Annals of Plant and Soil Research 26(4): 714-724 (2024)
https://doi.org/10.47815/apsr.2024.10422

Interactive effect of sulfur and nitrogen on growth and nitrogen assimilation of lettuce (*Lactuca sativa* L.) varieties

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Received, September, 2024; Revised accepted, November, 2024

ABSTRACT

Nitrogen (N) is one of the key elements for all living organisms and an indispensable constituent of many important macromolecules. Use of N containing fertilizers has dramatically increased over the years for increasing the crop productivity. However, the use efficiency of applied N is only 30-40%, and the rest is lost into the environment, causing severe environmental and health problems. Green leafy vegetables, especially lettuce, accumulate high nitrate content that adversely affects human health. Therefore, lettuce production with low nitrate content under the N fertilization is vital important, not only for health benefit but also for maintenance of environmental sustainability. Sulfur is the tenth most plentiful element in the universe, the nutrient that the plants overlook. Since, sulfate assimilation has metabolic coupling with nitrate assimilation, supplementation of sulfur (S) along with N fertilization has been recommended in many studies in various crops for improving the use efficient of N. In consideration of the need, a field experiment was carried out with combinations of N and S and split doses of N application to identify the most appropriate fertilizer (N and S) application rates in order to enhance lettuce productivity and to reduce nitrate accumulation. Two varieties of lettuce (Lactuca sativa), 'Pusa Kiran' (V_1) and 'Pusa Chaulai' (V_2) were chosen for the study. Six fertilizer treatments S_0N_0 (control), S_0N_2 (S_0N_{25+20}), S_0N_3 ($S_0N_{45+25+20}$), S_1N_0 ($S_{40}N_0$), S_1N_2 ($S_{40}N_{25+20}$) and S_1N_3 ($S_{40}N_{45+25+20}$) were conducted in a randomized block design with three replicates. Growth, nitrate assimilation and leaf nitrate content were assessed and compared at various stages of growth and development in both the varieties. The results suggested that the application of the combined doses of S and N (S_1N_3) in which N is applied in three split doses ($S_{40}N_{45+25+20}$) resulted in the maximum growth of the lettuce and accumulation of the least level of nitrate in leaves. Irrespective of the treatments, V_1 accumulated more nitrate in leaves than V_2 at all the phenological stages. Differential growth and leaf nitrate accumulation of lettuce varieties by the effect of supplementation of the N with S fertilization are evident with the change in the activities of the enzymes of nitrate assimilation. With this knowledge, a more economical and environmentally responsible approach to nitrogen management should be able to be developed for increased lettuce yield with lower nitrate levels.

Keywords: Nitrate; Nitrogen; Sulfur; Lettuce

INTRODUCTION

The growth and development of plants are significantly influenced by mineral elements. Six of the seventeen necessary elements must be present in significant amounts. For the last 50 years, fertilizers that supply mineral nutrients, particularly N, P, and K—have been regarded as crucial inputs in the agriculture sector for crop productivity. Among these mineral elements, nitrogen holds a special place because it is a crucial part of numerous genetic, structural and metabolic compounds found in plant cells. The contribution of the N fertilizer in raising crop productivity is remarkable as is evident from the approximately 20-fold increase in the global use of N fertilizer in the last 50 years (FAO, 2019). With this huge global N cycling, the human input of N fertilizers raises food production that significantly raises production to meet demand of raising population (Godfray *et al.,* 2010). This advancement, however, invited major ecological threat of disturbing N balance as the crops actually take up and assimilate 30-40% of N input, rest 70% of applied N is lost to the aquatic bodies, soil and atmosphere (Raun *et al.,* 1999). More of fertilizer N input significantly raises cost of production too (Rothstein, 2007). Excess N is lost in the atmosphere in the form of nitrous oxide through nitrification and denitrification processes. Though, this loss has small economic significance to the most of farmers, its loss is globally important because the nitrous oxide has 300 times more global warming effect than CO_2 (Griffis *et al.,* 2017). Some of the applied nitrogen is lost through leaching to

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ground water in the form of nitrate, if it is not absorbed by plant roots (Tamme *et al.,* 2009). Leached nitrate contaminates the ground water severely. Plants cannot assimilate whole of the nitrate that is taken up from soil because a part of it either remain non-assimilated or transport to the vacuole. Consumption of high nitrate either in the ground water or crop has serious negative effects on human health (Ikemoto *et al.,* 2002). Nitrogen is taken by the plants in the form of NO_3^- and as NH_4^+ (Leleu and Vuylsteker, 2004) which comes from biological N fixation through microorganism (as NH_4^+), natural N fixation (as NO_3^-) and major part from industrial N fixation in form of fertilizers (Marschner, 1995). Nitrate is usually the major form of inorganic nitrogen available to higher plants. It is taken up by the roots and moved to the shoot by the xylem for reduction and vacuolar storage, or it is reduced and stored in the vacuole. The enzymes nitrate reductase (NR) and nitrite reductase (NiR) work in tandem to reduce nitrate to ammonium (NH4+). The NH_4^+ is then incorporated into amino acids via the glutamine synthetase (GS) and glutamate synthase (GOGAT) cycle (Lea and Miflin, 2003). Improved yield as a result of increased nitrogenous fertilizer usage suggests that availability of nitrate is often a limiting factor in plant growth, however physiological studies suggest that it leads to an accumulation of nitrate in the plants, mainly in leafy vegetables, fodder and forage crops. The high level of nitrate in these plants is highly undesirable as it has serious implications on human and animal health. The most commonly reported toxic effect of nitrate consumption is methaemoglobinaemia in human beings. Meeting the needs for food, fibre and forage in a way that is economically and environmentally sustainable presents a challenge as efforts to boost productivity through more efficient use of resources. Efficiency of nitrogen use can be improved to some extent by supplementation of other nutrients that are not generally added for crop production.

According to a number of studies, the full potential of nitrogen fertilizer could not be realized in situations where sulfur (S) was deficient (Ahmad and Abdin 2000, Prosser *et al.,* 2001, Rathore *et al.,* 2015,). This is due to the fact that sulfur and nitrogen are found in all proteins, and it is expected that some metabolic coordination is necessary to ensure that the fluxes of S and N through their transport and assimilatory pathways fulfil the requirements for amino acids required for the synthesis of protein. It has been said that sulfur is the most overlooked plant nutrient, even though it is the tenth most abundant element in the universe and the fourth major nutrient (Scherer, 2010). Despite the fact that the S content of plants is comparable to that of phosphorous, there hasn't been a similar focus on applying S as fertilizers Consequently, reports of a widespread S deficiency in the agricultural field have appeared. Previously confined to small areas, sulfur deficiency has now spread to larger ones (Messick and de, 2001). The estimated total removal of S through various sources is 1.3 million tonnes/year at the present level of production, while estimated additions of sulfur through various sources is 0.80 million tonnes/year in India. Thus, the gap between addition and removal of sulfur is 0.5 million tonnes/year. As a result, more than 41% of the Indian soil has been reported to have deficiency of sulphur (Singh MV, 2001). There are reports showing that S deficiency causes variations in the concentration of N in plant tissue (Agrawal and Mishra, 1994). Accumulation of organic and inorganic nitrogenous compounds under S-deficient conditions has been reported (Hesse *et al.,* 2004). Amino-N concentration increased considerably in sugar beet with very low S supply (Hoffmann *et al.,* 20004). Similar results in S-starved sugar beet were also elucidated by Bell *et al.,* (1995), Hocking, (1995). It is suggested that the insufficiency of one or both of the two nutrients must affect a regulation that governs the assimilation of N and S.

