1 Introduction

1.1 Role of sulfur in plants

Sulfur (S) belongs to the secondary macronutrients (calcium, sulfur, magnesium), which are essential for plant growth, together with the primary macronutrients (nitrogen, phosphorus and potassium) that are required in much higher amounts, and the micronutrients. In some plants such as oil crops, legumes and forages, S requirement is even more important than phosphorus for optimal yield and quality. Usually cereal crops need 15 to 30 kg S/ha but the requirement for legumes and oil seeds is 25 to 50 kg S/ha (Sarda et al., 2014).

Sulfur has a vital role in the plant life cycle and for yield due to the presence of a wide range of sulfur-containing compounds including amino acids (cysteine and methionine), Fe-S cluster enzymes, glutathionine (GSH), thiols, vitamins, cofactors such as thiamine and coenzyme A, and sulfolipids in chloroplast membranes (Hell, 1997; Dubuis et al., 2005; Noctor, 2006; Begueret et al., 2007; Popper et al., 2011). Moreover, sulfur-containing secondary metabolites such as phytochelatins, phytoalexins, alliins, thionins and glucosinolates that are found in some plant species play important roles in plant physiology and plant defence processes against pests, pathogens and abiotic stresses. The sulfur enhanced defense (SED) mechanism was demonstrated to be present in virus infected tobacco and to suppress virus accumulation in the presence of sufficient sulfate. Also elemental sulfur limited the spread of infection in tomato with *Verticillium dahliae* infection (Thomma et al., 2002; Cooper and Williams, 2004; Durenkamp and De Kok, 2004; Falk et al., 2007; Holler et al., 2010; Bai et al., 2013).

Sulfur improves the efficiency of other macronutrients such as phosphorus and potassium in plants. An adequate sulfur status is important for the uptake and assimilation of micronutrients such as iron. In graminaceous plants growing under S deficiency condition, synthesis and secretion of mugineic acids were decreased and consequently the plants showed lower tolerance to Fe deficiency (Astolfi et al., 2006).

In crop plants such as brown rice, sulfur together with calcium, iron and zinc are important for reducing cadmium (Cd) uptake and accumulation in Cd contaminated soils (Khan et al., 1996; Nikiforova et al., 2003; Astolfi et al., 2006; Fan et al., 2010).

1.2 Sulfur access and assimilation in plants

In the soil, there are two main sulfur sources including organic (90-95%) and inorganic (5-10%). Plants mainly take up sulfate (SO_4^{2-}), which represents only 1% of total sulfur in the soil. Soil organic sulfur must be converted to inorganic sulfate (SO_4^{2-}) through microbiological and biochemical reactions prior to uptake by the plant.

First, sulfate is absorbed by epidermal and cortical root cells and subsequently is transported to different tissues by different sulfate transporters (ST) (Scherer, 2001; Hawkesford, 2003; Rouached et al., 2009; Kulhanek et al., 2011).

During sulfate assimilation into organic compounds, sulfate is reduced to sulfide in different steps (Fig.1). First, sulfate is activated by ATP sulfurylase (ATPS) and converted to adenosine-5'-phosphosulfate (APS). Then APS is reduced stepwise to sulfite by adenosine 5'-phosphosulfate reductase (APR), and sulfite is subsequently reduced further to sulfide by sulfite reductase (SiR). To form cysteine, sulfide is transferred to O-acetyl serine (OAS). Cysteine is the most important precursor for other organic sulfur containing compounds such as glutathione, coenzymes and methionine in plants (Leustek and Saito, 1999; Durenkamp and De Kok, 2004; Davidian and Kopriva, 2010; Hubberten et al., 2012b).
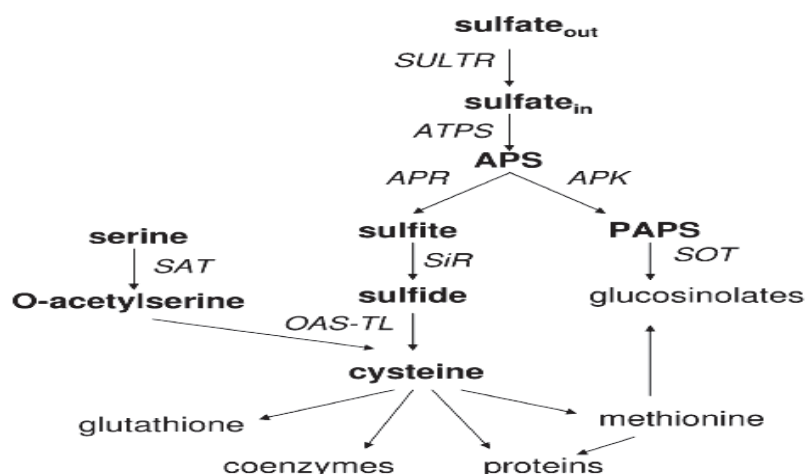

