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Metabolic regulation of Sulphur or/and Calcium under heavy metal stress

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Abstract: In the view of improving the adverse impact through abiotic stress, several scientist and researchers have joint hand and they are working with some minitechnology for improving the plants health and in this chain, plant mineral nutrients (calcium, nickel, potassium and sulphur) has been reported to be effective in counteracting environmental stimuli as they are involved in different aspects of plant metabolism. Among several mineral nutrients, sulphur (S) and calcium (Ca) have been recognized as signaling components and intensively investigated for their role in plant adaptation under stress condition. Herein, this review a brief highlight on role of S and Ca under heavy metal stress in plant system has been provided.

Introduction:

Sulphur and calcium as stress modulator

The food security of world is threatened due to low production of food grains as abiotic stressors severely affect the plants. In the view of improving the adverse impact through abiotic stress, several scientist and researchers have joint hand and they are working with some minitechnology for improving the plants health and in this chain, plant mineral nutrients (calcium, nickel, potassium and sulphur) has been reported to be effective in counteracting environmental stimuli as they are involved in different aspects of plant metabolism [1, 2, 3, 4]. Among several mineral nutrients, sulphur (S) and calcium (Ca) have been recognized as signaling components and intensively investigated for their role in plant adaptation under stress condition. In plants, about 90 % S is present as cysteine (Cys) and methionine [5]. The formation of sulphydryl (—SH) and disulphide (S—S) bonds in proteins and many enzymes is mediated by Cys (Saito, 2000). Moreover, it also serves as source for production of thiol peptides, like reduced glutathione (GSH) and phytochelatins (PCs) [6, 7, 8]. On the other hand, Ca is a well—established secondary messenger that mediates many aspects of cell metabolism. Typically 200 nM Ca is maintained in an unstimulated cell by Ca^{2+}—ATPases or H^{+}/Ca^{2+} antiporters [9] and it is released by Ca stores like cell wall and vacuoles, whenever it is required by the cell during stress.

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

Impact of sulphur and calcium on growth attributes

Sulphur has a striking role in improving the morphological attributes of plants. The FW, DW, RL and SL are important parameters, which significantly improved on 0.5 and 1.5 mM S exposure in *S. melongena* L. seedlings [10]. The enhanced plant growth under 1.5 mM S was supported by the decreased Cr accumulation that drastically declined the Cr/S ratio in comparison to 1.0 and 0.5 mM S [10]. Dixit et al. [2] reported S induced variable responses in rice seedlings; where 0.5 mM S enhanced the RL but declined the SL; however, 5.0 mM S dose reduced the RL than 3.5 mM S. Further, they have reported that higher dose of S changes spatial distribution of As thereby restricting (translocation factor; root to shoot ratio) greater amount of As in root than shoot [2].

The 6 and 9 mM S induced improvement in root and shoot biomass linked with the improvement in the N, K and Mg content of roots in Cd—stressed *Lactuca sativa* L. [11]. In a study on *O. sativa*, a remarkable increase in RL under 0.5 mM S in comparison to 3.5 mM S treated plants was found in order to increase the surface area of roots to fulfil the demand of S; while 5.0 mM S reduced the RL thereby improving root biomass [12]. Siddiqui et al. [13] have reported that the combined exposure of 40 mg S and 60 mg N Kg^{-1} sand considerably improved growth (shoot length, fresh weight, dry weight, leaf area etc.) in *Brassica* seedlings. Application of S also increases the grain and biomass yield of rapeseed under As toxicity [14]. Ostaszewska et al. [15] while working on continuous S starved *A. thaliana* for 9 weeks reported that plants showed smaller rosette size as well as number of leaves in comparison to control plants. When *B. juncea* L. were pre—treated with 100 and 200 mg S kg^{-1} soil before 10 days of sowing, improvement in plant DW, water use efficiency (WUE) and leaf area was observed and it was explained by the decrease in the Na^+ and Cl^- accumulation [16]. Sheng et al. [17] have reported that S at 5, 10 and 25 mM have differential role in RL, DW and relative water contents (RWC) of polish wheat with pronounced effect of 5 mM S.