Fertilizer application must be rationalized in order to make agriculture sustainable, as this can reduce the adverse effects of farming on the environment. The management of agricultural systems requires the assurance of both environmental quality and yield. The highest concentrations of nitrate are found in green leafy vegetables (Prasad *et al.,* 2004), and lettuce is categorized as having an extremely high nitrate content (Santamaria, 2006). Cultivating food crops with low nitrate content is crucial because ingesting large amounts of nitrate may exacerbate human pathologies (Mensinga *et al.,* 2003). The present study was, therefore, conducted to develop nitrogen fertilizer management protocol to improve the productivity of the lettuce with low nitrate content through the

supplementation of sulfur with nitrogen fertilizer, and application of N in split doses. Since nitrate assimilation and accumulation is not a simple matter but a complicated system involving many physiological processes, physiological investigations were carried out to ensure that the changes in the nitrate content of the produce are the result of change in the N and S assimilatory processes of the lettuce.

MATERIALS AND METHODS

Experimental Design, Treatments and Sampling

The experimental field of Aligarh Muslim University, situated at 27° 52′ N latitude, 78° 51′ E longitude, and 187.45 m altitude in Aligarh, India, was the site of the field experiments. The climatic conditions were semiarid and the soil was sandy loam. The seeds of the *Lactuca sativa* varieties 'Pusa Kiran' (V_1) and 'Pusa Chaulai' (V_2) were sown at the rate of 6 kg ha^{-1} in (2 m × 2m) plots in a randomized block design. Three replications of each treatment were followed for the study. At the time of sowing the plots were given 40 kg ha^{-1} each of phosphorous (P) and potassium (K) in the form triple super phosphate and muriate of potash, respectively. Sulfur in the form of calcium sulfate was applied to the field at the rate of 40 kg ha^{-1} in a single basal dose (S_1). Nitrogen as urea was applied at the rate of 90 kg ha^{-1} in two (N_2) or three (N_3) splits. Six treatments were designed, viz., S_0N_0 (control), S_0N_2 (S_0N_{45+45}), S_0N_3 ($S_0N_{45+25+20}$), S_1N_0 ($S_{40}N_0$), S_1N_2 ($S_{40}N_{45+45}$) and S_1N_3 ($S_{40}N_{45+25+20}$). S and N was given in three doses: the first at basal (time of sowing), the second at 20 DAS, and the third at 40 DAS. For the duration of the growth period, four irrigations were performed at various intervals. To keep weeds out of the crops, regular weeding operations were conducted. The seedlings were reduced in size to maintain a 25 cm intra-row spacing after two weeks of sowing. Days 20, 35, 50, 65, 80, and 95 after sowing (DAS) were sampled. Three plants from each pot were randomly chosen for the study. The plants were cut at the root shoot junction and weighed immediately after collection. To measure the activities of determine the activities of nitrite reductase (NiR), nitrate reductase (NR), glutamine synthetase (GS) and glutamate synthase (GOGAT). Nitrate and free amino acids concentration were measured in the leaves.

Determination of Activities Nitrate Assimilatory Enzymes

The activity of nitrate reductase (NR) was analysed through the earlier described procedure by Klepper *et al.* (1971), Nair and Abrol (1977). The NR activity was expressed as µmol NO_3^- produced g^{-1} FW h^{-1}. For analysing the nitrite reductase (NiR) activity, crude extract was prepared and enzyme assay was performed by the previously elucidated methods by Gupta and Beevers, (1984), Ida and Morita (1973). The NiR enzyme activity was expressed as µmol NO_2^- produced g^{-1} DW h^{-1}. The glutamine synthetase (GS) enzyme was extracted, and its activity was measured using an earlier technique (Rhodes *et al.,* 1975, McNally *et al.,* 1983). The GS enzyme activity was expressed as µmol γ-GHA produced g^{-1} DW min^{-1}. For glutamate synthase (GOGAT) enzyme activity extraction was performed and the enzyme activity was assayed by previously established techniques by McNally *et al.* (1983), Rhodes *et al.* (1975). The GOGAT enzyme activity was expressed as nmol NADH oxidized mg^{-1} protein min^{-1}.

Estimation of Concentration of Nitrate and Free Amino Acids

Method of Grover *et al.* (1978) was used to estimate the nitrate content of leaves. The value was given as µmol g^{-1} DW. The ninhydrin method was used to estimate the total free amino acid (Moore and Stein, 1948). The value was given in mg kg^{-1}.

Statistical Analysis

The data were analyzed statistically to find out the significance difference of sulfur supplementation along with nitrogen fertilization on the growth and other biochemical parameters of lettuce. The mean values of three replicates of all the parameters were calculated and analysis of variance was performed by the 'F' (variance ratio) test (Gomez and Gomez, 1984).

RESULTS

Fresh Weight per Plant

Fresh weight of both the varieties was significantly increased by the supplementation of

S with N, when compared with the treatments containing no S. Maximum fresh weight was observed when N was applied in three split doses with S (S_1N_3), followed by S_1N_2 (N applied in two split doses). Maximum response to the S supplementation and split doses of N was found with V2, when compared with V1. Irrespective of the treatments, maximum fresh weight was reported at 80 DAS (Figure 1).