Fig. 1 Plant sulfate assimilation (Adopted from Davidian and Kopriva, 2010), ATPS: ATP sulfurylase, APS: adenosine 5'-phosphosulfate, APR: adenosine 5' -phosphosulfate reductase, APK: APS kinase, PAPS: 3'-phosphoadenosine 5' -phosphosulfate, SAT: serine acetyltransferase, SiR: sulfite reductase, OAS-TL: OAS (thiol) lyase OAS: O-acetylserine, SOT: sulfotransferase.

In addition to pedospheric sulfur (SO_4^{2-}), plants are able to use atmospheric sulfur (mainly SO_2 and H_2S) to support their growth. Atmospheric sulfur is reported to be a significant sulfur source for white clover legumes under low sulfur conditions. SO_2 and H_2S as sole sulfur sources are foliar absorbed via stomates. SO_2 is oxidized to sulfate via sulfite oxidase, while H_2S is reduced to cysteine via O-acetylserine (Durenkamp and De Kok, 2004; Durenkamp et al., 2007; Varin et al., 2013).

1.3 History of plant S deficiency

With Europe`s industrialization in the nineteenth-century, atmospheric sulfur dioxide (SO_2) emissions highly increased because of sulfur-containing fossil fuels, domestic coal burning and vehicles. Symptoms of industrialization were ecosystem problems such as acid rains and human health problems. In London during fog or smog, e.g. during the "pea souper" smog in 1959, SO_2 levels reached up to 3500 $\mu g/m^3$ and caused 4,000 deaths. Furthermore, it is reported from Chinese cities, that smog led to 50,000 premature deaths in 2000 (Hedley et al., 2002; Zhao et al., 2003; Dubuis et al., 2005).

In response to critical health problems, international agreements in the 1970s led to a significant decline in environmental pollutions such as SO_2. The consequence of decreased SO_2 emissions was an increase in the frequency of S deficiency in plants during the last decades. The current sulfur input from the atmosphere is reduced to about 10 kg ha^{-1} $year^{-1}$ in most Western European countries. Furthermore, the improvement of agricultural methods with less utilization of S-containing fertilizers, also the breeding of crops with higher yield and therefore higher S demand, promoted the occurrence of S deficiency (Dubuis et al., 2005; Scherer et al., 2008a; Howarth et al., 2009).

1.4 Plant responses to S deficiency

The average sulfur content in plants is about 0.2% to 0.5% of the dry matter. When plants are grown under less than the optimal conditions, deficiency symptoms appear. Visual S deficiency symptoms are chlorosis and yellow leaves (as a consequence of a decrease in chlorophyll content). Yellowing is followed by necrosis and death in long term S deficiency (Hawkesford, 2000; khan and Mazid 2011). S deficiency has a negative effect on the crop yield, protein concentration,

and total RNA content. S deficiency can reduce seed quality due to a shift in N to S ratio in plants (Drew, 1975; Zhao et al., 1999a; Nikiforova et al., 2005; Pacyna et al., 2006; Lunde et al., 2008)

A general response to nutritional limitation is root proliferation to increase the nutrient uptake (Lopez-Bucio et al., 2003; Bai et al., 2013). With increasing the root sink capacity, transport of sulfate via the xylem to young leaves is reduced and results in strong sulfur starvation in young leaves. Indeed, expression of internal sulfate transporter genes is changed under S deficiency conditions and sulfate is remobilized from mature leaves to the roots instead of young leaves. This is consistent with an enhanced sink capacity of the roots for sulfate (Clarkson et al., 1992; Abdallah et al., 2010; Honsel et al., 2012).

The negative effect of S deficiency on the shoot biomass is more pronounced as compared to the root biomass, resulting in a significant increase in the root to shoot ratio.(Lopez-Bucio et al., 2003; Buchner et al., 2004b; Hawkesford and De Kok, 2006; Varin et al., 2010; Ciaffi et al., 2013).