Calcium has positive responses in morphological characters of plants, where the root, shoot and leaves are the majorly affected plant parts [18, 19]. The DW and water content in the root, shoot and leaf showed a positive response after Ca exposure in *S. lycopersicum* seedlings suffering from salt stress [20]. Similarly, a study on *O. sativa* showed a noteworthy improvement in plant height, FW, DW and leaf RWC when grown under salt stress [18]. However, Jiang and Huang [21] reported that external Ca^{2+} not affected ion homeostasis

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

during long —term exposure of heat stress in tall fescue (*Festuca arundinacea* L.) and Kentucky bluegrass (*Poa pratensis* L.). Moreover, Ahmad et al. [19] reported that Ca provoked root fresh weight, shoot fresh weight, root length, shoot length, root dry weight, shoot dry weight, number of pods and seed yield in mustard and *C. arietinum* seedlings and suggested that it might be due to enhanced water and minerals uptake. The similar stimulatory effect on length, FW and DW of root and shoot were also reported by Siddiqui et al. [22] on *V. faba*. Moreover, exogenous Ca have been shown to alleviate the Cd—induced cytotoxicity by efficiently enhancing the mitotic index and dropping chromosomal aberration rate in root tip cells [23]. Exogenous Ca caused significant improvement in length of main and lateral roots, their DW and leaf area in sesame plant [3] suggesting that Ca helps in displacing heavy metals to maintain the cellular metabolism. Calcium—induced stimulatory effects on morphological attributes of plants have also been reported in *Amaranthus hypochondriacus*, *Sedum alfredii* and *Arachis hypogaea* [4, 24, 25].

Impact of calcium on mineral nutrients status

Calcium plays a regulatory role in minerals uptake. Ahmad et al. [26] on *B. juncea* seedlings found that 50 mM Ca improved the uptake of S, Mn, Mg, Ca and K contents by root and their translocation to the shoots in Cd—stressed seedlings. In a study on *C. arietinum*, Ahmad et al. [19] reported that S, Mn, Mg, Ca and K contents were greatly accumulated in roots upon Ca treatment and a similar trend was observed for the accumulation of these examined mineral elements in shoots. Further, all the examined mineral elements which were decreased by Cd stress were partially recovered, when Ca was applied with Cd. In *V. faba*, Siddiqui et al. [22] have also reported that Ca and K accumulation increases with Ca addition.

Impact of sulphur and calcium on photosynthetic pigments

Sulphur exposure shows diverse responses for Chl contents in plants, which is a commonly reported phenomenon. Sulphur at 0.5 mM dose, decreases Chl *a*+*b* content; while at 1.5 mM noticeably raised the level of Chl *a* and *b* in *S. melongena* seedlings [10]. In polish wheat seedlings, Chl *a* and *b* contents were increased under 5 and 10 mM S, while sharply declined under 25 mM S [17]. Further, Chl *a* to *b* ratio showed a reverse trend. The increase in Chl *a* and *b* on exogenous exposure of S have been reported in earlier studies [13, 16, 22 27]. The decrease in Chl contents under S—starvation condition has also been reported in

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Vol-22-Issue-17-September-2019

several studies [28, 29], which may be due to strong oxidation of the photochemical apparatus. However, no change in the contents of Chl *a*, *b* and Car was noticed in *A. thaliana* grown under S—starved condition [15]. In *A. thaliana*, genes encoding for Chl *a*/*b* binding protein were repressed in response to S depletion [30, 31]. In *S. melongena*, Car has differential response under changing S doses; at 0.5 mM S, Car content was decreased; while at 1.5 mM S it was increased comparatively [10].