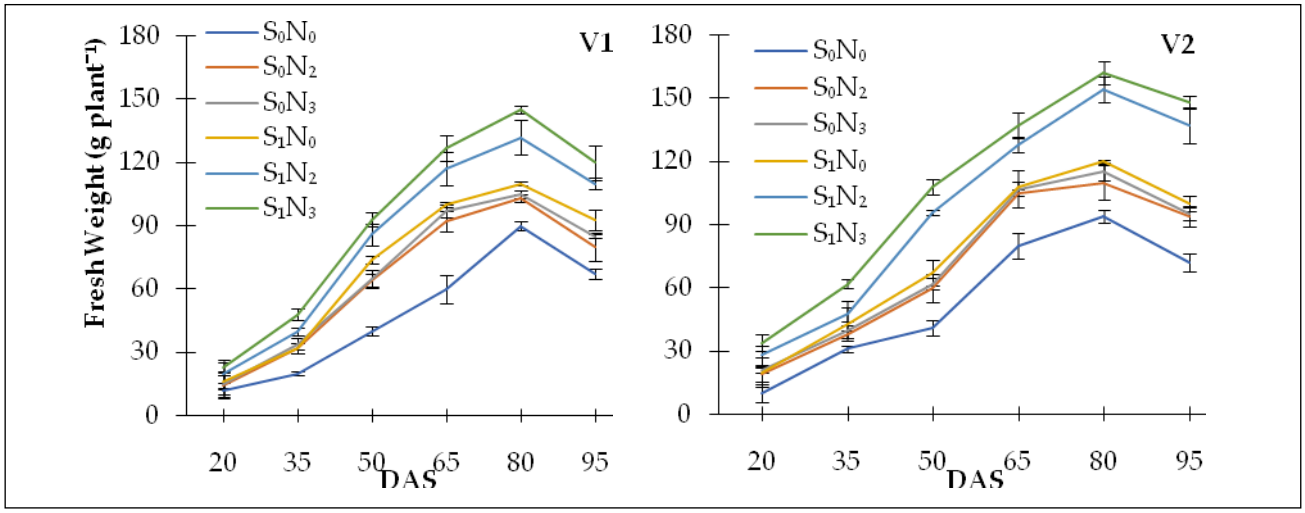

Figure 1: Fresh weight of *L. sativa* cv. Pusa Kiran (V_1) and Pusa Chaulai (V_2) at different phenological stages as influenced by the supplementation of sulfur with nitrogen fertilization and split application of N fertilizer. Values are mean of three replicates. The bars represent SE (n=3)

Nitrate Content

Irrespective of treatments, nitrate accumulation in the leaves of V1 was higher than V2 at all the stages of growth and development. Nitrate content in the leaves of the plants grown with N alone (S_0N_3>S_0N_2) was higher as compared to the plants grown in the presence of both S and N (S_1N_3>S_1N_2) (Figure 2). The result implies a reduced capacity of S_0N_3 and S_0N_2 plants for nitrate reduction. The incapacity of a plant in nitrate reduction has been associated with S deficiency which leads to the accumulation of N content in several species (Reuveny *et al.,* 1980). The treatment S_1N_3 resulted in a drastic reduction in the leaf nitrate content which was followed by S_1N_2 in both V_1 and V_2. In the presence of sulfur alone (S_1N_0) and the treatment without S or N (S_0N_0), dramatically low content of N was observed. Leaves accumulated N until the end of flowering in the plants treated with both S and N. However, no such observation was made for the plants grown with N alone.

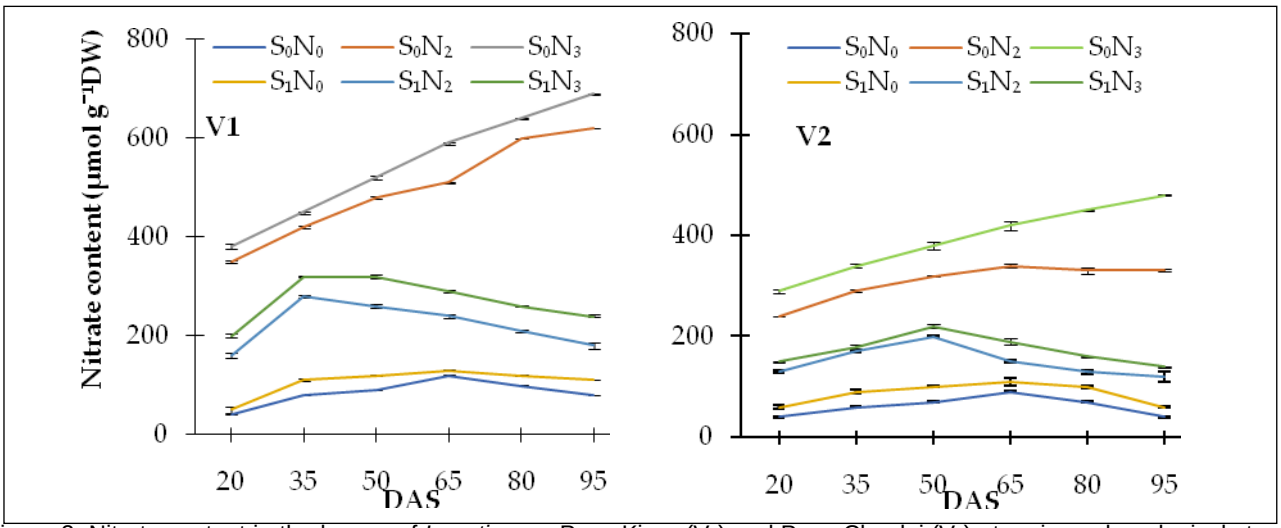

Figure 2: Nitrate content in the leaves of *L. sativa* cv. Pusa Kiran (V_1) and Pusa Chaulai (V_2) at various phenological stages as influenced by the supplementation of sulfur with nitrogen fertilization and split application of N fertilizer. Values are mean of three replicates. The bars represent SE (n=3)

Free Amino Acid Content

Irrespective of the treatments, V1 has higher level of free amino acids than V2 at all the phonological stages. The contents of free amino acids (FAA) in the leaves of the V_1 and V_2 varieties of lettuce were maximum at 35 DAS in S_0N_2 and S_0N_3 treatments, and thereafter, it remained at the same level throughout the phonological stages of the plants. Interestingly, maximum nitrate content with S_1N_2 and S_1N_3 treatments was found at 20 DAS, and there was sharp significant decline thereafter. Treatment containing S alone showed no considerable reduction in the free amino acids content (Figure 3).

