Review Article

Role of Sulphur Fertilization in Legume Crops: A Comprehensive Review

Abstract

Sulphur (S) is an indispensable macronutrient for plant growth and development, and its role in enhancing the productivity and quality of legume crops has garnered increased attention in agricultural research. This comprehensive review explores the multifaceted significance of sulphur fertilization in legumes, shedding light on the pivotal role it plays in optimizing crop performance. From its involvement in amino acid synthesis and protein formation to its impact on chlorophyll production and antioxidant defense mechanisms, sulphur is a key player in the intricate web of plant metabolic processes. The review delves into the intricate molecular mechanisms that underlie sulphur assimilation and its impact on various physiological functions in legumes. Additionally, it investigates the influence of sulphur on legume crop quality parameters, emphasizing its effect on protein content and amino acid profiles, as well as its role in improving crop resistance to abiotic and biotic stresses. The symbiotic relationships between legumes and nitrogen-fixing rhizobia further complicate the sulphur scenario, making it imperative to dissect the dynamic interplay between these crucial elements. With a backdrop of changing agricultural practices, environmental concerns, and the increasing demand for sustainable food production, this review addresses the challenges and opportunities in sulphur fertilization. It also highlights the need for precise nutrient management strategies to harness the full potential of sulphur in legume crop production. In conclusion, this comprehensive review serves as a valuable resource for researchers, agronomists, and policymakers, offering insights into the pivotal role of sulphur fertilization in enhancing the productivity, quality, and sustainability of legume crops in an ever-evolving agricultural landscape.

Key words: ATS, Amino acids, Fertilization,Legumes,Sulphur,

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Introduction

Sulphur (S) is a crucial plant nutrient and a fundamental component of various amino acids, which are the building blocks of proteins and essential for both plants and animals. It falls under the category of plant macronutrients and is utilized by crops in quantities similar to phosphorus (P). In the past, it was believed that there was an ample supply of sulphur available from the atmosphere. However, due to advancements in air quality standards following the implementation of federal clean air regulations, the risk of sulphur deficiency has increased. Sulphur is an indispensable element for all living organisms, including plants. It is found in protein-related amino acids like methionine and cysteine, as well as in vitamins such as biotin and thiamine, phytochelatins, chlorophyll, coenzyme A, and S-adenosyl-methionine**(Nakai and Maruyama, 2020).** Moreover, sulphur plays a role in the formation of disulfide bonds in proteins and contributes to the regulation of enzymes, particularly in redox control. Sulphur provides protection against oxidative damage through compounds like glutathione and its derivatives**(Aarabi*et al.*, 2020).** It is also a component of various secondary metabolites (SMs) in plants and is essential for their physiological functions, growth, and development. The amount of sulphur required by plants varies depending on the species and their growth stages. For example, more sulphur is needed during seed development and when plants are in their vegetative growth phase. **Yadav*et al.*~~,~~ *(*2021)~~,~~**also reported thatSulphur is essential for various biological processes, including photosynthesis, energy production, photoprotection, and metabolic reactions, and is a crucial component of compounds like iron-sulphur cluster-containing proteins.

Forms and sources of ~~S~~sulphur in soil

There are various sources of sulphur found in the soil. Organic matter being the primary contributor, accounting for approximately 95% of the total sulphur content in the soil. The decomposition of organic matter leads to the mineralization of organic sulphur, converting it into sulfate (SO_4^{2-}), which becomes available for plants. Apart from organic matter, various minerals within the soil also contain different forms of sulphur. Weathering or breakdown of these minerals results in the transformation of a portion of sulphur into sulfate. In industrial areas, there is a higher concentration of sulphur dioxide (SO^2) in the atmosphere, primarily due to activities such as fuel burning. This SO^2 dissolves in rainwater and eventually reaches the soil. Pesticides contribute relatively small amounts of sulphur to the soil, although some pesticides do contain sulphur, adding to the soil's sulphur content ref. Chemical fertilizers are another source of sulphur, as they typically contain a significant amount of sulphur in addition to nitrogen, potassium, and phosphorus. Sulphurparticipates in the soil functions in a manner similar to nitrogen. It naturally originates from the decomposition of organic matter and, to some extent, from soil minerals ref. Soils with high organic content or heavier texture tend to provide more adequate sulphur compared to lighter, less organic soils. Organic sulphur compounds undergo a transformation into inorganic forms that are usable by plants. The Sulphur Cycle illustrates these processes, which are driven by microbial activity, requiring moisture, warmth, and time.Organic sulphur in the soil is derived from organic matter, whether from applied organic materials or the remains of grazing or crops. To be taken up by plants, organic sulphur must be converted into the sulfate ion (SO_4^{2-}), these sulfate ions are present in the soil solution, making them susceptible to leaching, depending on soil texture and rainfall, similar to nitrates. This leaching risk must be considered when planning nutrient management, as discussed later.