Calcium plays a remarkable role in biosynthesis and functioning of photosynthetic pigments. In *V. faba*, the contents of Chl *a* and *b* were significantly enhanced by exogenous Ca [22]. Abd_Allah et al. [3] while working on *Sesamum indicum*, reported that exogenous Ca application promotes Chl *a*, *b*, Chl *a*+*b*, Chl *a*/*b*, Car and total pigments. According to Lechowski and Bialczyk [32], Ca acts as a secondary messenger in cytokinin action that promotes Chl biosynthesis; therefore, the maximum presence of leaf—Ca^{2+} increased the contents of photosynthetic pigments. Ahmad et al. [19] and [26] in mustard and chickpea leaves, respectively reported an increase of Chl *a*, Chl *b*, Car and Chl *a*+*b* contents upon Ca treatment. Further, flavonoids (mg catechin g^{-1} extract) and total phenolic contents (mg gallic acid equivalent (GAE) g^{-1} extract) in chickpea leaves were also increased by Ca treatment. Contrarily, in the leaves of *O. sativa*, the decrease in Chl *a*, *b* and Chl *a*+*b* was reported; however, Chl *b* was found greatly affected than Chl *a*, while Cars content was unchanged [18]. A similar decrease in pigment contents under Ca exposure was also reported by Rahman et al. [33] in rice leaves. Lu et al. [4] have reported that calcium silicate at 0.41, 0.83 and 1.65 g Kg^{-1} appreciably improved the Chl *a*, *b*, Chl *a*+*b* and total Car contents; while Chl *a* to *b* ratio showed no significant difference.

Impact of sulphur and calcium on photosynthesis and PS II photochemistry

Sulphur plays an important role in regulating the key metabolic process *i.e.* photosynthesis of plants, where *PN* is directly linked with the photosynthetic pigments status [10, 13, 16, 22, 27]. Singh et al. [10] in *S. melongena* L. seedlings reported that photosynthetic activity was declined under 0.5 mM S, while a considerable increment was noticed under 1.5 mM S. Sulphur induced ameliorating role in photosynthetic activity have also been reported by Fatma et al. [16] and [27] in *B. juncea* L. seedlings, where 200 mg S kg^{-1} soil showed higher values for photosynthetic attributes (F_v/F_m, *PN*, *gs*, *Ci*, PS II activity) and RuBisCO activity. The accelerated photosynthetic rate and growth by excess—S

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Vol-22-Issue-17-September-2019

application was justified with a similar increment in Chls, *gs*, RuBisCO, protein contents and carboxylation rates [34]. However, decreased F_v/F_m value occurred due to S deprivation that provoked photodamage to PS II RCs [35]. Fatma et al. [16] reported that plants fed with excess—S more efficiently did the formation of Fe—S clusters in electron transport system and the photosynthetic apparatus. Further, excess—S improved the *gs* along with RuBisCO activity that in turn increased the availability of CO_2 for RuBisCO enzyme [16]. Sheng et al. [17] in Mn stressed polish wheat seedlings have also reported that 5 and 10 mM doses of S appreciably increased *PN*, *gs* and *E* but reduced *Ci* as compared to control. The 25 mM S had no significant effect on the above—referred parameters. Further, 5 and 10 mM doses had a stimulatory effect on F_v/F_m, ΦPS II and *QP* [17]; however, 25 mM S suppressed these traits. The increase in photosynthesis with S may involve stomatal and non—stomatal limitations as the treatment increased *gs* that allow more exchange of *Ci* on one hand and increased RuBisCO activity on the other [36]. The Chl *a* fluorescence transient—JIP test in *S. melongena* L. seedlings were analyzed by Singh et al. [10] and reported that 1.0 mM S declined the attributes of JIP parameters: ΦP_o, Ψ_o, ΦE_o, PI_{ABS} and *QP*; while the reverse trend was observed in energy fluxes parameters: *ABS/RC*, TR_o/RC, ET_o/RC, DI_o/RC and *NPQ*. Further, S at 0.5 mM dose showed a reverse trend to that of 1.0 mM thereby showing the damaging effect on PS II photochemistry. Anjum et al., [37], reported that S maintains the redox status in plants and therefore recovers the damage to photosynthetic apparatus. Recently, D'Hooghe et al. [38] pointed out that S limitation caused plastocyanin (PC) and Ferredoxin—NADP reductase (FNR) reduction in *B. napus*. Further, Dixit et al. [39] reported that 3.5 and 5.0 mM S up—regulated the FNR level as well as the ATP synthase and they are involved in catalyzing the enhanced production of $NADPH+H^+$ required for CO_2 assimilation.