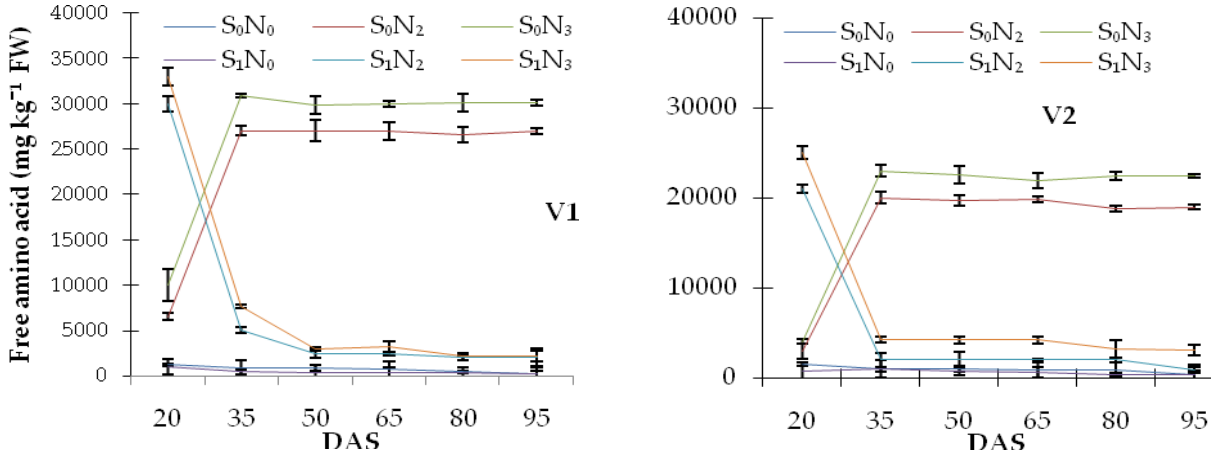

Figure 3: Free amino acid content in the leaves of *L. sativa* cv. Pusa Kiran (V_1) and Pusa Chaulai (V_2) at various phenological stages as influenced by the supplementation of sulfur with nitrogen fertilization and split application of N fertilizer. Values are mean of three replicates. The bars represent SE (n=3)

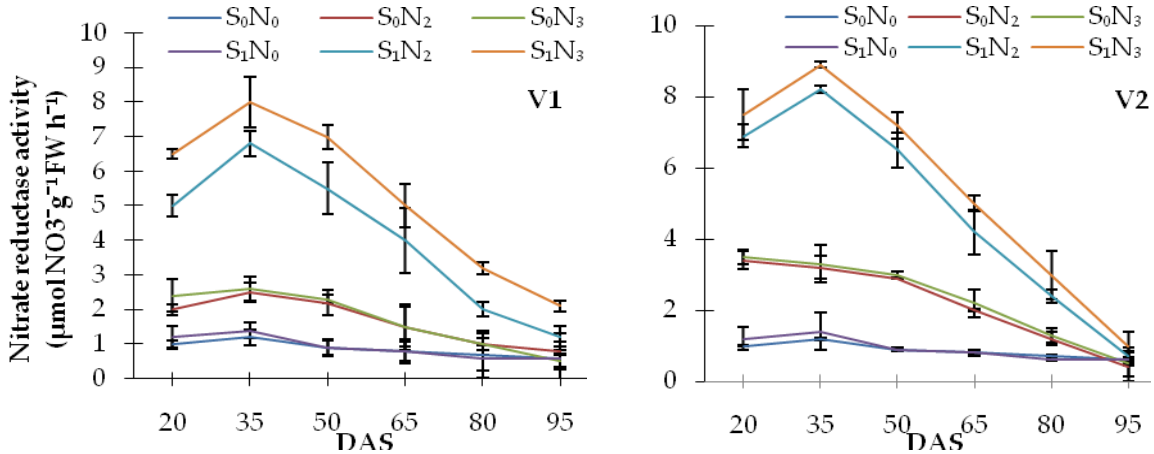

Figure 4: Nitrate reductase activity in the leaves of *L. sativa* cv. Pusa Kiran (V_1) and Pusa Chaulai (V_2) at various phenological stages as influenced by the supplementation of sulfur with nitrogen fertilization and split application of N fertilizer. Values are mean of three replicates. The bars represent SE (n=3)

Nitrate Reductase Activity

Irrespective of treatments, the nitrate reductase (NR) activity was higher in V2 than V1. The Pusa Chaulai (V2) variety of the lettuce responded better than V1 to the supplementation of S with N. In all the treatments, the highest NR activity was reported at 35 DAS. The NR activity in the leaves of the *L. sativa* cultivars was maximum with the treatment S_1N_3 ($S_{40}N_{45+25+20}$), followed by S_1N_2 ($S_{40}N_{25+20}$) (Figure 4). The NR activity of S_0N_3 ($S_0N_{45+25+20}$) and S_0N_2 (S_0N_{25+20}) treated plants was lesser than that of the S_1N_3 and S_1N_2 treatments. The S_0N_0 (control) and S_1N_0 ($S_{40}N_0$) treated plants also showed minimum NR activity. The highest NR activity of S_1N_3 indicated that the NR activity was dependent on an adequate supply of both N and S. The S_0N_3 ($S_0N_{45+25+20}$) and S_0N_2 (S_0N_{25+20}) treated plants had lower NR activity as

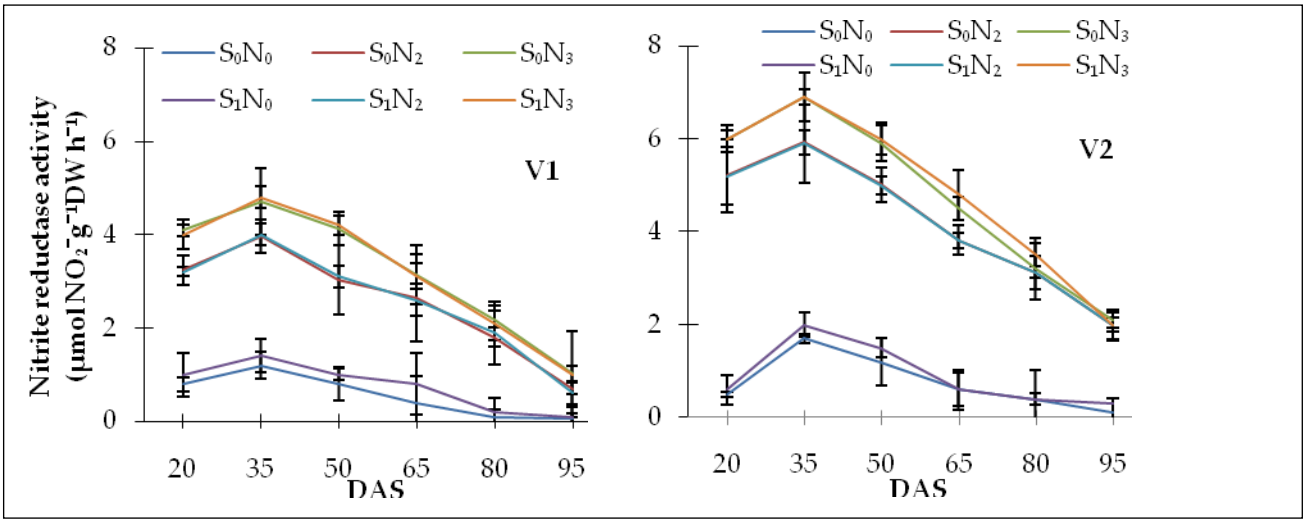

Figure 5: Nitrite reductase activity in the leaves of *L. sativa* cv. Pusa Kiran (V_1) and Pusa Chaulai (V_2) at various phenological stages as influenced by the supplementation of sulfur with nitrogen fertilization and split application of N fertilizer. Values are mean of three replicates. The bars represent SE (n=3)

compared to S_1N_3 and S_1N_2, preferably due to the lack of S. In the S_1N_0 treated plants, the NR activity remained very low due to the lack of N. The obtained result conformed to the findings of adequate amount of S requirements for NR synthesis (Pal *et al.,* 1976).

