Enhancing Nitrogen Use Efficiency in Crops for Sustainable Agriculture

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Abstract

The last few decades witnessed the heavy application of nitrogenous fertilizers in the field. The utilization of nitrogenous fertilizers is needed to enhance grain production and to fetch the food demand of the exponentially growing population. Moreover, the production of nitrogenous fertilizer requires a huge amount of energy and unused fertilizer is detrimental to the ecosystem. The identification of candidate genes/QTLs for nitrogen use efficiency is quite challenging to the researchers and breeding crops to improve nitrogen use efficiency is a complex task and breeders still struggling to get nitrogen-use-efficient crops. Therefore, a targeted and holistic approach prerequisites at the time. The hunting and modulation of genes for nitrogen uptake, assimilation, and mobilization would play a key role in improving crop cultivars for nitrogen use efficiency. The regulatory genes that play a crucial role in nitrogen metabolism, amino acid biosynthesis, regulation of carbon, and nitrogen cycle could be employed for engineering crop plants for enhanced nitrogen use efficiency and grain yield.

Introduction

Many nutrients are essential for plant growth and development. Among all nutrients, nitrogen (N) plays a pivotal role that limiting crop productivity. N is involved in cellular metabolism and is required by the plant in a larger amount. The N losses in the environment are more as compared to other nutrients revealing its essential requirement in crop production. Leguminous crop species can fix gaseous N into utilizable form but this fixed N is not sufficient to meet the crop production demand of our growing population.

In the last four decades, cereal production has doubled. The increased cereal production is driven mainly by the use of high-yielding varieties and fertilizers in agriculture. Nitrogenous fertilizer constitutes 70% of total fertilizer consumption in agriculture and it is being manufactured at a large scale to cater to demand in agriculture. About 111.59 million metric tonnes of nitrogenous fertilizers are applied in world agricultural land to meet the crop's N requirement (FAO 2022). However, the tremendous application of nitrogenous fertilizer results in increased cereal production and also leaves behind a detrimental effect on soil and water quality. However, the heavy application of synthetic fertilizers in the soil leads to the depletion of soil N (Mulvaney et al., 2009). The rising demand and high cost of nitrogenous fertilizer in agriculture call for a second green revolution and that could be accomplished by designing nitrogen-use efficient (NUE) crops. These NUE crops could uptake, translocate, and assimilate N better and allow a lesser quantity of reactive N into the ecosystem (Zeigler and Mohanty 2010; Figure 1).

Constraints in Crop Production

According to the Future of Food and Farming reports, the food demand worldwide will increase by 40% by 2030 and the sustainable limit for the surface area of the earth that could be utilized for crop production is estimated to be around 15%. As a result, advanced and rapid solutions are required to enhance the productivity of the limited land in agriculture and to fulfill the demand arising due to the exponential growth of the population. The major factor in crop production that limits the yield is abiotic stress including flood, drought, extremes of temperature, high salinity, biotic stresses, and limitations of minerals available for plants in the soil. The unfriendly conditions reduce crop yield and agricultural production by inhibiting growth, development, and vigor ultimately resulting in plant death (Foley et al., 2011). Crop yields were analyzed globally in the regions where malnutrition in humans is prevalent and there is less availability of inorganic nutrients and water in the soil of such regions resulting in low crop productivity (Mueller et al., 2012).

Importance of Nitrogen for crops

N plays a significant role in the growth and development of plants. It is a constituent of important biomolecules such as nucleic acid, enzymes, protein, ATP, chlorophyll, alkaloids, etc. Also, N enhances root system architecture leading to better absorption of water and nutrients from the soil (Fageria and Barbosa 2001).

Deficiency of N leads to altered metabolism in the plant. The typical symptoms of N deficiency in plants are stunted growth, leaf chlorosis, reduced tiller number in cereal crops, and lesser pod in legumes. The N deficiency in crops ultimately results in reduced yield and biomass. In chronic N deficiency, older leaves undergo senescence and detach from plants (Barbieri et al., 2000).