Phosphoenolpyruvate carboxylase (PEPc) is a stromal enzyme involved in the photo—fixation of CO_2 in plants and enables net C skeleton synthesis. In *Z. mays*, whether S up— or down—regulates the PEPc activity was mainly decided by S status in plants [28]. Carbonic anhydrase (CA) catalyzes the reversible interconversion of CO_2 and HCO_3^- thus, actively participates in facilitating CO_2 supply around RuBisCO. Siddiqui et al. [13] and Mohammad et al. [40] stated that S improved CA activity in *Brassica* plants.

Calcium plays a vital role in electron transport in photosynthetic process and the light—driven metabolic reactions of plants [41]. According to Miqyass et al. [42], H_2O

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Vol-22-Issue-17-September-2019

oxidation in PS II needed Ca as obligatory activators. Further, Ramalho et al. [43] in *Coffea arabica* established that Ca played a critical role in the Chl maintenance and photochemical efficiency of PS II. Andosch et al. [44] in Cd stressed *Micrasterias,* noticed that pre—treatment with Ca lowered the structural damage of chloroplast and the decline of O_2 production and thus, reformed photosynthetic activity in plants. The pre—treatment with $CaCl_2$ recovered *PN*, the quantum yield of PS II and *QP* under low night temperature (LNT) stress in tomato leaves [45]. Yang et al. [25] reported that in Ca^{2+}—treated plants, F_v/F_m was improved as compared to the heat and high irradiance (HI) stressed *Arachis hypogaea*, which was escorted by lower *NPQ* values. Further, they have also reported the higher content of D1 protein in similar condition in peanut [25]. Turner et al. [46] and Rocha and Vothknech [47] in their studies showed that calcium—binding protein (CAS) was essential for the maintenance of activity, recovery and turnover of PS II, and also in driving high light acclimation. In tomato leaves, pre—treatment with Ca relaxed the chloroplast from overloaded proton gradient formation across the thylakoid membranes, which was required for efficient *QE*: the energy—dependent component of *NPQ* [45]. Furthermore, Turner et al. [46] and Rocha and Vothknech [47] reported that Ca^{2+} enhances the binding of CaM with NADK2, which modulates NAD/NADP balance. Exogenous Ca also promotes ATPase activity [45]. Chen et al. [48] reported that Ca application mitigated *QP*, F_v/F_m and ΦPS II, which were decreased in salt—stressed *Rumex* leaves. Similarly, in tobacco, a significant improvement in *PN*, *gs* and carboxylation efficiency (*CE*) was noticed by Tan et al. [49], which suggested that pre—treatment with Ca improves photosynthesis by improving *gs* and maintaining thermostability of OEC in tobacco.

Impact of sulphur and calcium on nitrogen metabolism status

The effect of S and Ca in nitrogen metabolism of plants is less focused. In a study, Astolfi et al. [28] have reported that after 5 days of S—starvation, the NR and GS activities in leaves were greatly decreased; however, in S—sufficient plants, the activities of the same were found to be improved than control. Contrarily, GDH activity responds negatively to S—sufficient; while positively to S—deficient plants suggests that NH_4^+ assimilation pathway may be lithe and synthesis of glutamate may occur through alternative pathways for PCs production [28]. Results achieved by Robison et al. [50] and Fox et al. [51] illustrate that GDH has positive role in glutamate catabolism but plays no role in NH_4^+ assimilation. On the other hand, Melo—Oliviera et al. [52] also pointed towards the potential role of GDH in

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

NH_4^+ assimilation; and investigation by Masclaux et al. [53] shown that GDH plays role in the recycling of organic N, obtained as a by—product from protein degradation. Siddiqui et al. [13, 22] showed that the activities of NR, NiR, GS and GOGAT were increased when 20 and 40 mg S kg^{-1} sand were supplied to *B. juncea*. However, under same situations when 60 mg S kg^{-1} sand was supplied, the activities of referred enzyme get lowered but were still higher than control [13].