Nitrite Reductase Activity

The nitrite reductase (NiR) activity was higher in V2 than V1, irrespective of the treatments. Maximum NiR activity was observed at 35 DAS. The NiR activity was the highest in the both the varieties treated with S_1N_3 and S_0N_3, when compared with the other treatments. No significant difference in the NiR activity of S_1N_3 and S_0N_3 treated plants was found, indicating that high N application irrespective of S, showed high nitrite reductase activity (Figure 5). Similarly, S_1N_2 and S_0N_2 treated plants showed lesser NiR activity than S_1N_3 and S_0N_3. However, no significant difference was observed in the nitrite reductase activity of S_1N_2 and S_0N_2 treated plants. The lowest nitrite reductase activity was observed in S_0N_0 plants and S_1N_0 plants. The results showed the dependence of NiR solely on N in both V_1 and V_2 as previously reported (Kaur *et al .,* 2018) .

Glutamine Synthase Activity

The V2 has higher GS activity than V1 irrespective of the treatment. The highest GS activity was reported at 20 DAS at all the treatments, except S_1N_2 and S_1N_3 where the activity of the GS was maximum at 35 DAS. Supplementation of S with N resulted in the significantly higher GS activity in both varieties, when compared with the treatments containing no S (S_0N_2, S_0N_3). Application of N in three split doses resulted in the higher GS activity than two split doses of N (Figure 6). Interestingly, no significant difference in the GS activity of both varieties was observed at other treatments containing N alone (S_0N_2, S_0N_3) or S alone (S^1N_0) at all the phonological stages. The result showed that a balanced supply of both N and S is required for higher GS activity and the lack of either N or S has a pronounced negative effect on the activity of the GS. Previous studies showed similar reports of decline in GS activity in S-starved plants due to the accumulation of amino acids which inhibit the activity of GS as a result of feedback inhibition (Redinbaugh *et al.,* 1993).

Glutamate Synthase Activity

The glutamate synthase (GOGAT) activity was higher in V2 than V1 at all the phonological stages of the plant. Maximum GOGAT activity was reported at 35 DAS. The trend of decline in GOGAT activity after 35 DAS was the same in both the varieties, irrespective of the treatments. The maximum increase in the GOGAT activity was seen by the application of S_1N_3 and S_0N_3

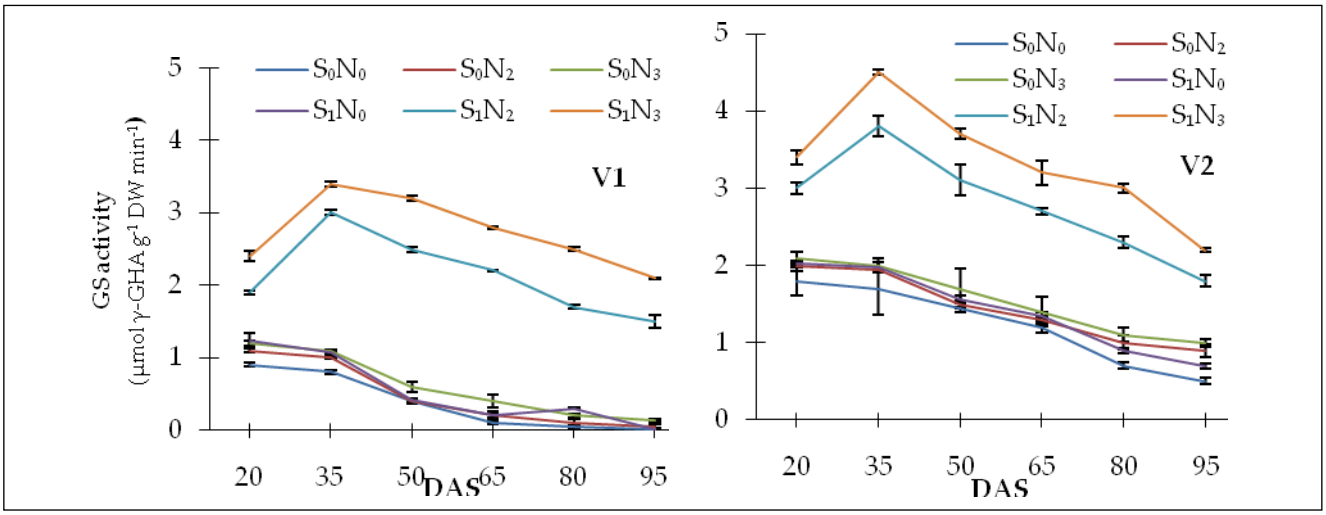

Figure 6: Glutamine synthase activity in the leaves of *L. sativa* cv. Pusa Kiran (V_1) and Pusa Chaulai (V_2) at various phenological stages as influenced by the supplementation of sulfur with nitrogen fertilization and split application of N fertilizer. Values are mean of three replicates. The bars represent SE (n=3)

treatments, followed by S_1N_2 and S_0N_2 treatments, and S_0N_0 and S_1N_0 (Figure 7). Interestingly, there was no significant difference between the GOGAT activity of the plants (V1, V2) treated with S_1N_3 and S_0N_3. Similar observation was found with S_1N_2 and S_0N_2 treatments and S_0N_0 and S_1N_0 treatments.

DISCUSSION

Nitrogen and sulfur play an essential role in the process of protein synthesis. Nitrate is the predominant form of nitrogen that plants take up from the soil; however, dissolved amino acid or ammonia can also carry nitrogen from the root to the shoot. The nitrogen assimilation in plants takes place through the activities of various enzymes. First, nitrate reductase in the cytosol reduces the absorbed nitrate to nitrite, which is then further reduced to ammonium by nitrite reductase in the cell's chloroplasts by Tischner *et al.,* (2007), Hanke *et al.,* (2004). The generated ammonia is then incorporated into amino acids through the GS-GOGAT pathway (Masclaux-Daubresse *et al.,* 2006). Glutamine synthetase uses glutamate as a substrate and incorporates the ammonia as the amide group of glutamines. The GOGAT then produces two glutamates by transferring the amide group to a 2-oxoglutarate molecule and leads the transamination reactions for the production of all the other proteinaceous amino acids [40]. Further, in the sulfur assimilation pathway the sulfur transporters take up the available sulphur (McGrath and Zhao, 1996) which is first activated by the ATP sulfurylase to form adenosine-5-phosphosulfate which further leads to a series of steps wherein O-acetylserine (thiol)lyase (OAS) produces cysteine which together with the amino acids obtained from N assimilation pathways is utilized by the plants for protein synthesis (Lee *et al.,* 2011).