Soil Nitrogen and its uptake

Plants capable of utilizing N in the form of simple inorganic N compounds such as Ammonium (NH_4^+), and Nitrate (NO_3^-) as well as complex polymeric N molecules like protein. Plant species including mycorrhizal and non-mycorrhizal types have the potential to take up amino acids from soil strata. In soil, the production, and concentration of N in the form of amino acids and inorganic molecules depend on the efficiency of proteolytic enzymes, and the availability of surface organic horizon (Berthrong and Finzi 2006). Proteolytic activity is exhibited from exudates containing proteolytic enzymes secreted by plant roots, mycorrhizae, and free-living microbes (Bajwa and Read 1985). Inorganic N is found in soils commonly in the form of NO_3^- and its concentration in soils is governed by oxygen availability and pH. The NO_3^- transporters belonging to the *NRT1* and *NRT2* family of a transporter are involved in low and high-affinity NO_3^- transport in the plant (Krapp et al., 2014). Other classes of transporters such as NH_4^+transporters are responsible for transporting NH_4^+ and *AtAMT1;1* was the first report of NH_4^+ transporter that is isolated from *Arabidopsis thaliana* through complementation with yeast mutant defective in NH_4^+ uptake. However, NH_4^+ gets assimilated in chloroplast by the GS/GOGAT cycle and NO_3^- is converted into Nitrite (NO_2^-) by nitrate reductase enzyme in the cytosol of both root and shoot. In contrast, NO_2^- is converted into NH_4^+ by nitrite reductase in chloroplast (Ninnemann et al., 1994).

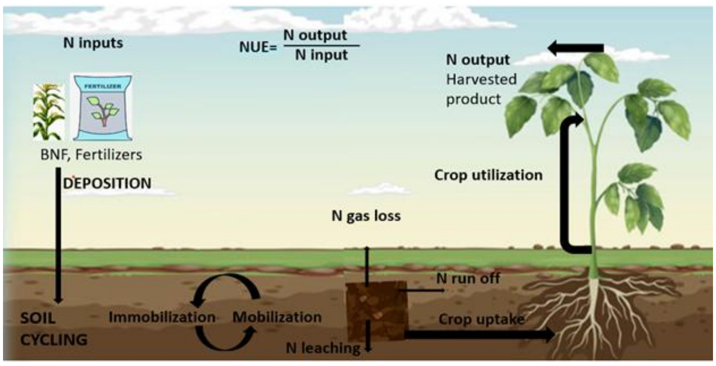

Fig. 1 Nitrogen cycle and nitrogen use efficiency.

Nitrogen assimilation

NO_3^- enters the plant system, is reduced to NO_2^- by enzyme nitrate reductase, and gets assimilated inside plants through chloroplastic enzymatic machinery. Moreover, NO_2^- formed in cytosol gets transported to the plastid and chloroplast of the root and shoot and converted into NH_4^+ by nitrite reductase. Further, NH_4^+ assimilated in different amino acid biosynthesis (Ruiz et al., 2007).

GS/GOGAT isoforms are present in cytosol and chloroplast. GS1 and NADH-GOGAT are found in the cytosol whereas GS2 and Fd-GOGAT are found in the chloroplast. Glutamate amino acid involved in the GS/GOGAT pathway gets converted into another amino acid by aminotransferases. Another important player in N assimilation is Asparagine synthetase (*AS*) which forms asparagine and glutamate after reacting aspartate and glutamine. Also, NADH–glutamate dehydrogenase (*GDH*) found in mitochondria captures NH_4^+ under stress, and glutamate gets converted into α-ketoglutarate. Another important enzyme, carbamoyl phosphate synthase (*CPSase*) located in plastid synthesized carbamoyl phosphate using precursor molecules NH_4^+/amide group, bicarbonate, and ATP. This heterodimeric enzyme in *Arabidopsis* is formed by two small subunits and a large subunit (Potel et al., 2009; Figure 2).

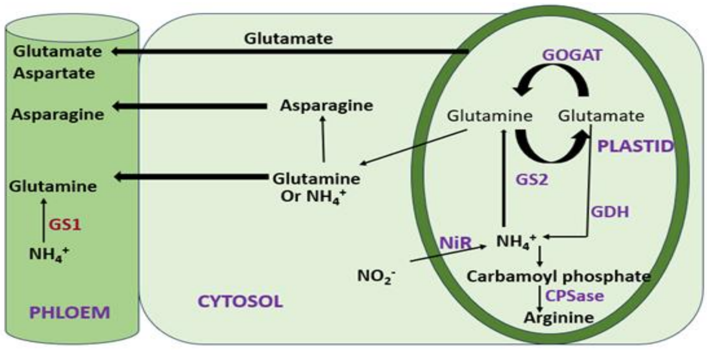

Fig. 2 Nitrogen assimilation in plant cell.

