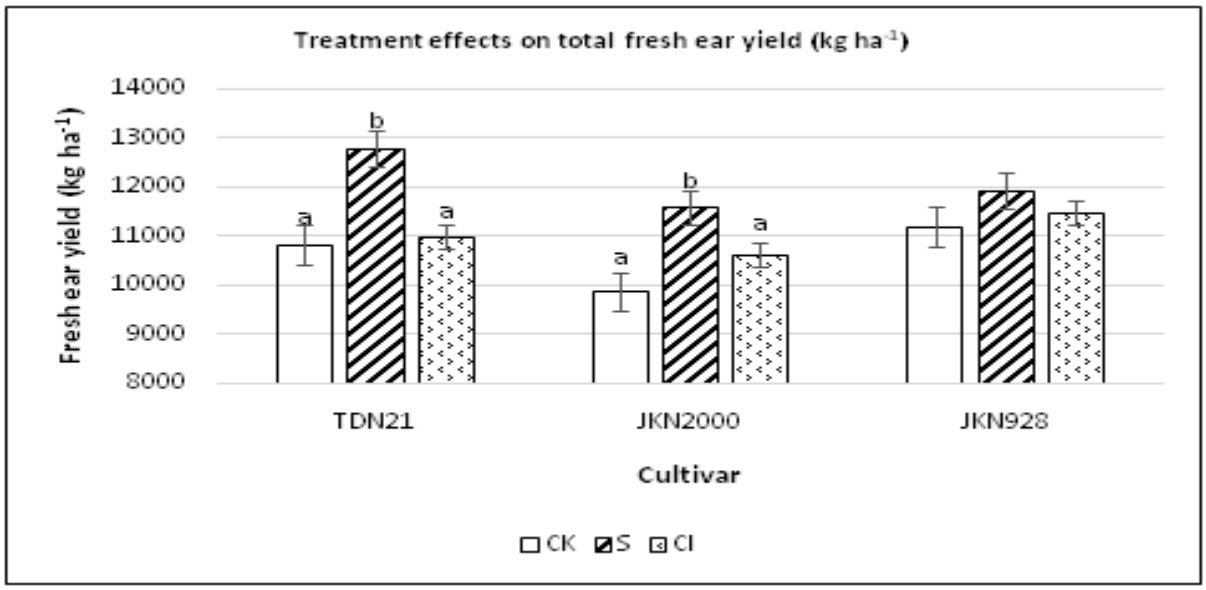

Fig. 5. Treatment effects on total fresh ear yield (kg ha^{-1}) in different fresh corn cultivars. Bars with similar letter(s) for a particular cultivar means treatments are not statistically significant at $p \leq 0.05$.

Treatment effects on the level of lipid peroxidation (MDA content)

Both S and Cl could not significantly influence MDA content at jointing (56 DAS) stage. However, at 70 DAS stage, S significantly ($p \leq 0.05$) decreased MDA content by 16.13%, 7.93 % and 13.08 % in cultivars 1, 2 and 3 respectively whereas Cl significantly increased MDA content by 12.91 % in TDN21 cultivar. At the milky dough (85 DAS) stage, sulphur further significantly decreased MDA content in TDN21 and JKN2000 cultivars, whilst Cl significantly decreased MDA content in TDN21 and increased it in JKN928 (Fig. 4). TDN21 S-treatment at 85DAS recorded the lowest (6.70 µg g^{-1} FW) and TDN21 Cl-treatment at 56DAShad the highest (46.36 µg g^{-1} FW) MDA contents (Fig. 4).

These results may suggest that S plays a key role in alleviating cell degradation from oxidative damage in fresh corn plants, especially during flowering to milk stages. Sulphur act as a major modulator of GHS-mediated control of plant stress tolerance. It is incorporated into organic molecules and is located in thiol groups in proteins (cysteine/cys-residues) or non-protein thiols (GHS), mantains homeostasis of GHS and oxidized glutathione ratios, and protects plants from oxidative damage. Glutathione (or its precursor, GHS) functions as a ligand and GHS peroxidases are induced in plant cells in response to abiotic stress. These enzymes are involved in detoxification of lipid peroxidases (Mazid *et al.*, 2011a; Anjum *et al.*, 2012).

Our present findings may suggest that it is normally during reproductive to maturity stages when the maize plants are subjected to serious abiotic stresses and more lipid peroxidation occurs during this stage. In response, plants show high levels of SOD, POD and lipid peroxidases to counteract the effects, with sulphur addition providing a better cushion to plants than non-S addition.

