INTRODUCTION

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Among the ten essential elements (C, H, O, N, P, S, K, Ca, Mg, Fe), which were the first to be recognized as necessary for all higher plants six (C, H, O, N, P, S) are light, nonmetallic elements. Three of these (C, H, O) which are furnished by carbon dioxide and water respectively contribute most of the structures and substances that are involved in nature. However it is as difficult to conceive of the properties of protoplasm without nitrogen as it would be to conceive of organic chemistry and biochemistry without the properties of carbon. Nitrogen, only slightly heavier than the carbon atom and in an adjacent position in the periodic table, has very peculiar properties. These properties include a wider range of valence states in compounds which may be used in the metabolism of plants. The combining power of nitrogen may be 3 as in ammonia, 5 as in ammonium chloride, but nitrogen may exist in oxidation or reduction states which range +7 to -3. Although compounds in which two nitrogen atoms are combined together are fairly common, compounds in which three nitrogen atoms are so combined are rare, and they are usually very unstable. However, when nitrogen enters into combination with carbon and oxygen, it forms an integral part of the complex repeating structures that are important in the molecular architecture, which is the basis of so much biological structure and function. The examples here are the proteins, built up from amino acids and the nucleic acids built up from nucleotides which contain both

nitrogen and phosphorus. Whereas the fragments (amino acids, nucleotides) from which these macromolecules are constructed were recognized much earlier, the intimate knowledge of the role of these macromolecules (Proteins, nucleic acids) in cells is much more recent. It is now very well established that proteins form chemical basis of life. The crucial importance of nitrogen for the existence of human race on this planet is further highlighted by the fact that the conversion of inorganic nitrogen nitrate or elementary nitrogen, to organic form is essentially a characteristic of plants, whereas to build proteins, animal cells essentially require already elaborated organic nitrogen compounds. In other words nitrogen nutrition as well as nitrogen assimilation in the crop plants play a key role in protein nutrition of the animal kingdom on this planet. It is because of this reason that attempts are continuously made for fulfilment of nitrogen demands of crop plants. This is mainly achieved by manufacture of nitrogen fertilizers. The total world production of fertilizer nitrogen is some 40 million tons at present and is rising by nearly 2 million tons per year and this adequately speaks about the importance of nitrogen nutrition in agriculture.

Nitrogen assimilation by plants is a catenary process beginning with root uptake and ending in the formation of a protein, nucleotide or other nitrogenous biochemical compounds. Nitrogen is available from the soil as an exchangeable cation, ammonium or nitrate dissolved in soil water and moving by mass flow depending partly on the rate of transpiration by plants. It is now very well established that nitrate is the

principal nitrogen source for all higher plants. Although nitrate is the main ultimate source of nitrogen, it is rarely accumulated as such in tissues of higher plants. The entering nitrate is, therefore, rapidly converted by plants to organic forms. Assimilation of nitrate depends on activity of nitrate reductase (NADH nitrate oxidoreductase, EC 1.6.6.1). Nitrate reductase is an enzyme complex of high molecular weight varying from 2,30,000 to 5,00,000 depending on the organisms in which it occurs. It contains several prosthetic groups such as FAD, cytochrome 557 and molybdenum that seems to be ubiquitous constituents of this protein in eukaryctes. Although the nature and properties of this enzyme have been studied by employing conventional techniques followed for most of the enzyme systems, application of in vivo technique has added a new dimension to the study of this enzyme system in higher plants. It has been observed that this enzyme is substrate inducible and the efficiency of enzyme induction and the actual enzyme activity in the plants depend on numerous intrinsic and external factors.

The fact that the enzyme nitrate reductase catalyzes the first step of nitrogen assimilation thereby determining nitrogen use efficiency in higher plants, has attracted attention of several plant physiologists in recent years. Considerable work has been done on the behaviour of this enzyme under different environmental and ontogenic conditions as well as on the factors affecting the rate of nitrate reduction process. However, most of those studies have been

centered around plants like barley, wheat, soybean, and cotton and other crop species such as rice, sugarcane and sorghum have not been investigated in this respect. The legumes have not paid due attention in this respect, perhaps due to the fact that they possess the ability to establish a nitrogen fixing symbiosis with Rhizobium species which reduces their requirement for fixed nitrogen from the soil. Symbiotic nitrogen fixation by legumes does make a valuable contribution to N-inputs, especially in developed countries where effective Rhizobia inoculation techniques have been developed in the context of an extensive agricultural system. But the same cannot be said about developing countries like India. Further, nitrogen fixation in grain legumes like soyabean, pea and peanut seems to be limited by the amount of photosynthate available, which appears in sufficient to support simultaneously the reduction process and grain production and various environmental stresses. Thus, under field conditions symbiotic nitrogen fixation accounts for only 25-30% of the total nitrogen accumulated by these legumes at harvest (Fowden, 1978). Thus, it is clear that in legumes also nitrate reductase can play an important role in controlling overall nitrogen economy of the plants. Crotalaria juncea or sunhemp is one of the very popular legumes employed on a large scale as a green manure in Indian cropping system. The plant is also an useful source of fibre. The plant is also reputed for lectins in it's seeds and leaf proteins. Notwithstanding

it's promising economic value, very little work has been done on physiology of this legume. This situation has prompted us to undertake studies of enzyme nitrate reductase in this legume.

In order to understand the basic problems involved in study of nitrate reductase a brief resume of the literature on this enzyme has been taken and it is included in chapter I. This is also supplemented with a brief account of Crotalaria juncea and physiological studies carried out in this plant species. The methodology followed for the present study is covered under the chapter 'Materials and Methods'. In the present investigation an in vivo technique for assay for enzyme nitrate reductase has been employed since it was realised in the preliminary experiments that this technique is comparatively simple, reliable and easy to follow for large number of samples. Further, in the preliminary experiments not much variation in enzyme activity was evident in in vitro and in vivo methods. The important findings of the present investigation have been discussed in the light of available literature in chapter III, 'Results and Discussion'. The present investigation can be broadly divided into two parts. In the first part activity of enzyme nitrate reductase in different Crotalaria species, in different plant parts of Crotalaria juncea, during different phases of growth and during leaf ontogenesis has been investigated. An attempt is also made to study daily variations in the activity of the enzyme in stem and leaves

of Crotalaria juncea. The second part deals with possible regulation of the enzyme and for this study germinating seeds have been employed as an experimental material. This study was aimed at investigating the influence of light, nitrates, growth promoters, growth inhibitors, salt stress and water stress on the activity of the enzyme nitrate reductase. An attempt has been also made to see whether presowing soaking treatments of the seeds with growth regulators has any beneficial effect on the enzyme activity. The important findings of the present investigation are briefly summarized under the heading "Summary and conclusions".

The present study was persued to have preliminary idea about the behaviour of nitrate reductase in the legume The above investigation represent only one attempt in understanding basic process of nitrate reduction in the local variety of Crotalaria juncea. It must be admitted that many more such attempts on other facets of nitrogen metabolism such as nitrogen fixation, ammonia assimilation and amino acid interconversion are essential to derive a clear idea about nitrogen nutrition in this legume.