Role of sulphurin plants

Introduce figure 1.

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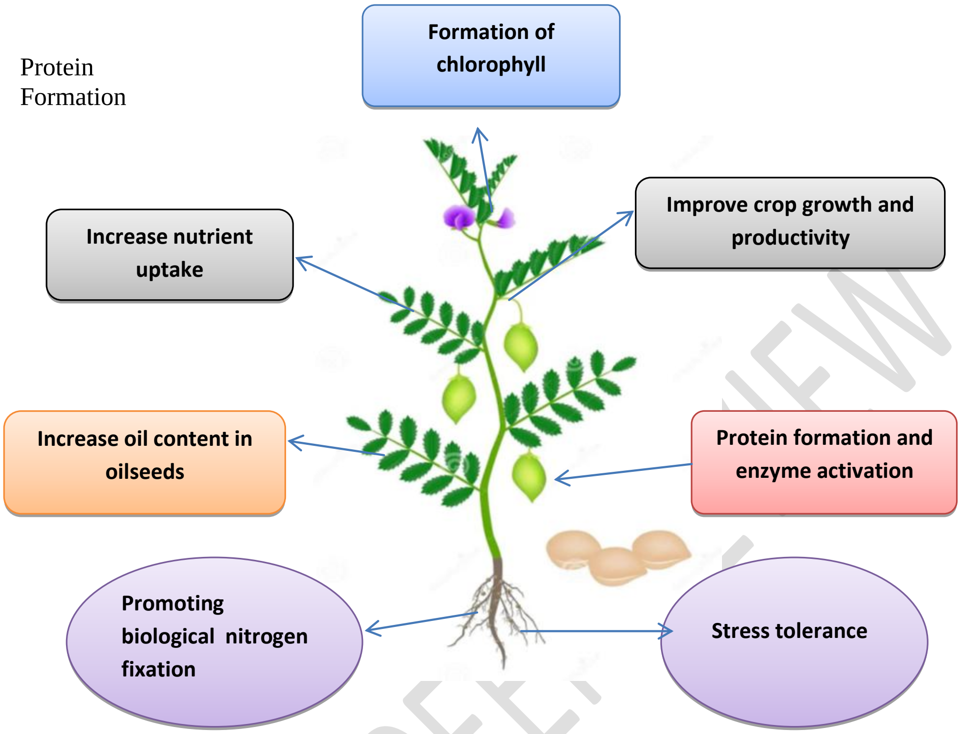

Fig. 1: Role of ~~S~~sulphur in plants

Source

Sulphur deficiency in plants

Sulphur deficiency in plants refers to a condition in which plants do not receive an adequate supply of sulphur, which is an essential nutrient for their growth and development. When plants lack sufficient sulphur, they can exhibit various symptoms and physiological problems that can negatively impact their overall health and productivity.