The current research exhibited that the split application of N along with supplementation of S ($S_{40}N_{45+25+20}$) fertilizers resulted in the reduction of the nitrate accumulation in *Lactuca sativa* cultivars 'Pusa Kiran' (V_1) and 'Pusa Chaulai' (V_2). The cultivar V_2 showed lower accumulation of nitrate as compared to V_1. However, the nitrate content in both the cultivars showed high nitrate accumulation in the presence of N alone as compared to the treatment comprised of both N and S (S_1N_2 and S_1N_3) (Figure 2). Substantial amount of nitrate accumulation especially in the family *Brassicaceae* was earlier reported under limiting conditions of S nutrition by McGrath and Zhao (1996) and Koralewska *et al.* (2009). Figure 2 directs towards the fact that a close link exists between the nitrogen and sulfur metabolism by Fazili *et al.* (2008) and Khan *et al.* (2015). In plants, a regulatory interaction between the assimilatory S and N reduction has been recorded in different studies by Rennenberg *et al.* (1979), Haller *et al.* (1986), Ahmad and Abdin (2000), Koprivova *et al.* (2000) and Baishya *et al.* (2015). A possible reason for the nitrate accumulation in the absence of S could be the accumulation of free amino acids in the plants due to the inhibited activity of NR and GS-

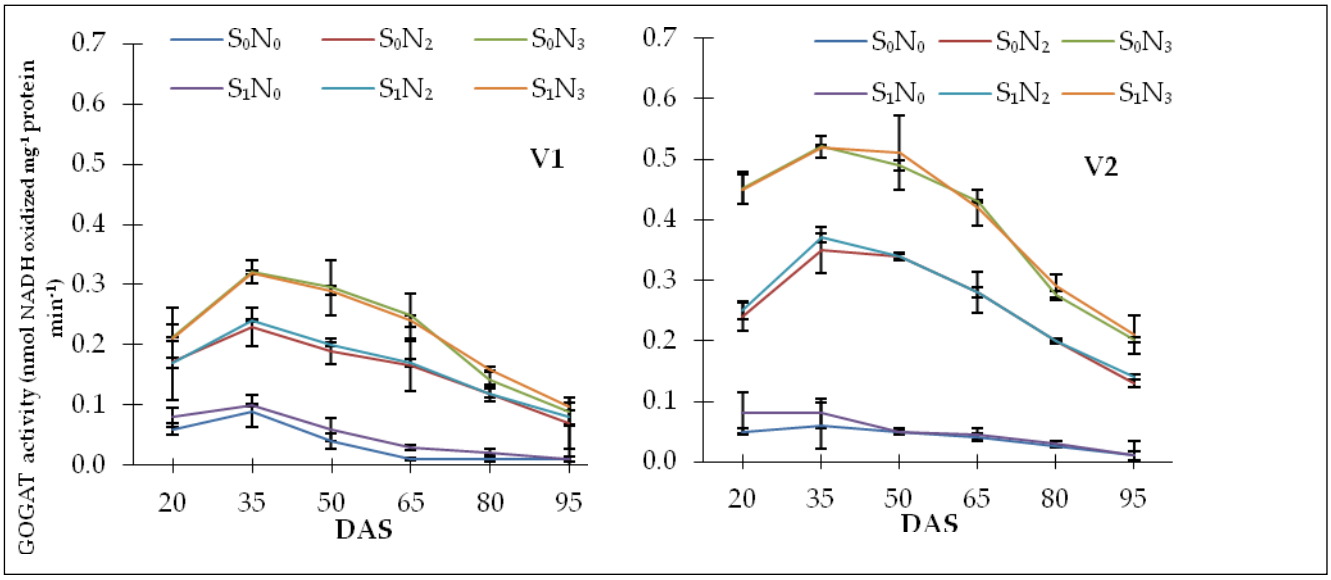

Figure 7: Glutamate Synthase activity in the leaves of *L. sativa* cv. Pusa Kiran (V_1) and Pusa Chaulai (V_2) at various phenological stages as influenced by the supplementation of sulfur with nitrogen fertilization and split application of N fertilizer. Values are mean of three replicates. The bars represent SE (n=3)

GOGAT cycle as a result of feedback inhibition. In conformity to the fact, the figure 3 demonstrated the lower free amino acid content at the S_1N_2 and S_1N_3 treatments, and higher free amino acid accumulation at the S_0N_2 and S_0N_3 treatments. High accumulation of free amino acids suggests the inefficiency of the crop to incorporate the amino acids into protein which implies high nitrate accumulation as a result of inhibited NR activity. A previously reported study conducted on cultured maize cells grown under S-deficit conditions showed an increased pool size of glutamine (Amancio *et al.,* 1997).

It has also been reported that in sulfur deprived tobacco, glutamine and asparagines accumulation in the leaves negatively regulated the NR activity (Migge *et al.,* 2000). Both sulfur and nitrogen are required by the plant to support the process of protein synthesis. As demonstrated in figure 4, the S was found to regulate the activity of the NR. High NR activity was found in the S_1N_2 and S_1N_3 treatments as compared to the treatments without S (Figure 4). A positive effect of S in the NR regulation was found earlier in maize seedlings (Kredich, 1971) and tobacco cells (Pal *et al.,* 1976). Increased OAS content was reported under S-deficit conditions and it was concluded that OAS accumulation reduced the NR activity which reduced the process of nitrate assimilation for maintaining the constant concentration levels of organic and S compounds [38]. Further, as seen from the results, the GS activity is low in the absence of either N or S, or both N and S (Figure 6). But higher GS activity was reported when the plant received both N and S supply. The possible reason for the retarding activity of GS at the treatment containing no S/N could be the feedback inhibition of GS caused by the accumulation of free amino acids as reported in different studies by Kaur *et al.* (2011), Redinbaugh and Campbell (1993), Scheible *et al.* (2004) and Ruffel *et al.* (2008). The activity of NiR and GOGAT, however, did not show any considerable changes in the absence of S (Figure 5, 7). The current research also showed that the activity of NiR, NR, GOGAT and GS were high prior to the flowering period after which their activities declined. It suggests that the application of the fertilizers should be carried out before the flowering period.

