CHAPTER - ONE

INTRODUCTION
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Sulphur is one of the essential nutrients required for plant growth, physiological functioning and adaptation to changes in the environment, including stress resistance. It is considered as the fourth major important nutrient after nitrogen, phosphorus and potassium for agricultural crop production. Inspite of its crucial role in various metabolic processes, sulphur is a neglected nutrient for crop plants. As a result, its deficiency is increasing in agricultural soils, resulting in decreased yield and inferior quality produce. Several studies including ours, have shown that deficiency of sulphur in soil also affects the uptake of other nutrients, especially of nitrate (Ahmad et al., 1999). Sulphur deficiency is linked with increase in plant disease incidence (Bearchell et al., 2005). Plant sulphur nutrition affects not only the crop yield but its quality also (Falk et al., 2007; Taylor et al., 2008). Appropriate applications of fertilizer can remedy deficiencies in many instances, but there remain considerable uncertainties regarding the timing and type of S-application, which in turn influence the persistence of S in the soil and its availability to the plant. In general, there is a substantial seasonal variation in S available to the plant. Ideally, crops must be engineered to maximize the uptake when S is abundant so that they are able to tolerate periods of low S-availability in a better way. Studies on the mechanisms for controlling sulphate uptake and assimilation suggest approaches for genetic manipulation of expression of the transporters to engineer crops with
improved S-utilization efficiency and S-deficiency-stress tolerance (Clarkson and Hawkesford, 1993; Hawkesford and Smith, 1997). Therefore, increasing the sulphur-utilization efficiency of plants is becoming an important issue. A genetic approach can be useful in defining the basis of important agronomic traits as well as in basic research.

Relative to other major nutrients, little is known about the regulation of uptake and assimilation of sulphate by plants from the soil. A knowledge of the underlying molecular mechanisms is essential to understand the regulation of sulphur metabolism and its interaction with assimilation of other nutrients, especially nitrogen, and to improve such agronomic traits as yield, nutritional quality and environmental stress resistance. The recent resolution and analysis of genes encoding the transporter proteins involved in the uptake and distribution of sulphate in plants and that of the enzymes involved in assimilation of sulphur have provided due impetus to the efforts to understand how sulphur metabolism is managed by plants.

The predominant proportion of sulphur in the plant, which is generally taken up as sulphate by the root, is reduced in the assimilatory sulphate-reduction pathway and assimilated into structural and functional organic sulphur compounds (De kok et al., 2002; Ahmad et al., 2005a; Hawkesford and De kok 2006; Haneklaus et al., 2006). The uptake of sulphate by roots and its transport to the shoot appear to indicate two primary sites of regulation of sulphur assimilation (Hawkesford and De Kok, 2006). Distinct sulphate transporter proteins mediate the uptake, transport and subcellular
distribution of sulphate, and are encoded by a gene family consisting of 14 members in *Arabidopsis* and probably a similar number in other species. Although root plastids contain all sulphate-reduction enzymes, sulphate reduction takes place predominantly in the leaf chloroplasts (Haneklaus et al., 2006). The reduction of sulphate to sulphide occurs in three steps. At first, sulphate needs to be activated to adenosine 5’-phosphosulphate (APS), catalyzed by ATP sulphurylase. Subsequently, APS is reduced to sulphite, catalyzed by APS reductase. The latter reaction is assumed to be one of the primary regulation points in sulphate reduction, since the activity of APS reductase is lowest among the enzymes of sulphate-reduction pathway and it has a fast turnover rate. Sulphite is reduced by sulphite reductase with ferredoxin as a reductant and the sulphide thus formed is incorporated into cysteine, catalyzed by O-acetyl serine (thiol) lyase, with O-acetylserine as the substrate. The synthesis of O-acetylserine is catalyzed by serine acetyltransferase and together with O-acetylserine(thiol) lyase it is associated as enzyme complex named cysteine synthase. The formation of cysteine is the direct coupling step between sulphur and nitrogen assimilation in plants. Cysteine is the precursor or reduced sulphur donor of most other organic sulphur compounds in plants. The predominant proportion of the organic sulphur is present in protein fraction (upto 70% of total S), as cysteine and methionine residues. In the proteins, cysteine and methionine are highly significant in the structure, conformation and function of proteins (Hawkesford and De Kok, 2006; Haneklaus et al., 2006). The thiol groups of
the cysteine residues in proteins can be oxidized resulting in disulphide bridges with other cysteine side chains (and from cystine) and/or linkage of polypeptides, and make an important contribution to the structure of proteins. The thiol groups are also of great importance in substrate binding of enzymes, in metal-sulphur clusters in proteins (e.g. ferredoxins) and in regulatory proteins (e.g. thioredoxins). The present work seeks to elucidate the mechanisms of regulation of sulphate uptake and assimilation. Besides the well-known molecular and genetic advantages of Arabidopsis thaliana, it provides an excellent model organism for the investigation of plant sulphur metabolism. The present work was conducted with the following objectives:

1. Identification of signal molecule(s) for regulation of sulphate uptake.
2. Identification of signal molecule(s) that mediate between the cellular-sulphur status and the differential expression of sulphate-assimilation genes.
3. Evaluation of the relative contribution of plant organs and tissues in the source-sink relationships of sulphate assimilation.