Fig. 2: Sulphur deficient plants: Chlorosis

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Common signs and symptoms of sulphur deficiency in plants may include:

i. Chlorosis: The most common symptom of sulphur deficiency is the yellowing of the leaves, known as chlorosis. This typically starts with the younger leaves and can progress to older leaves as the deficiency worsens.**(Hawkesford et al., 2016; Jobe et al., 2019)**

ii. Stunted Growth: Sulphur-deficient plants often show reduced growth, resulting in smaller overall plant size**. (Narayan et al ., 2022)**

iii. Delayed Maturity: Sulphur deficiency can cause a delay in flowering and fruiting, which can lead to reduced yields in crop plants.**(Dobermann, 2000)**

iv. Reduced Protein Synthesis: Sulphur is a critical component of amino acids, which are the building blocks of proteins. Inadequate sulphur can result in reduced protein synthesis, leading to poor plant structure and function.**(Yu et al., 2018)**

v. Impaired Photosynthesis: Sulphur is necessary for the formation of chlorophyll, the green pigment responsible for photosynthesis. Sulphur deficiency can hinder photosynthesis, reducing a plant's ability to produce energy and carbohydrates.**(Terry,1976)**

vi. Increased Susceptibility to Pests and Diseases: Sulphur deficiency can weaken a plant's natural defenses, making it more susceptible to pests and diseases.**(Criollo-Arteaga et al ., 2021)**

vii. Altered Nutrient Uptake: Sulphur deficiency can disrupt the uptake of other essential nutrients, such as nitrogen and phosphorus, leading to further nutrient imbalances.**(Mehmood., 2021)**

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The severity of sulphur deficiency symptoms can vary depending on the plant species, soil conditions, and environmental factors. To address sulphur deficiency in plants, it is important to conduct soil tests to assess sulphur levels and consider sulphur fertilization if necessary. Proper nutrient management practices can help ensure that plants receive the sulphur they need for optimal growth and development.Sulphur deficiency symptoms in pulses and oilseeds crops are summarized in Table 1.

Table 1. Sulphur deficiency symptoms in pulses and oilseeds crops~~.~~

Crops	**Deficiency Symptoms**
Chickpea	Plants appear erect, premature drying, and withering of young leaves.
Groundnut	Small Plant height. A “V” shaped petiole appearance. New leaves, the area around the main vein may be pale. Seed maturity delayed.
Pea	Chlorosis in young leaves. Flowering and yield are reduced.
Green gram	Chlorosis in young leaves. Flowering and yield are reduced.
Soybean	Foliage becomes pale green to yellow with non-prominent veins, growth and maturity is delayed, protein formation is reduced.
Pigeon pea	Young and middle leaves turn yellow, branching; leaf size and flowering are suppressed. Flowers lack normal yellow color and shed early. Pod formation and seed development is retarded.
Rapeseed and mustard	Cupped leaves and a reddening of the underside of leaves and stem are observed. Flowers abort prematurely resulting in poor pod formation. Sulphur deficiency reduces oil content in seeds
Sesame	Growth is retarded, leaves are smaller and fully emerged leaves first turn pale and then golden yellow. Number of flowers and pods, hence yield is reduced.
Sunflower	Leaves and flowers become pale. Plants are smaller with shorter internodes. Reduced number and size of leaves.
Linseed	Yellowing, curling and premature drying of tips of young terminal (top most) leaves is evident. Chlorosis gradually spreads on old leaves. The stem remains slender with poor branching. Number of floral buds is reduced and most of these fail to open.
French bean	Plants have short internodes, fewer and smaller leaves. The entire foliage appears pale green. Growth is poor and yield is low.

Source?

Source of Sulphur

Introduce table 2

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Table 2.Source of sulphur fertilizer and S content (%)

S.No	Source	S content (%)
1	Elemental sulfur	>90
2	Ammonium thiosulfate	26
3	Gypsum (calcium sulfate)	19
4	Ammonium sulfate	24

Source?

