Nitrogen Metabolism

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The forms of nitrogen available to the plant may be divided into 4 groups — nitrate nitrogen, ammonia nitrogen, organic nitrogen and molecular nitrogen. Although most plants utilise the nitrate from nitrogen, several plants can assimilate ammonia and certain forms of organic nitrogen. The utilization of molecular nitrogen is confined to a certain few groups found among the pro-karyotic forms of plant life including certain species of free living bacteria (Azotobacter, Clostridium) and BGA (Anabaena, Nostoc)

Nitrate reduction or ammonification: The roots of most higher plants absorb. Nitrogen from the soil in the form of nitrate (NO_3^-). Nitrogen induced from, is notn directly used by the plant, but must be reduce to ammonia before it may inco-operated into the nitrogenous compounds of the plant. The reduction of nitrate to ammonia requires the energy of respirations. The carbohydrate of the plant not only provide skeleton needed inco-operation of NH_3 (ammonia) but also provide energy through their breakdown in respiration.

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The first step in nitrate reduction the conver-
-sion of nitrate to nitrite (NO_2^-). One of the impor-
-tant enzyme in this regard is nitrate reducta-
(NR). Since, the formation of nitrite from nitrate
requires the transfer of e^-s to nitrate, scientist
originally believed that the compound hyponitrite
(HNO_2) was involved as an intermediate in the
transfer of e^-s. Later, it was found that hypo-
-nitrite was not detected in plant tissue
because of its high instability. Further it was
believed that another compound (Hydroxyl amine
(NH_2OH) was an intermediate in the sequence lea-
-ding from nitrate to ammonia accumulated
evidences rules out these compound also in the
reduction of nitrate to ammonia (Mengel, 1978).
Thus the current idea is that the reaction
proceeds as follows —

$$NO_3^- \xrightarrow[\text{nitrate reductase}]{2e^-} NO_2^- \xrightarrow[\text{nitrite reductase}]{6e^-} NH_3$$

Because of the wide spread occurrance of
the above intermediates in plants and the detec-
-tion of the enzyme. This pathway appears to be
important breakthrough in the conversion of nitrate
to ammonia

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Nitrate and Nitrite Reductase

Nitrate reductase is a match of flavo protein that catalyses the reduction of nitrate to nitrite and was isolated in highly purified form (Evans, Nason 1956). The enzyme system includes a reduced pyridine nucleotide ($NADPH_2$ or $NADH_2$) as an e^- donor, flavin adenine dinucleotide (FAD) and molybdenum (Mo). Electrons are passed from reduced pyridine nucleotide to FAD and produce reduced $FADH_2$.

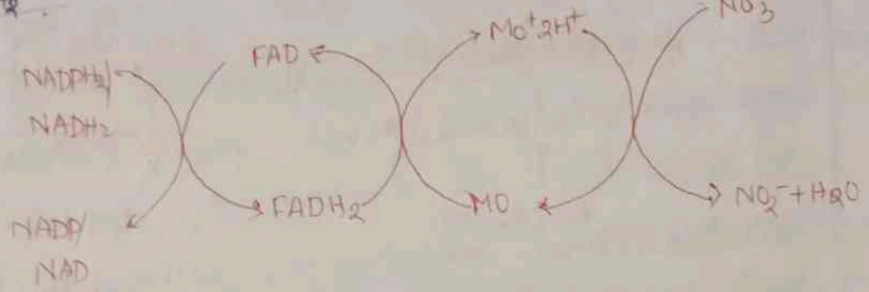

Fig :- Sequence of e^- transport in NO_3^- reduction

The e^-s are in turn passed from $NADH_2$ to oxidised molibodenum and produce reduced molibde-num, which passes e^-s to nitrate and reduces it to nitrite.

Nitrate reductase is an inducible enzyme. An inducible enzyme is distinguish from a constitutive enzyme in that it is only aparent in

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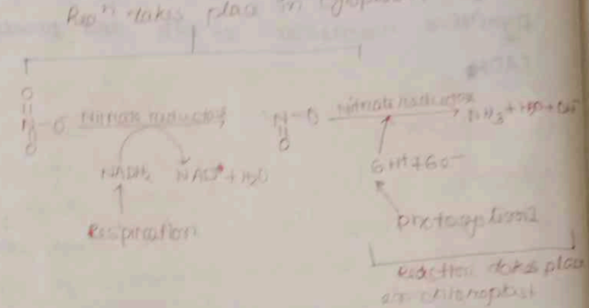
in the presence of its particular substrate

Nitrite reductase is also a metallo flavo protein. Murphy and others (1974) suggest that the nitrite reductase is a single protein that catalises reduction of NO_2 to NH_3 directly from a reductant of photosystem-1. The generalised scheme of nitrate reduction as follows—