Further, we observed that ears harvested from S-treated plants exhibited reduced insect and disease damage compared to CK, especially in sweet corn (TDN21) cultivar. This realization, coupled with improved plant stands in S-treated plots as compared to CK, may further suggest that sulphur confers some tolerance to both biotic and abiotic stresses in fresh corn plants grown under field conditions.

However, Cl significantly increased MDA content in TDN21 at 70 DAS and JKN928 at 85DAS, but significantly decreased MDA content in TDN21 at 85 DAS (Fig. 4). The influence of Cl on plant growth and response to stimuli depends on the plant variety and the balance of other available anions (Fixen, 1993).

In our present study, the issue of plant variety and growth stage differences may have played a part, although Cl has shown a tendency to increase MDA content, suggesting its role in ameliorating oxidative stress damage in fresh corn plants is not apparent. However, Cl element has been posited to operate as a counter ion for cation transport and as an osmoticum (Lovett *et al.*, 2005).

Treatment effects on total fresh ear yield

Sulphur significantly ($p \leq 0.05$) increased total fresh ear yield (TY)in TDN21 and JKN2000 cultivars. Compared to control, Sresulted in 18.12%, 17.53% and 6.58% increases in TY in TDN21, JKN2000 and JKN928, respectively. The highest (12 783 kg ha^{-1}) yield was recorded in TDN21 sulphur-treatment, whilst the least (9 858.9 kg ha^{-1}) was realized in JKN2000 CK-treatment (Fig. 5). However, chlorine treatment caused slight non-significant increases in TY of 1.43%, 7.71% and 2.4% in cultivars 1, 2 and 3 respectively.

In addition, we realized that sulphur significantly increased total fresh ear yield principally by influencing ear diameter and ear weight. We speculate that the increased growth and vigour of maize plants caused by sulphur addition leads to higher dry matter production and consequently yield, as sulphur, working in synergy with nitrogen, results in greater translocation of photosynthates from vegetative parts to developing ear and grains in a source-sink relationship. The enhanced photosynthates in developing ear will lead to increased ear diameter, consequently increasing average ear weight and total fresh ear yield. Previously, Channabasamma *et al. (*2013) posited that corn grain yield is the manifestation of yield attributing characters, and consequently, manipulation of those characters, either exogenously or genetically, contribute to higher yield. In addition, Tiwari and Gupta (2006) reported that fertilization at 30kg ha^{-1} S can increase yield by about 21.85% in field maize through sulphur`s enhancing effect to nitrogen Further, S fertilization significantly increased the fresh ear yield by influencing the total ear number ha^{-1} especially in sweet cultivar TDN21.

Ear number is equally important to whole ear weight in determining total yield in vegetable corn because ear number is used as a commercial unit (Worrajinda *et al.*, 2013). Here, we hypothesise that the realized positive influence on ear number ha^{-1} was probably because of S conferring some abiotic and biotic stress tolerance compared to control plants. This is supported by the observed better plant stands and low incidences of pest and disease attack in S treated plots as compared to control plots. Thus, in the wake of huge demand for high yield and quality cereal and vegetable diets, sulphur can play a role in enhancing fresh corn production.

However, Cl effect on yield was not significant. Chlorine is generally a non-limiting factor for plant growth, and in most cases, it`s effect on yield is negligible (Fixen, 1993).

Conclusion

We reveal that S significantly increases total leaf chlorophyll content and net photosynthetic rate in different fresh corn cultivars, particularly at the jointing stage. Chlorine also enhances leaf chlorophyll content from jointing to milky dough stage, particularly in sweet corn cultivars. Further, S significantly enhances SOD and POD activities at all the growth stages in all cultivars, whilst Cl effect is not significant. Our results also reveal that S can significantly reduce MDA content in fresh corn cultivars, prominently from the reproductive to milky dough stages, whereas Cl shows a tendency to increase MDA content and being less significant. We, therefore, conclude that S confers antioxidative and protective physiological functions against general abiotic stress in fresh corn grown under field conditions. This, coupled with S nutrient`s enhancement effect to N, leads to increased fresh ear yield. However, Cl influence on yield is not significantly apparent. However, we recommend further investigations, first, to determine the optimum fertilization levels, and secondly, to unravel S and Cl effects to specific abiotic stresses.

Acknowledgements

This research was supported by the Science and Technology Project of Food Production in Hebei Province, Ministry of Science and Technology of China (Grant No. 2013BAD07B05).

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