Regarding the role of Ca in N—metabolism, Tyerman et al. [54] evidenced that in soybean (*Glycine max*) nodules, transport of NH_4^+ through the symbiosome membrane may take place *via* an ion channel; which are very sensitive for Ca that may be inhibited by very small doses. Further, Streeter et al. [55] reported that exogenous addition of Ca to infected cells of root nodules, elevated Ca levels 2—3 folds, which consequently depressed N content of shoots by 17—30 %. In addition, the approximates of nitrogenase activity were in consistent with the findings for N content of shoots [55]. On the other hand, Banath et al. [56] reported that the addition of Ca increases N levels in plant tissues. In a study on welsh onion, Yi et al. [57] stated that appropriate doses of Ca dramatically increased the activities of NR, GS, GOGAT and GDH. Further, a noteworthy increase in NO_3^-, NH_4^+, free amino acids and soluble protein was observed suggesting that Ca improves the contents of different N forms and the activities of N—metabolism enzymes.

Impact of sulphur and calcium on reactive oxygen species (ROS) and oxidative stress biomarkers

Sulphur and Ca play a protective role against environmental changes in plants. Singh et al. [10] while working on Cr stressed *S. melongena* L. seedlings reported that the contents of ROS and oxidative stress biomarkers ($O_2^{\bullet-}$, H_2O_2, MDA equivalents and electrolyte leakage) varied with varying doses of S. Sulphur at 0.5 mM dose decreased the contents of $O_2^{\bullet-}$, H_2O_2 and MDA equivalents; while 1.5 mM S exposure more efficiently declined these traits even less than that of control (medium S; 1.0 mM). Further, Singh et al. [10], by performing *in—vivo* visualization of $O_2^{\bullet-}$, H_2O_2 and MDA contents in leaf tissues of *S. melongena* confirmed their biochemical findings, where leaves supplemented with 1.5 mM S showed the lowest intensity of NBT formazan, brown, pink and blue precipitates, respectively. Similarly, Dixit et al. [12] and [2] in rice plant have reported that H_2O_2 accumulation was decreased with increasing S treatment (1.5> 3.5> 5.0 mM S) both in root

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

and shoots. In a study on mustard seedlings, Khan et al. [36] have reported that S (1.0 mM) reduces TBARS and H_2O_2 content compared to control and *in—vivo* visualization for $O_2^{\bullet-}$ contents in leaves followed a similar trend. In contrast, Sheng et al. [17] by histochemical detection of $O_2^{\bullet-}$ and H_2O_2 reported that 5 and 10 mM S did not show any change but the same were enhanced under 25 mM S in root and leaf tissues of the polish wheat plant. The 5 and 10 mM doses of S alleviate Mn—induced lipid peroxidation and loss of plasma membrane integrity in roots, while 25 mM S exposure increased ROS in root as compared to control. Liang et al. [58] showed that both $O_2^{\bullet-}$ and MDA contents were declined in roots on added S exposure (4 mM), while increased in the leaves of polish wheat. The ameliorating effect of S was also reported by Fatma et al. [16] and [27], where 200 mg S kg^{-1} soil more efficiently reduced the H_2O_2 and TBARS contents in *Brassica* plant, which was further confirmed by histochemical staining in leaves [27]. In contrast, S—deficiency promotes TBARS content; however, the presence of S promotes the growth of *B. juncea* and *A. thaliana* seedlings even under stress by reducing TBARS content [29]. Siddiqui et al. [13] reported that 20 and 40 mg S kg^{-1} sand showed a significant reduction in H_2O_2 and MDA contents, while 60 mg S kg^{-1} sand showed reverse trend under stress in genotypes of *B. juncea*.