$NO_3^- \rightarrow NO_2^- \rightarrow NH_3$

Molecular nitrogen fixation

The conversion of N_2 to NH_3 is accomplished by asymbiotic nitrogen fixation (nitrogen fixation by so called free living organisms not in association with another). N_2 may also be incorporated into aas by symbiotic N_2-fixation, i.e, N_2 fixation by an organism

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living in close association with another. Thus N_2 is made available to plants by N_2 fixation, which is the reduction of N_2 to NH_3 and always appears to be carried out by prokaryotic organisms. $N_2 \longrightarrow NH_3$

Asymbiotic nitrogen fixation:— Credit for actually showing that on living organism is involved in N_2 fixation must be given to Winogradsky who in 1894 isolated that N_2 fixating anaerobic bacterium Clostridium pastorianum. Two more important free living N_2 fixing organisms were isolated by Beijerinck in 1901. These two our Azobacter chroococcum and Azobacter agile.

In contrast to the first one these two are aerobes. Free N_2 can also be fixed by a large no. of BGA (nostoc, Anabaena)

Symbiotic Nitrogen fixation:—

Leguminous plants obtain free nitrogen through a symbiotic association with soil bacteria of the genus Rhizobium. In Alder symbiotic relationship with certain

invasion of the genus Actinomycete. In both cases nitrogen fixation is done to fix the
The relationship between legumes and Rhizobium is assumed to be species specific.
The actual site of nitrogen fixation is the nodules formed on the roots of the legume plant as a result of invasion of the Rhizobium. In this relationship the bacteria provide the host plant with fixed nitrogen and the host plant provides the micro-organisms with soluble carbohydrates. The accumulation of Rhizobia in vicinity of plant root occurs probably because plant roots excrete certain growth factors into the soil. The bacteria either penetrate the relatively soft root hairs or an immature, damaged or broken root hairs. Wipf and Cooper (1938) found that the number of chromosomes in the nodule cell contains double the no. of chromosomes found in the normal somatic cells. He also postulated the successful formation of nodules occurs only when the root nodule bacteria infect cells containing

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-ing double the normal somatic complement of chromosomes

Mechanism of symbiotic nitrogen fixation:—

One of the important factor for the purpose of N_2 fixation is Leg Haemoglobin present in the nodules. It is the result of 'Rhizobium-legume complex' nodules that lack leg haemoglobin are unable to fix N_2. Leg haemoglobin is an oxygen carrier. The oxygen is necessary for the e⁻ trans-port chain of the rhizobium. Because of its high affinity for oxygen, Leg Hb provides oxygen to root nodules bearing quickly. It also believed that Leg Hb keeps level of molecular oxygen low in the basteroid. This function is particularly important because nitrogenase is sensitive to O_2 and looses activity in its presence. Nodules lacking Hb may be unable to fix oxygen, due to this condition whole reaction takes place as follows ⟶

i) The electron and hydrogen appears to be donated through ferredoxin or other reducing agent of the electron transport system (ETS) and Kreb cycle of the bacteria.

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2. This Bacteriod provides ATP by oxidative phos-phorylation.

3. ATP is required in the transfer of e^- from an iron protein complex through the Fe, Mo of the nitrogenase system to the reduction process.

4. The Kreb cycle of the bacteriod produces the keto acids that are incorporated into reactions with ammonia to form acetic acid. Most of these amino acids are transporated to the bacteriod.

5. The nitrogenase enzyme, which is an iron-protein complex, mediates the generation of ATP.

6. The nitrogenase enzyme, which is an iron-protein complex, mediates the transfer of e^- from ferredoxin to an iron and molybdenum, protein complex, where possibly N_2 reduction takes.

Certain micronutrients, such as iron, copper, cobalt and molybdenum appears to be essential. The iron requirement is accounted for its presence in legHb, Cu is required in legHb synthesis. Cobalt is essential part of Vitamin B-complex.

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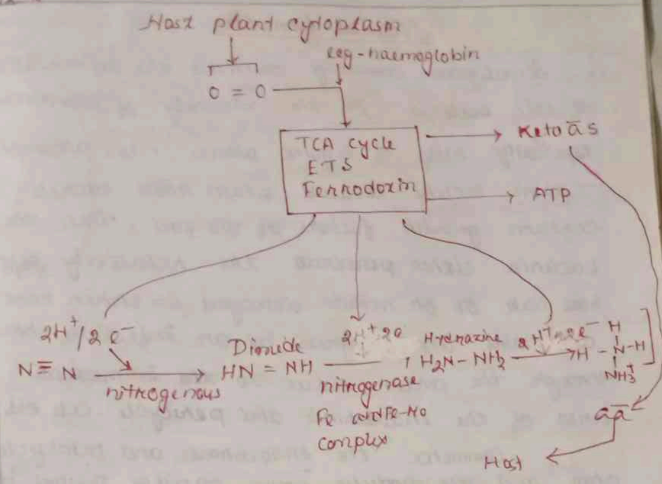