Ammonium thiosulphate (ATS~~)~~

Factors affecting sulphur deficiency in plants

Sulphur deficiency has become more prevalent in recent years due to the decrease in atmospheric inputs. This reduction in industrial sulphur emissions, primarily resulting from pollution control regulations, has led to a decreased deposition of sulphur into the soil from the atmosphere. As a consequence, soils may have lower sulphur levels, making it more challenging for plants to access the sulphur they need for healthy growth and development. Some of the reasons that cause sulphur deficiency include: Intensive cultivation, sulphur free high analysis fertilizer, high yielding cultivar~~.~~**(Camberato and Casteel, 2010; Gao et al., 2016; Craig, 2019).**

Several factors can influence sulphur deficiency in plants. The sulphur content in the soil itself plays a critical role, with soils naturally low in sulphur or depleted due to intensive farming practices being more susceptible to sulphur deficiency. Soil pH levels also affect sulphur availability, as highly alkaline soils limit sulphur uptake. Additionally, soil organic matter, which contains sulphur, contributes to availability, with higher organic matter content enhancing sulphur uptake. Soil texture plays a role, with sandy soils having lower sulphur retention capacity and a higher risk of leaching **(Scherer, 2001).** Microbial activity is vital for transforming organic sulphur into usable forms, but adverse conditions like excessive dryness or waterlogging can hinder this process (**Zhao & McGrath,1994**).Weather and climate conditions, including rainfall, impact sulphur availability, with excessive rainfall leaching sulphur and arid regions experiencing reduced sulphur mineralization. Industrial and environmental pollution can deposit sulphur dioxide (SO2) in the soil, affecting sulphur levels but potentially causing soil acidification **(López-Bucio et al., 2019).** The use of sulphur-containing fertilizers can mitigate deficiencies **(Hawkesford, 2012).** Lastly, understanding the sulphur uptake capabilities of specific plant species or varieties is essential for preventing sulphur deficiency.

Sulphur uptake and assimilation by legume crops

Sulphur is absorbed by plant cells in the form of sulfate through sulfate transporters and must undergo a reduction process to convert into organic sulfide. The process of sulfate assimilation begins with the activation of sulfate into adenosine-5'-phosphosulfate (APS) catalyzed by ATP sulphurylase. The APS is subsequently reduced into sulfite and further into sulfide through the actions of APS reductase (APSR) and sulfite reductase (SIR), respectively. Sulfide is then integrated into O-acetylserine, resulting in the formation of cysteine (Cys). O-acetylserine is synthesized from serine and acetyl-coenzyme A by the enzyme serine acetyltransferase (SAT). Notably, some of these enzymes exist in different isoforms localized within distinct cellular compartments. In Arabidopsis thaliana, for example, OAS-A1, OAS-B, and OAS-C are found in the cytosol, chloroplasts, and mitochondria, respectively **(Heeg et al., 2008).** Similar homologous OAS isoforms have been identified in legume nodules. Additionally, multiple SAT isoforms exist, including cytosolic SAT5 and plastidic SAT1 **(Krueger et al., 2009)**. Although transcripts of SAT1 and SAT5 can be detected in nodules according to the Gene Expression Atlas of model legumes Lotus japonicus (LjGEA1) and Medicagotruncatula (MtGEA2), proteomic analyses have not detected these enzymes, suggesting their low abundance in nodules. On the contrary, methionine (Met) is produced from cysteine (Cys) within the plastids through a sequential process involving cystathionine γ-synthase (CGS), cystathionine β-lyase (CBL), and methionine synthase (MetS). However, in both Arabidopsis thaliana and legumes, there are cytosolic isoforms of MetS that play a role in providing Met for the synthesis of ethylene, polyamines, and nicotianamine. This metabolic pathway necessitates the prior conversion of Met into S-adenosylmethionine by S-adenosylmethionine synthase (SAMS), as discussed in detail by **Bürstenbinder and Sauter (2012).** While all the enzymes involved in Met, ethylene, and polyamine synthesis have been identified in nodules. A quantitative proteomic approach has shown that the levels of MetS and SAMS, as well as ethylene production, decrease during drought stress in nodules. However, it's noteworthy that the levels of Cys and Met remain consistent. This observation suggests that maintaining the balance of sulphur-containing amino acids is crucial for symbiotic nitrogen fixation (SNF) under such conditions **(Larrainzar et al., 2007).**

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Methods of sulphur application in legume cultivation