Calcium—induced reduction in ROS accumulation and consequently damage in plant cells have been linked with a protective mechanism [59]. In Lentil seedlings, the addition of Ca notably declined the levels of H_2O_2 upon Cd exposure [60]. Farzadfar et al. [61] reported that $CaCl_2$ exposure considerably reduced the $O_2^{\bullet-}$ and H_2O_2 concentration in chamomile plant. Moreover, with the help of fluorescence imaging technique by using specific probes for $O_2^{\bullet-}$ and H_2O_2, Tian et al. [24] reported that exogenous Ca treatment resulted into comparatively low fluorescence of $O_2^{\bullet-}$ and H_2O_2 pointing towards lower ROS production in root tips of *Sedum alfredii*. In *O. sativa* leaves, Rahman et al. [33] and [18] reported that upon Ca application, no marked difference for $O_2^{\bullet-}$ and H_2O_2 was found in between control and Ca treated seedlings, which was further confirmed by histochemical detection. Contrarily, MDA contents were found to reduce after Ca exposure to salt stressed *Solanum lycopersicum* exposure [20]. The role of Ca in reducing stress biomarkers have also been detailed in *S. indicum*, *V. faba* and tobacco plants (3, 22, 49).

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

Impact of sulphur and calcium on the antioxidant defense system

The role of varying doses of S on enzymatic antioxidants such as SOD, POD, CAT, GST, APX, DHAR and GR were extensively studied in *Luffa*, rice, wheat, gram and mustard seedlings [17, 62, 63; 64]. In *S. melongena* L., Singh et al. [10] reported an increment in the activities of APX, GST and GR under 1.5 mM S; while a substantial decline under 0.5 mM S except for APX. Dixit et al. [2] reported the increase in APX and GPX activities with rising doses of S (0.5, 3.5 and 5.0 mM) in both root and shoots except for GPX in the shoot. In *B. chinensis*, Liang et al. [58] showed that the activities of enzymes of AsA—GSH cycle *i.e.* APX, DHAR and MDHAR were appreciably augmented in response to 4 mM S as compared to control. Sheng et al. [17] while studying the activities of SOD, APX and GR in polish wheat plant reported the activities were higher upon 25 mM S treatment; while there were no significant differences under 5 and 10 mM S treatments than control. In soil conditions, S treatment individually at 100 and 200 mg kg^{-1} soil, enhanced the activities of CAT, APX and GR as compared to control but with constant rate; however, when supplied to salt stressed *B. juncea* L. seedlings, 200 mg S kg^{-1} soil proved more effective in enhancing the activities of referred enzymes [16, 27]. Similarly, Siddiqui et al. [13] reported that 20 and 40 mg S kg^{-1} sand significantly enhanced the SOD, CAT and GR activities in dose—dependent manner in *B. juncea*; however, 60 mg S kg^{-1} sand showed a negative impact. In gypsum (S 23.3 % and Ca 8.6 %) treated *Cicer arietinum*, an increment was noted for SOD, APX and CAT activities in both 10 and 20 days after treatments [65]. Bashir et al. [29] have reported that the activities of SOD, GST, APX and GR were decreased under S—deficient, while increased under S—sufficient conditions in *A. thaliana* and *B. juncea* seedlings, respectively.

The role of Ca in regulating ROS metabolism via antioxidants system has been well documented in earlier studies. Ahmad et al. [19] and [29] studied the effect of Ca on Cd—induced toxicity in *B. juncea* and *C. arietinum* seedlings and reported that SOD, CAT and GR activities were increased markedly after exogenous Ca exposure to Cd—stressed seedlings, suggesting that Ca—induced higher antioxidant enzymes activities, which consequently lowered H_2O_2 and lipid peroxidation to maintain the structural and functional integrity of cell membranes [66]. Similarly, the addition of Ca to culture media significantly alleviated Cd—induced oxidative injuries in root and shoots of *Lens culinaris* by regulating CAT, DHAR and GR activities [60]. Besides this, Cho et al. [67] reported that Ca deficiency in Cd—stressed rice seedlings, further aggravated the toxicity by down—regulating the CAT, APX

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Vol-22-Issue-17-September-2019

and GR activities, which authorized the imperative role of Ca in antioxidant defense networking of cell. Further, Rahman et al. [33] and [18] noticed that activities of SOD, GPX, GST, MDHAR and GR were almost stable upon Ca exposure alone but when supplied to salt treated rice seedlings, a noteworthy improvement was found. However, in a study on *S. indicum*, Abd_Allah et al. [3] reported an increment in the activities of SOD, POD, CAT, APX and GR upon Ca exposure. Tian et al. [24] while studying with *S. alfredii* reported that total SOD along with its isoforms: Mn—SOD, Fe—SOD and Cu/ Zn—SOD were expressed upon Ca exposure, where Mn—SOD was maximum; while Cu/ Zn—SOD was least expressed. Studies on tobacco [49] and *V. faba* [22], proved that Ca addition improves the enzymatic antioxidant activities.

