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A COMPREHENSIVE PROCESS OF NITROGEN FIXATION IN PLANTS

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SUMMARY

Nitrogen is a component of several biomolecules that are essential for all organisms' growth and development. Nitrogen fixation is the biological process that converts molecular nitrogen to ammonia. Biological nitrogen fixation is mediated by diazotroph microorganisms that use nitrogenase enzymes to enhance atmospheric nitrogen. Much of this is accomplished through a symbiotic interaction between plants and diazotrophic bacteria. Microbiology and plant biology are discussed in symbiotic nitrogen fixation discussions. Some of the nitrogen fixation mechanisms mentioned in this paper begin with the formation of nodules, the action of the nitrogenase enzyme in reducing nitrogen to ammonia, and the presence of rhizobia in nodules. This study provides a comprehensive overview of the nodule formation process, the role of the nitrogenase enzyme in reducing nitrogen to ammonia, and the presence of rhizobia in nodules. A more complete literature review on biological nitrogen fixing in plants is required to obtain more specific information.

keywords; fixation, nitrogen, nodule, nitrogenase

INTRODUCTION

As an element found in many required biomolecules that play an important role in life (Ferguson, 1998; Smil, 2004), nitrogen (N_2) is the biggest gas available in the Earth's atmosphere (Frank *et al.,* 2003). N_2 can be present in proteins and amino acids, as well as many other organic molecules derived from nitrogen fixation (Egamberdieva and Kucharova, 2008; Ma'ruf *et al.,* 2016).

N requirements in plants are met by fertilizers and manure, which are not absorbed by the plants and are discharged into the atmosphere as nitrogenous greenhouse gasses (Flechard *et al.,* 2007) or soil leaching (Stout et al., 2000; Trindade et al., 2001). Despite the fact that plants require a substantial amount of N for growth. As a result, alternate N sources are required to enable more sustainable farming methods. Legumes have the potential to meet this demand because of their unique capacity to fix N from the atmosphere organically, benefiting not just the legume but also other plants around (Liu *et al.,* 2011; Ma'ruf *et al.,* 2022). Nitrogen fixation has a significant economic, ecological, and economic impact since the availability of N fixed is the element that most frequently limits agricultural production worldwide (Smil, 2004).

According to what is known so far, Prokaryotes catalyze biological nitrogen fixing. *Eubacteria* and archaea are two diverse categories of procaryotes (Widmer et *al.,* 1999; Zehr *et al.,* 2003). Nodulation is the first step in biological nitrogen fixation. The root nodule of some plants develops into a nitrogen-fixing habitat for bacteria in the microaerobic environment. This process is carried out by bacteria from the genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Bradyrhizobium*, and *Azorhizobium* (together known as rhizobia) and *Frankia*. Except for *Frankia*, all of the genera evolved from the *α-proteobacteriall Rhizobiaceae* family and induced nodules in plants of the Leguminosae family. *Frankia* is a filamentous gram-positive actinomycete that causes nodules in the families *Betulaceae, Casuarinaceae, Myricaceae, Elaegnaceae, Rhamnaceae, Rosaceae, Coriariaceae,* and *Datisticaceae* (Benson and Clowson, 2000).

An enzyme is required in the process of N fixing. Nitrogenase is a complicated enzyme that is permanently inactivated by

oxygen and is responsible for nitrogen reduction. As a result, this activity necessitates anoxic or near-anoxic conditions (Gage, 2004). Nitrogenase works as a catalyst in processes that break down nitrogen molecules and convert them to ammonia in the presence of energy and electrons (Postgate, 1982).

According to the above explanations, legume nodules are very complex organs that consist of several interactions with several processes that operate at different levels, including nodule formation, carbon metabolism, oxygen supply, transmembrane transportation, and cellular redox (Udvardi and Pool, 2013; Nasr Esfahani *et al.,* 2014; Terpolilli and Hood, 2012). The following part will go into further depth on the intricacy of legume nodules, beginning with the process of nodule formation and progressing to the subsequent mechanism in biological nitrogen fixation.

MECHANISM OF NODULE FORMATION

The most typical symbiotic connection for nitrogen absorption is the interaction of bacteria with diverse plant groupings. This connection involves bacteria from various physiological backgrounds, including gram-negative proteobacteria such as *Rhizobia sp*. and *Burkholderia sp*., gram-positive *Frankia sp.,* and filamentous or unicellular cyanobacteria (Benson and Silvester, 1993; Rai *et al.,* 2000).

Infection Initiation

Rhizobia has the ability to adhere securely to host root hairs, with *Rhizobium leguminosarum* forming a two-step connection. The first is a weak binding, Ca_2^{+-} dependent binding phase to root hair mediated by rhicadhesin, a protein found in most rhizobia (Smit et al., 1995; Smit et al., 1989). The bacterial production of cellulose fibrils mediates the tight binding stage after the weak binding. *R. leguminosarum* requires fibril production to produce biofilms such as caps on the terminals of pea root hairs (Smit et al., 1995; Smit et al., 1989). Host lectins have also been implicated in rhizobial adhesion. Lectin attaches to both the plant cell wall and the saccharide section on the surface of suitable bacteria at the same time. Cellular contact and specific binding of bacteria compatible with root tips are important for infection and infection thread formation because they result in exposure of the tip of easily infected root hair to the right symbionts and thus the concentration of Nod factors required to trigger root hair curling and infection thread formation (Hirsch, 1999; van Rhijn *et al.,* 2001).