There are a lot of methods of sulphur application legumes crops but some of the methods like; basal application at the time of sowing, foliar fertilization and fertigation are very effective that increase the use efficiency of sulphur in crop production. Recommended dose of 30 kg/ ha S application recorded significantly higher growth and yield **(Patel et al., 2012; Muhammadet al., 2016; Makol et al., 2020).** Foliar application in legumesobtained yield and yield attributes at different interval and different dose **(Ankit et al., 2021).**

Sulphur Requirements of Crops

Plant species exhibit significant variability in their sulphur (S) requirements, and maintaining an adequate and balanced S nutrition is critical for their overall production, quality, and overall health **(Till, 2010; Zhao et al., 2008; Khan and Mazid, 2011).** The S requirement of plants varies depending on their developmental stage and species, with S concentrations in plants typically ranging from 0.1% to 1.5% of dry weight (**Anjum et al., 2012**). Despite its lower abundance compared to nitrogen (N), S in plants plays essential roles in numerous metabolic pathways. Under subsistence agriculture, the removal of S by an average crop varies; it's approximately 3-4 kg in cereals, 5-8 kg in sorghum and millets, 8-12 kg in pulses, and exceeds 12 kg in oilseeds. When comparing the S requirements of crops to phosphorus (P), the typical ratio (P:S) is around 1.3 for cereals, 0.8 for legume forages, and 0.6 for cruciferous crops. Notably, oilseeds, such as Brassicas like oil rapeseed and mustard, have a S uptake that is nearly twice that of P uptake. Consequently, Brassicas are more sensitive to S deficiency compared to cereal crops As a result, crop plants are generally categorized into three classes based on their S requirements: high (including oil rapeseed, mustard, Cruciferous vegetables, alfalfa, etc.), medium (including cotton, grasses, sugarcane, coffee, etc.), and low (including cereals, groundnut, sugar beet, etc.) S-requiring species**(Clarkson and Hanson, 1980).**

Role of sulphur fertilization in legumes

Sulphur plays a crucial role in pulse crops **(Vidyalakshmi et al., 2009),** with pulses containing approximately 0.24% to 0.32% sulphur content. Sulphur is responsible for various essential processes, including protein synthesis, chlorophyll formation, and overall growth and metabolism **(Parashar and Tripathi, 2020).** When soils in regions where pulse crops are grown lack sufficient sulphur, it can result in stunted plant growth and a decline in both photosystem 1 and photosystem 2 activities. This decline leads to reduced chlorophyll levels, resulting in lower rates of photosynthesis **(Giordano et al., 2000).** Consequently, there is a decrease in chlorophyll a/b binding protein and Rubisco levels (Jamal, 2006). In sulphur-deficient plants, there is a significant reduction in Rubisco content and the number of monosaccharides, coupled with an increase in starch content. However, there are no observed changes in metabolite levels within the Calvin cycle or the tricarboxylic acid (TCA) cycle **(Lunde et al., 2009).** The sulphur content in the soil has a positive impact on yield, particularly when sulphur is readily available in the soil (Eriksen, 2009). In regions with low sulphur content in the soil, biological nitrogen fixation, nodulation, and peanut crop yields are adversely affected **(Jamal et al., 2010).**Application of sulphur in the form of sulphate to the soil results in an increase in whole plant dry mass, nodule biomass, and root length, particularly when expressed on a root length basis. This increase can be attributed to improved nodule development, as well as enhanced nitrogenase and leghemoglobin synthesis. As a result, nitrogen fixation is significantly improved in sulphur-deficient plants following sulphur application **(Eriksen, 2009).Yadav et al.,(2011),**reprted that 20 kg/ha S application gave the higher growth and yield attribues in clusterbean. The significantly higher growth attributes like: plant height, number of branches per plant, leaf area index, etc are increased with increasing sulphur levels. The significantly higher growth attributes recoerded at 30 kg/ha S application in different pulses crops (chickpea, moongbean, urdbean**) (Khurana , 2007; Kumar and Mehera, 2022; Das et al., 2016).Rasool et al**.(yr) observed increases in growth parameters, yield characters, seed yield, oil content and yield with high applications of S fertilizer. Application of S at 60 kg ha−1 recorded 2.3%, 23.5%, and 12% increases in plant height, LAI, and dry matter production, respectively, over 30 kg S ha−1. This was attributed to S regulating the metabolic and enzymatic processes including photosynthesis and respiration. Yield characters were significantly influenced by S application of 60 kg S ha −1 over 30 kg S ha−1. Seed yield increased significantly with higher S application, probably due to better partitioning of photosynthate to the reproductive parts of the plants being influenced by higher rates of S. As compared to 30 kg S ha^{-1} rate, 6 0 kg S ha^{-1} offered a 2% and 10.55% increase in oil content and yield, respectively. S fertilization had significant effect on yield, yield attributes