CONCLUSIONS

Based on the detailed study of the growth and nitrate assimilatory capacity of two varieties of the lettuce throughout the growth stages, it is suggested that the supplementation of S with N fertilizer and application of N in split doses before the flowering period is optimum for the improving the productivity and quality in terms of reduction of nitrate accumulation of the *L. sativa* varieties. The $S_{40}N_{45+25+20}$ treatment was found to be the best treatment for the better productivity and low accumulation of nitrate for the cultivation of lettuce varieties. Between the two varieties,

Pusa Chaulai (V_2) responded better than cv. Pusa Kiran (V_1). The close link between the S availability and N reduction explained the trend in the enzymatic activities of the different enzymes involved in N assimilation. In the presence of sulphur, high NR and GS activity was found that positively regulated the reduction of nitrate. High NR activity was also linked to low free amino acids accumulation and nitrate accumulation. The availability of S regulated the activity of the NR.

REFERENCES

Agrawal HP, Mishra A.K. (1994) Sulphur nutrition of soybean. Comm Soil Sci Plant Anal **25:** 1303-1314.

Ahmad, A.; Abdin, M.Z. (2000) Photosynthesis and its related physiological variables in the leaves of Brassica genotypes as influenced by sulfur fertilization. *Physiol Plant **110***, 144-149.

Amancio, S.; Clarkson, D.T.; Diogo, E.; Lewis, M.; Santos, H. (1997) Assimilation of nitrate and ammonium by sulfur deficient Zea mays cells. *Plant Physiol Biochem **35***, 41-48.

Baishya, L. K., Rathore, S. S., Singh, D., Sarkar, D., and Deka, B. C. (2015) Effect of integrated nutrient management on rice productivity, profitability and soil fertility. *Annals of Plant and Soil Research*, ***17***(1), 86-90.

Bell MA, Fisher RA, Byerlee D, Sare K (1995) Genetic and agronomic contributions to yield gains: a case study for wheat. Field Crop Res **44:** 55-65.

FAO. (2019). World fertilizer trends and outlook to 2022. Rome.

Fazili, I.S.; Jamal, A.; Ahmad, S.; Masoodi, M.; Khan, J.S.; Abdin, M.Z. O. (2008) Interactive effect of sulfur and nitrogen on nitrogen accumulation and harvest in oilseed crops differing in nitrogen assimilation potential. *J Plant Nutr. **31**,* 1203-1220.

Fowler, M.W.; Jessup, W.; Sarkissian, G. S. (1974) Glutamate synthetase type activity in higher plants. *FEBS Lett. **46**,* 340-342.

Godfray, H.C.J., Beddington, J.R., Crute, IR., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. (2010) Food security: the challenge of feeding 9 billion people. *Science **327***, 812-818.

Gomez, K. H. and Gomez, A. A. (1984) Statistical Procedures for Agricultural Research. Second Edn. Wiley- Inter Science publication, JohnWiley and Sons, New York.

Griffis, T. J., Chen, Z., Baker, J. M., Wood, J. D., Millet, D. B., Lee, X., Venterea, R. T., and Turner, P. A. (2017) Nitrous oxide emissions are enhanced in a warmer and wetter world. *Proceedings of the National Academy of Sciences of the United States of America***, ***114***, 12081–12085.

Grover, H.L.; Nair, T.V.R.; Abrol, Y.P. (1978) Nitrogen metabolism of the upper three leaf blades of rice at different soil levels: I. Nitrate reductase activity and content of various nitrogenous constituents. *Physiol. Plant. **43*****,*** 287-292

Gupta, S.C.; Beevers, L. (1984) Synthesis and degradation of nitrite reductase in pea leaves. *Plant Physiol*, ***75***, 251-252.

Haller, E.; Suter, M.; Brunold, C. (1986) Regulation of ATP-sulfurylase and adenosine 5′-phosphosulfate sulfotransferase by the sulfur and the nitrogen source in heterotrophic cell suspension cultures of Paul's scarlet rose. *J Plant Physiol **125***, 275-283.

Hanke, G.T.; Kimata-Ariga, Y.; Taniguchi, I.; Hase, T. A (2004) Post Genomic Characterization of Arabidopsis Ferredoxins. *Plant Physiol. **134***, 255-264.

Hesse H, Nikiforova V, Gakiere B, Hoefgen R (2004) Molecular analysis and control of cysteine biosynthesis: Integration of nitrogen and sulphur metabolism. J Exp Bot **55**: 1283–1292.

Hocking PK (1995) Effects of nitrogen supply on the growth, yield components and distribution of nitrogen in Linola. J Plant Nutri **18:** 257-275.

Hoffmann C, Stockfisch N, Koch HJ (2004) Influence of sulphur supply on yield and quality of sugarbeet (*Beta vulgaris* L.)-determination of a threshold value. Euro J Agron **21**: 69–80.

Ida, S.; Morita, Y. Purification and general properties of spinach leaf nitrite reductase. *Plant Cell Physiol.* **1973**, *14*, 661-671.

Ikemoto, Y.; Teraguchi, M.; Kaneene, Y. (2002) Plasma level of nitrate in congenital heart disease: Comparison with healthy children. *Pediatr. Cardiol.* ***23*****,** 132–136.

Jones-Mortimer, M.C. (1968) Positive control of sulfate reduction in Escherichia coli. The nature of the pleiotropic cysteineless mutants of *E. coli* K 12. *Biochem J.****110,*** 597-602.

Kaur, G.; Chandna, R.; Pandey, R.; Abrol, Y.P.; Iqbal, M.; Ahmad, A. (2011) Sulfur starvation and restoration affect nitrate uptake and assimilation in rapeseed. *Protoplasma* ***248***, 299-311.

Khan, M.I.R.; Nazir, F.; Asgher, M.; Per, T.S.; Khan, N.A. (2015) Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *J Plant Physiol.* ***173***, 9-18.

Klepper, L,A.; Flesher, D.; Hageman, R,H. (1971) Potential for nitrate reduction in wheat (*Triticum aestivum* L.). *J. Plant Nutr.* ***3***, 843-852.

Koprivova, A.; Suter, M.; Op den Camp, R.; Brunold, C.; Kopriva, S. (2000) Regulation of sulfate assimilation by nitrogen in Arabidopsis. *Plant Physiol.* ***122****,* 737-746.

Koralewska, A.; Buchner, P.; Stuiver, C.E.E.; Posthumus, F.S.; Kopriva, S.; Hawkesford, M.J.; De Kok, L.J. (2009) Expression and activity of sulfate transporters and APS reductase in curly kale in response to sulfate deprivation and re-supply. *J Pl. Physiol.* ***166****,* 168-179

Kredich, N.M. (1971) Regulation of L-Cysteine Biosynthesis in Salmonella Typhimurium I. effects of growth on varying sulfur sources and o-acetyl-l-serine on gene expression. *J Biol Chem.* ***246***, 3474-3484.

Lea, P.J.; Miflin, B.J. (2003) Glutamate synthase and the synthesis of glutamate in plants. *Plant Physiol. Biochem.*, ***41***, 555–564.