Nitrogen Remobilization

During senescence, NH_4^+, NO_3^-, and urea contribute to the remobilization of N from senescing leaves to grain. However, urea released during nucleic acid and protein metabolism in senescing leaves gets transported to sink utilizing the *DUR3* urea transporter in Arabidopsis. Also, a higher level of expression has been shown by *NRT1.6*, *NRT1.5*, and *AMT1.5* transporters at the time of senescence revealing their role in NH_4^+ and NO_3^- remobilization (Masclaux-Daubresse et al., 2010).

Leaf senescence and protein degradation produce a lot of amino acids that get converted to other amino acids and transported to the sink via the phloem. The concentration of glutamine and asparagine in phloem sap is enhanced during senescence and these amino acids are available as a transport form of N for remobilization. *Arabidopsis* contains 67 transporters for amino acid transport belonging to 11 gene families and plant cells get NH_4^+ through the uptake of NH_4^+ from the soil and also from amino acid catabolism and photorespiration processes. A larger quantity of NH_4^+ is generated in mitochondria during photorespiration (Rentsch et al., 2007).

Consequences associated with low nitrogen use efficiency

The movement of N in the environment is governed by the biogeochemical cycle and it involves dynamic interaction of soil and plant systems. This interaction is regulated by various processes including N fixation, nitrification, denitrification, leaching, etc.

Water pollution: Leaching of N into the soil causes groundwater pollution. N losses rely on different factors like the type of nitrogenous fertilizer, soil physical and chemical properties, and agronomical management practices. Moreover, leaching in the light texture of sandy soil is more due to the enhanced permeability of dissolved NO_3^- in water.

Eutrophication: It involves the enrichment of Inland and marine water bodies with nutrients especially N and phosphorus, which in turn results in excessive algal and phytoplankton growth leading to low oxygen and toxic chemical accumulation. The polluted water consumption causes harmful effects on fish, livestock animals, and human beings.

Nitrogen Accumulation: Ammonia is released into the environment through volatilization losses from the agricultural land area. Moreover, chemical fertilizers contribute significantly to anthropogenic N deposition in river basins. Also, the deposition and enrichment of N are higher in water bodies and forest ecosystems leading to an imbalance in the ecosystem.

The Greenhouse effect: The denitrification process releases nitrous oxide (N_2O) which is an important greenhouse gas responsible for global climate change. Approximately two-thirds of N_2O is released from agricultural soils. Further, the emission of N_2O in the atmosphere results in the degradation of the ozone layer.

Breeding and genetic engineering approaches utilized for enhancing nitrogen use efficiency

NUE in crop plants has been enhanced by several methods but the efficient approach could be the genetic manipulation of the transporter, assimilatory, remobilization genes, and transcription factors involved in N metabolism. The key assimilatory genes like glutamine synthetase (*GS*), Glutamine oxoglutarate aminotransferase (*GOGAT*), Alanine aminotransferase (*AlaAT*), Glutamate dehydrogenase (*GDH*), Aspartate aminotransferase (*AspAT*) play a vital role in N and plant metabolism. The GS-GOGAT cycle GS imparts an important role in ammonia assimilation and recycling and GS1.1 and GS2 capture precursors of N compounds, and NH_4^+ in cytoplasm and chloroplast respectively. There could be an enhancement in NUE, if the N metabolism genes are modulated in a specified way either through breeding or genetic engineering. The breeding approach is tedious and time-consuming and it suffers from linkage drag. The other targeted approach including transgenic and genome editing seems to be the prominent approach for enhancing NUE. Further, crops could be designed by specifically modulating key genes through genetic engineering including insertion, modification, or deletion of N metabolism genes through precise molecular scissors and glues.

Conclusion

Nitrogenous fertilizers are essential for increasing grain production but are energy-intensive and environmentally harmful. Improving NUE in crops involves identifying and modulating key genes for nitrogen uptake, assimilation, and mobilization. N is crucial for plant growth, and its deficiency impacts yield. While legumes fix N, it's insufficient for global needs. Traditional breeding is slow; genetic engineering offers precise solutions. Enhancing NUE reduces environmental issues like pollution and greenhouse gas emissions. Hence, developing nitrogen-efficient crops is vital for sustainable agriculture. Further, Geneticists and plant biotechnologists' role is crucial for modifying the pathway in a specific and tailored way to create "super crops" with improved NUE, paving the way for a sustainable agricultural revolution.

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