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and protein content of soybean. Application of S at 20 kg ha^{-1} in soybean produced the highest plant height, seed yield (1000-seed weight) and straw yields as compared to the lowest responses realised from the control treatment (**Farhad et al., 2010**).

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Interaction of S with Other Nutrients

In the plant biology, sulphur (S) and nitrogen (N) play interconnected and synergistic roles in protein synthesis. The availability of these nutrients in plants is intricately linked **(Mazid et al., 2011)**. The S requirements and metabolism in plants are intimately tied to their N nutrition, and conversely, the S status of a plant significantly influences N metabolism. Just as the carbon assimilation pathway is closely associated with nitrate assimilation in plants, S and N assimilatory pathways are also intertwined, with the abundance of one element affecting the other. Numerous researchers have reported that a deficiency in sulphur can limit the efficient utilization of added soil nitrogen **(Kaya et al., 2009; Jamal *et al*., 2011).** Consequently, to achieve the maximal utilization of added nitrogen, the addition of sulphur to the soil becomes imperative. For instance, studies have shown that an ideal ratio of available nitrogen to available sulphur is 7:1, with ratios falling below 7 resulting in reduced yields in oilseed rape crops **(Janzen and bettany., 1984)**. Furthermore, in field-grown oilseed rape crops alongside mustard crops, it was observed that 27-31% of added sulphur was recovered in the absence of nitrogen, but this recovery increased to 37-38% when 60 kg/ha of nitrogen was applied **(Jamal *et al*., 2011)**.

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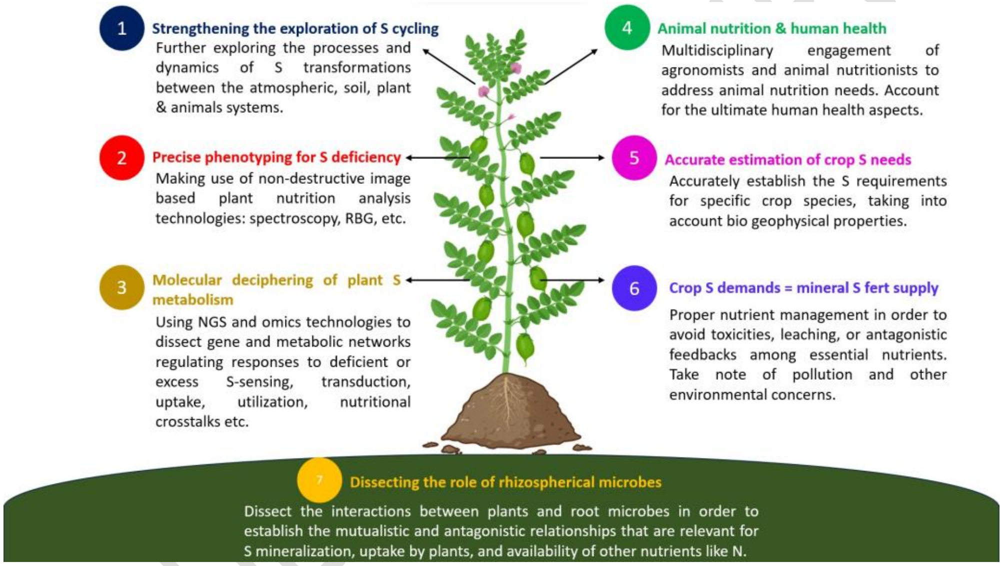
Future crop sulphur research