Impact of sulphur and calcium on non—enzymatic antioxidants

Singh et al. [10] have reported that the contents of Cys and NPTs were significantly increased under 0.5 and 1.5 mM S treatment than 1.0 mM. Similarly, Dixit et al. [2, 12, 39] in rice seedlings reported that Cys, GSH and PCs contents were increased with rising doses of S in root and shoots. In contrast, the level of GSSG was in the order: 0.5> 1.0> 1.5 mM S that lead to the increased GSH to GSSG ratio in rice; while high S exposure restored this ratio [2]. These studies suggest towards the major role of PCs in chelating As^{III} over the GSH [68, 69]. In series, Karimi et al [70], reported higher content of PCs in roots of *Isatis capadocia than GSH.* In a study, Liang et al. [58] have reported that S treatment alone noticeably increased the NPTs, PCs and GSH contents and GSH/GSSG in two cultivars of *B. chinensis*, but decreased GSSG contents, thereby indicating that thiol compounds demand more S for their synthesis [71]. Further, the AsA pool *i.e.* AsA and DHA contents in both cultivars were increased as compared to the control. Bashir et al. [29] and [72] while comparing in between S—deficient and S—sufficient *A. thaliana* and *B. juncea* seedlings, reported that AsA and AsA/DHA were declined at 7 and 14 DAT in S—deficient plants; while DHA showed increasing trend in both the seedlings. In contrast, the opposite trend in S—sufficient plant was found [29]. Further, Bashir et al. [29] reported that GSH, GSSG and PCs contents and GSH/GSSG ratio were decreased in S—deficient; while increased in S—sufficient seedlings both at 7 and 14 DAT. Moreover, Anjum et al. [37], Siddiqui et al. [13] and Bashir et al. [72] reported that contents of AsA, GSH and Pro were better during S—sufficient conditions in *Arabidopsis* and mustard seedlings; while decreased under S—deficient conditions. The increment in AsA, Cys, Pro, GSH/GSSG, PCs and NPT contents upon S addition were

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Vol-22-Issue-17-September-2019

reported in earlier studies [13, 17, 36, 37]; however, sometimes higher doses were found deleterious for these non—enzymatic traits [17, 27]. AL—Huqail et al. [65] in *C. arietinum* plant reported that gypsum increased AsA and DHA contents, while AsA:DHA showed a reverse trend in 10 and 20 DAT seedlings. Further, GSH, GSSG, GSH:GSSG and PCs along with Pro content were sharply enhanced under similar conditions.

Exogenous Ca, differentially modify the antioxidant defense system in plants [73]. The exogenous use of $CaCl_2$ in *Pisum sativum* L. seedlings was found to be highly effective in counteracting the oxidative damage credited by Cd by improving AsA, carotenoids and tocopherol contents [74]. In Ca treated roots of *S. alfredii* suffering from Cd—toxicity, Tian et al. [24] showed that the NPTs and GSH levels were increased and H_2O_2 content was declined, suggesting that Ca promotes GSH biosynthesis under stress condition. A study by Lopez—Climent et al., [75], confirmed the role of GSH in improving citrus plant tolerance against Cd toxicity by raising PCs biosynthesis. While studying with Cd—stressed *O. sativa* seedlings, Srivastava et al. [76] and Rahman et al. [18] and [33] reported a noteworthy increase in AsA and GSH, while decrease in DHA and GSSG contents and DHA/AsA and GSSG/GSH ratio, suggesting a Ca mediates gradual shift from oxidized cellular redox status towards maintenance of the cellular redox homeostasis. Calcium also showed to increase Pro content in *V. faba*, *P. sativum* and *C. arietinum* [19, 22, 74] when added to stressed plants.

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ISSN: 0971-1260

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