Another response to S deficiency is early flowering in plants. A rebalancing of metabolism and a general reduction in metabolic activity, i.e. decrease in the total RNA, chlorophyll, biomass and protein content, indicates the plant's priorities to save resources for survival. Consequently, S deficiency leads to earlier flowering although with retarded growth (Nikiforova et al., 2005; Hoefgen and Nikiforova, 2008; Watanabe et al., 2010).

1.5 S deficiency and nitrogen fixation of legumes
1.5.1 Leguminous plants and nitrogen fixation

Leguminous plants are cultivated approximately on 250 million ha worldwide. Symbiotic nitrogen fixation is one of the main characteristics of legumes. Bacterial symbiosis fixes about 9×10^{10} kg of N_2 per year which is about 80% of the total biologically fixed N_2 (Vance, 1998; Graham and Vance, 2000). Gram-negative bacteria in the soil like *Rhizobium*, *Mesorhizobium*, and *Allorhizobium* can undergo a symbiotic association with legumes by means of a molecular dialogue or cross talk between both partners. This leads to the formation of a new plant organ, the so-called nodule.

1.5.2 Nodule formation

In symbiotic associations with rhizobial bacteria (Fig. 2), plants secrete first flavonoids into the rhizosphere which triggers the release of rhizobial *Nod* factors (lipochitooligosaccharide signals). Then *Nod* factor signals are detected by receptor-like kinases *(Nod* factor receptors) in the root where they induce a number of physiological and morphological responses. Subsequently, cell division initiates nodule formation in the root cortex. Simultaneously, *Nod* factors stimulate the formation of an infection thread thorough root hair curling around the bacteria to encapsulate one or more bacteria. Afterwards bacteria enter the plant cell via an infection thread and are later surrounded by a plant plasma membrane-derived membrane in the symbiosome where they differentiate into their endosymbiotic form called bacteroids to fix nitrogen (Stougaard, 2000; Long, 2001; Oldroyd and Long, 2003; Oldroyd and Downie, 2008; Dolgikh et al., 2011; Oldroyd, 2013).
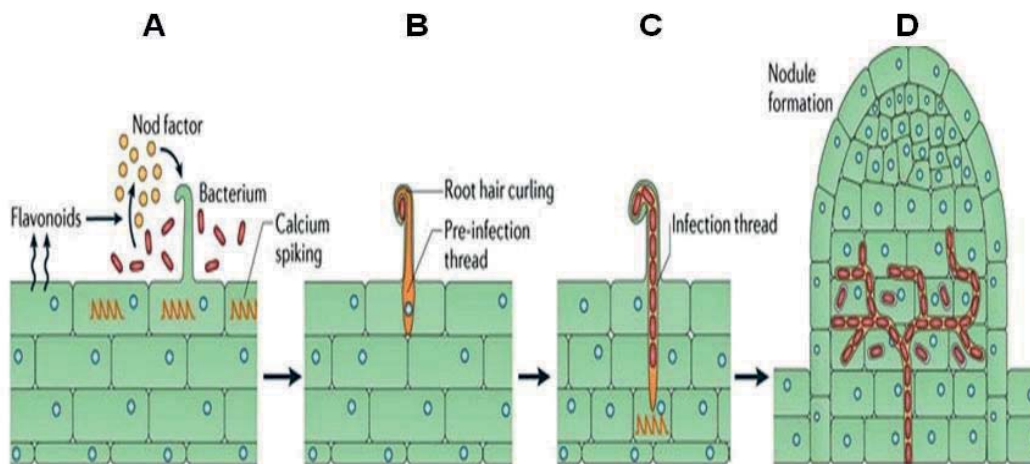

Fig. 2 Nodule formation (Oldroyd, 2013), **A**: The initial signaling in Rhizobium-legume interaction, **B:** Curled root hair formation and encapsulation of Rhizobium into root hair, **C**: Root hair invasion by development of the infection thread, **D**: Differentiated bacteroids and nodule formation.

Two main types of nodules have been described: determinate and indeterminate types. Determinate nodules lose meristematic activity with maturation and cannot be infected anymore by rhizobia. Determinate nodules grow due to cell expansion which leads to a spherical shape particularly in tropical legumes (for example in *Glycine* and *Phaseolus)*. Indeterminate nodules