Lee, B.R.; Koprivova, A.; Kopriva, S. (2011) The key enzyme of sulfate assimilation, adenosine 5′-phosphosulfate reductase, is regulated by HY5 in Arabidopsis. *Plant J.* ***67*****,** 1042-1054.

Leleu, O., Vuylsteker, C. (2004) Unusual regulatory nitrate reductase activity in cotyledons of *Brassica napus* seedlings: enhancement of nitrate reductase activity by ammonium supply. *J. Exp. Bot.* ***55*****,** 815–823.

Marschner, H. (1995) Mineral Nutrition of Higher Plants. Academic Press Limited London,

Masclaux-Daubresse, C.; Reisdorf-Cren, M.; Pageau, K.; Lelandais, M.; Grandjean, O.; Kronenberger, J.; Valadier, M.H.; Feraud, M.; Jouglet, T.; Suzuki, A. (2006) Glutamine Synthetase-Glutamate Synthase Pathway and Glutamate Dehydrogenase Play Distinct Roles in the Sink-Source Nitrogen Cycle in Tobacco" *Plant Physiol.* **140**, 444-456.

McGrath, S.P.; Zhao, F.J. (1996) Sulfur uptake, yield responses and the interactions between nitrogen and sulfur in winter oilseed rape (*Brassica napus*). *J. Agri. Sci.* ***126*****,** 53-62.

McNally, S.F.; Hirel, B.; Gadal, P.; Mann, A.F.; Stewart, G.R. (1983**)** Glutamine synthetases of higher plants-Evidence for a specific isoform content related to their possible physiological role and their compartmentation within the leaf. *Plant Physiol.*, ***72***, 22-25.

Mensinga, T.T.; Speijers, G.J.; Meulenbelt, J. (2003**)** Health implications of exposure to environmental nitrogenous compounds. *Toxicol. Rev.*, ***22***, 41–51.

Messick DL and de Brey C (2001). The global sulphur situation and outlook. In: Proceeding of the 51st Fertilizer Industry Round Table, St. Peter Beach, Florida.

Migge, A.; Bork, C.; Hell, R.; Becker, T,W. (2000**)** Negative regulation of nitrate reductase gene expression by glutamine or asparagine accumulating in leaves of sulfur-deprived tobacco. *Planta.*, *211*, 587-595.

Mohanty, B.; Fletcher, J.S. (1980) Ammonium influence on nitrogen assimilating enzymes and protein accumulation in suspension cultures of Paul's scarlet rose. *Physiol. Plant.*, *48*, 453-459.

Moore, S., Stein, W.H. (1948**)** Photometric methods for use in the chromatography of amino acids. *J. Bio. Chem.*, *176*, 367-388.

Nair, T.V.R.; Abrol, Y.P. (1977) Studies on nitrate reducing system in developing wheat ears. *Crop Sci.*, *17*, 438-442.

Pal, U.R.; Gossett, D.R.; Sims, J.L.; Leggett, J.E. (1976) Molybdenum and sulfur nutrition effects on nitrate reduction in burley tobacco. *Can J Bot.*, *54,* 2014-2022.

Prasad, S.; Chetty, A.A. (2008) Nitrate-N determination in leafy vegetables: Study of the effects of cooking and freezing. *Food Chem.*, *106*, 772–780.

Prosser IM, Purves JV, Saker LR, Clarkson DT (2001) Rapid disruption of nitrogen metabolism and nitrate transport in spinach plants deprived of sulphate. J Exp Bot **52:** 113–121.

Rathore, S. S., Shekhawat, K., Kandpal, B. K., Premi, O. P., Singh, S. P., and Chand, G. (2015) Sulphur management for increased productivity of indian mustard: a review. Annals of Plant and Soil Research, , *17*, 1-12.

Raun, W.R.; Johnson, G.V. (1999) Improving nitrogen use efficiency for cereal production. *Agron. J.***,** *91*, 357-363.

Redinbaugh, M.G.; Campbell, W.H. (1993) Glutamine synthetase and ferredoxin-dependent glutamate synthase expression in the maize (Zea mays) root primary response to nitrate. *Plant Physiol.*, *101*, 1249-1255.

Rennenberg, H.; Schmitz, K.; Bergmann, L. (1979) Long distance transport of sulfur in *Nicotiana tabacum*. *Planta.*, *147*, 57-62.

Reuveny, Z.; Dougall, D.K.; Trinity, P.M. (1980) Regulatory coupling of nitrate and sulfate assimi lation pathways in cultured tobacco cells. *Proc. Natl. Acad. Sci. USA*., *77*, 6670-6672.

Rhodes, D.; Rendon, G.A.; Stewart, G.R. (1975) The control of glutamine synthetase level in *Lemna minor* L. *Planta*, *125*, 201-211.

Rothstein, S.J. (2007) Returning to our roots: making plant biology research relevant to future challenges in agriculture. *Plant Cell***,** *19*, 2695–2699.

Ruffel, S.; Freixes, S.; Balzergue, S.; Tillard, P.; Jeudy, C.; Martin-Magniette, M.L.; Van Der Merwe, M.J.; Kakar, K.; Gouzy, J.; Fernie, A.R.; Udvardi, M. (2008) Systemic signaling of the plant nitrogen status triggers specific transcriptome responses depending on the nitrogen source in Medicago truncatula. *Plant Physiol*, *146,* 2020-2035.

Santamaria, P. (2006) Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J. Sci. Food Agr.*, *86*, 10–17.

Scheible, W.R.; Morcuende, R.; Czechowski, T.; Fritz, C.; Osuna, D.; Palacios-Rojas, N.; Schindelasch, D.; Thimm, O.; Udvardi, M.K.; Stitt, M. (2004) Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. *Plant Physiol*, *136*, 2483-2499.

Scherer H.W. (2008) Impact of sulfur on N2 fixation of legumes. In *Sulfur assimilation and abiotic stresses in plants*, Khan N.A., Singh S., Umar S. Eds. Springer, Berlin, Germany pp. 43–54.

Scherer HW (2001). Sulfur in crop production. Euro J Agron **14:** 81–111

Singh MV (2001) Importance of sulphur in balanced fertilizer use in India. Fertilizer News **46:** 13-35.

Tamme, T.; Reinik, M.; Roasto, M. (2009**)** Nitrates and Nitrites in Vegetables: Occurrence and Health Risks. In *Bioactive Foods Promoting Health: Fruits and Vegetables*; Watson, R.R., Preedy, V.R, Eds.; Academic Press: Salt Lake City, UT, USA,; pp. 307–321.

Tischner, R.; Kaiser, W. (2007) Nitrate Assimilation in Plants. In *Biology of the Nitrogen Cycle*, Bothe, H., Ferguson, J.S., Newton, E.W. Eds.; Elsevier: Amsterdam, Netherlands, pp. 283–301.