Fig :- Mechanism of symbiotic nitrogen fixation

Enzyme nitrogenase :-

Nitrogenase consists of two distinct proteins after called component - I and component II. Component-I is an FeM Fe-Mo protein, apparantly with two(2) Mo and 28 iron atoms. Component II is an Fe protein contain 4 atoms of iron (Yates 1980) Both Mo and Fe become reduced and then oxidised as nitrogenase accept electrons from ferrodoxin and transfers them to nitrogen to form ammonia.

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Nodules formation

Investigator commonly observed the assimilation of soil bacteria in the vicinity (near) of plant roots, especially roots of legume plants. This accompli-cation occurs because plant roots excrete certain growth factors of the soil. Then the bacteria either penetrate the relatively soft root hair tip or invade damaged or broken root and hairs and progress in an infection thread through the cortex tissue to the immediate area of the endodermis and pericycle. Cell ~~ets~~

commerce the endodermis and pericycle area, and the nodules grows rapidly pushed it may to the surface of the roots. One rather remarkable observation, first made by wift and Cooper (55) in 1938, is that the nodule cell certain double the no. of chromosomes found in the normal somatic cells of the plant. wpf and Copper, in a later study of the nodules formation in pea and vetch (56) showed that successful nodule bacteria invade cells contain-ing double the normal somatic complement of chromosomes. These wel are stimulated into

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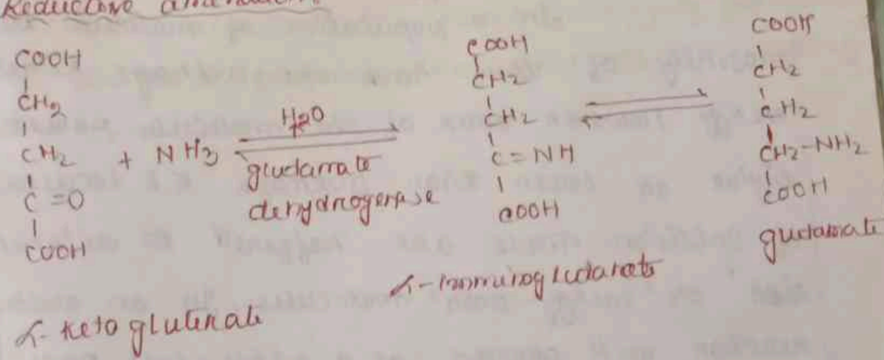
meris amatic activity the invasion and form the nodule. If there are no cells with dauble normal chromosome no. in the area of the root penetrated by the infection thread with nodules will form. Fig shows clover roots with nodules and Figs is a scanning e⁻ micrograph of infected alternative root, nodules.

The factor or factors causing the profuge growth of the cells that from nodules is at present unknown rhizobia are known to produce the plant hormone Indole acetic acid (IAA), However, many other soil microorganism are able to produce IAA but are not able to cause nodule formation.

1. Reductive amination:–



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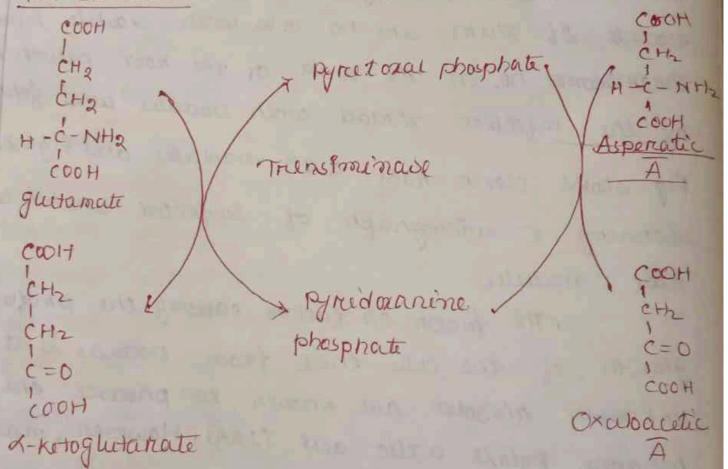
Translocation :-



The Mechanism of Enzyme Action

1. Lowering the energy of activation of reaction:-

In a population of molecules the majority of them have the average kinetic energy. However some of the molecules possess higher or lower than average K.E because of collision. These are reffered to as 'energy rich' or 'energy-poor' molecules. In an ordinary reaction will proceed at a very slow pace