Over the past two decades, substantial progress has been made in sulphur (S) research, focusing on enhancing crop yields, improving nutritional quality, understanding S uptake and metabolism, identifying S nitrate transporter genes, elucidating S's role in abiotic and biotic stress responses, and recommending S application rates for different crops. However, several critical knowledge gaps persist, warranting further investigation. Firstly, the intricate dynamics of S transformation within the atmospheric, plant, and soil systems, including its interactions with other atmospheric gases (~~e.g.,~~for example greenhouse gases), need comprehensive exploration. Additionally, investigations into the symbiotic relationships between plants and rhizospheric microbes, particularly rhizobia and arbuscularmycorrhizal fungi, regarding S mineralization and nitrogen (N) fixation, are essential. Furthermore, the interactions between S and other plant nutrients should be advanced to determine S's contributions (synergistic or antagonistic) to nutrient uptake and metabolism by plants. Proper soil nutrient balancing, accounting for residual S amounts in soil and plant residues, is crucial. In the realm of plant biology, utilizing next-generation sequencing technologies and omics approaches is essential to delve into the molecular mechanisms underlying plants' responses and tolerance to S deficiency or excess. This includes characterizing key genes, proteins, and metabolic networks involved in S perception, signaling, uptake, utilization, and nutrient crosstalk. These findings can aid in transgenic breeding for highS use efficiency (SUE) plants. Additionally, S nutrition's impact on animal nutrition and performance should not be overlooked. S plays a critical role in enhancing the protein value of forage and silage, which is essential for animal grazing and feeding. However, balancing S fertilization to increase protein levels without causing leaching losses or toxicity in animal systems requires careful consideration. Lastly, precision phenotyping of crop plants for S deficiency or toxicities using high-throughput plant phenotyping technologies, including spectroscopy imaging, fluorescence imaging, and RGB imaging, can provide non-destructive insights into nutrient concentrations and their phenotypic effects. This approach can significantly contribute to optimizing S management in crop production.



1 **Strengthening the exploration of S cycling**
Further exploring the processes and dynamics of S transformations between the atmospheric, soil, plant & animals systems.

2 **Precise phenotyping for S deficiency**
Making use of non-destructive image based plant nutrition analysis technologies: spectroscopy, RBG, etc.

3 **Molecular deciphering of plant S metabolism**
Using NGS and omics technologies to dissect gene and metabolic networks regulating responses to deficient or excess S-sensing, transduction, uptake, utilization, nutritional crosstalks etc.

4 **Animal nutrition & human health**
Multidisciplinary engagement of agronomists and animal nutritionists to address animal nutrition needs. Account for the ultimate human health aspects.

5 **Accurate estimation of crop S needs**
Accurately establish the S requirements for specific crop species, taking into account bio geophysical properties.

6 **Crop S demands = mineral S fert supply**
Proper nutrient management in order to avoid toxicities, leaching, or antagonistic feedbacks among essential nutrients. Take note of pollution and other environmental concerns.

7 **Dissecting the role of rhizospherical microbes**
Dissect the interactions between plants and root microbes in order to establish the mutualistic and antagonistic relationships that are relevant for S mineralization, uptake by plants, and availability of other nutrients like N.

Fig.3: Future prospects of Sulphur research

Source

Conclusion

Sulphur plays vital roles in the fundamental processes of plant metabolism, providing essential antioxidant and protective functions against various environmental stresses. As we confront the growing global demands for food, animal feed, and biofuels to support an increasing population, sulphur, much like other macronutrients, can play a pivotal role in promoting sustainable soil fertility management, enhancing crop yields, and producing nutrient-rich crops. Consequently, as we look ahead, there is a need to refocus our attention on sulphur nutrition. Our future efforts should be concentrated on deepening our comprehension of the molecular mechanisms and dynamics governing sulphur availability and utilization in plants. Additionally, we should strive to unravel the intricate interactions between sulphur and other nutrients. Furthermore, exploring the involvement of soil rhizospheric microbes in the transformation of sulphur within plants is essential.

Institutional Review Board Statement

Not applicable.

Data Availability Statement

Not applicable.

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