Purified nod factors from suitable Rhizobial species caused harm to the host plant's root hair. The most susceptible root hair is the one that has nearly stopped developing (root hair zone II). Nod factor-induced deformation of Zone II root hairs begins with isodiametric swelling at the root hair tip. It is also followed by the formation of a new growing tip that mimics root hair tips in the polarized and active zone I (Miller et al., 1999; Sieberer and Emons, 2000). The presence of pure Nod factor of *S. dililate* in *M. truntacula* root hair shows that the source site of Nod factor is a structure resembling shepherd's crooks (Esseling *et al.*, 2003). Plant hormones such as ethylene can influence the response of root hair to the presence of Nod factor. Ethylene inhibits Nod factor signal transmission, increasing the frequency of productive infections and influencing the degree of root hair deformation (Oldroyd *et al*., 2001).

An infection thread frequently begins at the intersections of branching root hairs or when two root hairs are pushed together. Enzymes in rhizobial bacteria may breakdown cellulose and other cell polysaccharides (Zorreguieta *et al.,* 2000). The study's findings revealed S. meliloti and R. leguminosarum bv. Trifolii causes the pit of each host root to develop (Mateos *et al.,* 2001).

Threads of Infection Extend Via Root Hairs

The nucleus is a common thread infection and active streaming column of cytoplasm during infection thread

development in root hair. The plant cytoskeleton plays a significant role in the formation of infection threads due to active cytoplasmic streaming at the ends of the expanding threads. The significant amount of cytoplasm in the tip area and the young infection thread cell wall in alfalfa make distinguishing tip thread growth difficult (Gage, 2004). As previously stated, curling and infection cause changes in the organization of microtubule arrays in root hair. Arrays shift from helical to cortical, with new nucleus-associated endoplasmic microtubules and cortical microtubules aligned with the long axis of the root hair. The endoplasmic reticulum then appears to connect the nucleus to the tip of the root hair. As curling begins, the microtubules become focused on the curl and eventually get detached from the root hair tip and dispersed between the root hair nucleus and the infection thread's developing tip (Timmers *et al.,* 1999).

Bacteria within infection threads are topologically outside the root hair, and the infection thread wall is continuous with the root hair cell wall (Rae *et al.,* 1992). The matrix within the thread's lumen contains material that is generally present in the extracellular matrix of plant cell walls. A glycoprotein found in homogenized pea nodules, the lumens of infection threads in pea nodules, the cell walls and apoplastic spaces of rapidly expanding pea root cortical cells, and the material released from pea root tips has been shown to react with MAC 265 (Rae *et al.,* 1991; van Den Bosch *et al.,* 1989). The antibody detects one or more members of a particular group of extensin proteins found solely in legumes, according to partial sequencing of the MAC 265 antigen and cloning of its cDNA (Rathbun *et al.,* 2002).

NITROGENASE

Nitrogenase is a metalloenzyme complex that comprises a number of centers made up of Fe, FeS, and Mo that are required to transfer electrons to diatomic nitrogen. This has solidified (Georgiadis *et al.,* 1992; Chan *et al.,* 1993). Because nitrogenase comprises several complicated metallo-centers, its tertiary structure is dependent on a variety of chaperones that are in charge of the proper assembly of each structural component. Many of the genes that encode these chaperones have been genetically identified as nif genes (Dixon and Kahn, 2004). NifU, NifS, NifB, NifE, NifN, and NifV are the well understood chaperones that are required for the assembly of the FeS centers and their correct insertion into NifH (Hu and Ribbe 2013). The overall generalized nitrogen reduction mechanism may be characterized as the interaction of two cycles: the Fe protein cycle and the MoFe protein cycle. The Fe protein cycle involves the protein binding two molecules of ATP while acquiring one electron. Following that, the Fe protein interacts with the MoFe protein, resulting in a redox transfer of electrons from the Fe protein to the MoFe protein at the cost of two ATP/electrons. The Fe protein is then released, and the cycle begins again. The single-electron addition to the MoFe protein occurs eight times in what is known as the MoFe protein cycle. This cycle depicts the complex's eight single-electron additions and predicts the locations at which H2 and NH3 are released (Thornley and Lowe, 1985).

CARBON TRANSPORT TO THE NODULE

Reductant and ATP are the direct energy needs for reducing nitrogen to ammonia. Both reducing agents and ATP are created by bacteria and translocated to bacteroids via the factory's carbon compound metabolism (Geddes and Oresnik, 2016). Sucrose is broken down by glycolysis to phosphoenolpyruvate in infected nodule cells, according to enzyme evidence from isolated nodule tissue (Vance and Heichel 1991). The presence of phosphoenolpyruvate carboxylase and cytosolic malate dehydrogenase in cytosol infected cells suggests that phosphoenolpyruvate is converted to oxaloacetate and subsequently transformed to malate, which is then provided to repair active bacteria (Miller *et al.* 1998). Carbon must be delivered across plants and symbiotic membranes to enter the bacterial

cytoplasm since simbiosomes are surrounded by plant derivative membranes. Dicarboxylic acids are actively transported across these membranes, according to evidence from trials utilizing gently isolated symbiosomes (Udvardi *et al.* 1990). In addition, it was shown that bacterial mutants that were unable to transport dicarboxylic acids could form symbiosomes but could not fix nitrogen (Geddes and Oresnik, 2016).

CONCLUSION

Nitrogen fixation is the biological process that converts molecular nitrogen to ammonia. Biological nitrogen fixation is mediated by diazotroph bacteria that use nitrogenase enzymes to enhance atmospheric nitrogen. Microbiology and plant biology are discussed in symbiotic nitrogen fixation discussions. This review merely provides a broad overview of the nodule formation process, the role of the nitrogenase enzyme in converting nitrogen to ammonia, and the presence of rhizobia in nodules. A more complete literature review on biological nitrogen fixing in plants is required to obtain more specific information.

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