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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/17210
DOI URL: http://dx.doi.org/10.21474/IJAR01/17210

RESEARCH ARTICLE

DEMYSTIFYING THE ROLE OF NITRATE TRANSPORTERS IN PLANT DEFENSE

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Manuscript Info

Manuscript History
Received: 05 May 2023
Final Accepted: 09 June 2023
Published: July 2023

Key words:-
Nitrate Transporters, NUE, Nitrate, Ammonium, Pathogen

Abstract

Plants are frequently exposed to a variety of soil-borne or airborne diseases during its life, which pose a threat tothe safety and security of the world's food supply. Due to millions of years of evolution, plants have developed multi-layered defense mechanisms to detect and offend the invasion of these diverse harmful microbes, such as viruses, bacteria, nematodes, and fungi.Plant defense mechanisms are greatly influenced by nitrogen nutrition. The form of nitrogen nutrition, either nitrate (NO_3^-) or ammonium (NH_4^+), plays a very important role in plant defense. Both these forms of N, have tight coordination with a group of transporter proteins called nitrate transporters. The critical role of NO_3^- transporters in mediating the absorption and its long-distance transport have been the subject of several researches; however, little is known about the transport systems involved in NO_3^- re-allocation under the influence of biotic and abiotic stressors. NO_3^- transporters are essential to know how plants react to challenging environmental conditions. In fact, when less NO_3^- is sent to the shoot, plants adapt to environmental stress better. The role of NO_3^- transporters in helping plants thrives in several challenging environmental cues. As a matter of fact, under different stresses, the amount of NO_3^- that is translocated from roots to shoots fluctuates, which may have an impact on plant's NUE either favorably or unfavorably.

Introduction:-

Nitric oxide- a critical messenger in plant defense

Upon pathogen attack, a number of biochemical elements are elicited which activates many signaling pathways (Kaur et al., 2022). When there is no pathogen, plants grow and develop by utilizing the available oxygen. But, during pathogen infection, reactive oxygen species (ROS) are generated in plant tissues (Singla et al., 2019), which in turn leads to photo-oxidative damage and internal cellular structures (Mittler, 2017).Among ROS, superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2)are rapidly produced. This causes rapid oxidative burst and stimulates genes involved in cellulardefense and protection during hypersensitive response (HR). HR is another crucial defensemechanism against the spread of microbial diseases. It is defined as rapid programmed cell death in response of an infection and is specifically localized. This prevents the transmission of pathogen to other regions of the plant (Balint- Kurti, 2019).

During HR, another important signaling molecule is rapidly produced, i.e., nitric oxide (NO) (Delledonne et al., 1998). NO functions in both abiotic as well as biotic stress (Khan et al., 2023, Fancy et al., 2017). The relationship

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of NO with nitrogen (N) is well known since many decades. N is an important macronutrient and nitrate (NO_3^-) and ammonium (NH_4^+) are its two readily available forms taken up by the plants and affects plant's resistance (Gupta et al., 2013). Nitrate uptake and metabolism may regulate both NO generation and scavenging (Mur et al., 2013).

However, apart from N, NO has a complicated relationship with other minerals such as phosphorus (P), potassium(K), zinc (Zn), and magnesium (Mg). Graziano et al., (2002) provided the first evidence suggesting a protective rolefor NO in plant mineral nutrition when Fe-deficient maize plants were supplied with NO exogenously. Similarly, the role of NO was also evidenced in plants which grow in nutrient deprivation. During alterations in nutrient supply, plant tissues induce NO production and therefore, a new mechanism involving the role of NO in nutrient deficiency and plant growth was explained (Buet et al., 2019). Nitrate is readily absorbed by the roots and its concentration plays a decisive role in lateral root elongation and development (Alvarez et al., 2012). NO is directly involved in modulating root growth and have a metabolic connection with nitrate.

Nitrogen Use Efficiency

The demand for the use of chemical fertilizers based on N has steadily increased over time because N is a crucial ingredient needed for plant growth and total productivity. According to Mohanty et al., (2020), 60–70% of applied N fertilizers are lost leading to serious environmental problems such as pollution, global warming, biodiversity loss, and significant plant physiological abnormalities. A good crop yield must be maintained while using very little fertilizer because the rate at which N is being applied is alarmingly high. Therefore, increasing plants' nitrogen usage efficiency (NUE) is one of the natural solutions to solve agricultural difficulties. In other words, a key element in increasing crop yield is effective N use.

Depending on the specific plant species, NUE is a measurement of the amount of seeds, grains, or fruits produced per unit of available soil nitrogen. According to Bharati and Mandal (2019), NUE can also be described in terms of N uptake efficiency (NUpE), N transport efficiency (NTE), N remobilization efficiency (NRE), and N utilization (assimilation) efficiency (NUtE), all of which are significant determinants of NUE in plants.

NO_3^- and NH_4^+ are the two forms of N that are made available to plants. Because NO_3^- is easily leached, its availability to plants is mostly limited (Jin et al., 2015).NO_3^- acts as a signaling moleculeand triggers the activation of NO_3^-related genes involved in its absorption, transport, assimilation, vegetative and reproductive development. According to Iqbal et al., (2020), plants absorb NO_3^- from the root, assimilate it, and then move it to the shoot where it can be remobilized to sink organs. The primary forces behind the absorption of $NO_3^-$to the remobilization stage are NO_3^-transporters.

Nitrate transporters and NUE

In fact, various researchers have addressed the mechanisms involved in transport, sensing, and signaling processes while discussing the relationship between NO_3^-uptake and transport activities in plants (Fan et al., 2017; Zuluaga and Sonnante, 2019; Vidal et al., 2020). Plants in order to use N sources, maximize the activities of NO_3^- transporters. These NO_3^- transporters' functions in the enhancement of plant NUE. The impact of necessary nutrients and environmental signals especially in biotic stress and the expression and activity of NO_3^- transporters, however, is less understood.

Nitrate transporters along withcoordinated expression with other transcription factors, have an indisputable influence on crop productivity and NUE. Not only N, NO_3^- transporters have impact on the uptakeand utilization efficiency of other plant nutrients thereby improving NUE in crop plants (Aluko et al., 2023).

Two main NO_3^- uptake systems have evolved in plants. The low-affinity transport system (LATS) promotes nitrate uptake in conditions of high soil nitrate (millimolar concentration; > 0.5 mM), whereas the high-affinity transport system (HATS) drives nitrate in conditions of low soil nitrate (micromolar range) (Iqbal et al., 2020; Raddatz et al., 2020). It is well known that four families of NO_3^- transporters, including chloride channel (CLC), slow anion channel-associated homologs (SLAC/SLAH), nitrate transporter 1/or peptide transporter NPF (NRT1), nitrate transporter 2/nitrate-nitrite-porter NRT2/NNP, participate in the uptake and transport of nitrate in plants.

NPF (NRT1), NRT2, NRT3 and their homologs have been identified as the main channels involved in root nitrate uptake and long-distance transport between and among plant organs (Hsu and Tsay, 2013; Wang et al., 2021b).Members of the NRT2 family, in contrast to the NRT1 family, are high-affinity NO_3^- transporters (HATs).

Seven members of the NRT2 family have been described. Out of the seven identified NRT2 transporters, four (NRT2.1, NRT2.2, NRT2.4, and NRT2.5) have been shown to be directly involved in the influx of NO_3^- into Arabidopsis root cells (O'Brien et al., 2016).

As a result, enhanced plant growth under N shortages and challenging circumstances depends on NO_3^- redistribution in plants (Fan et al., 2017). Stressed plants tend to store more nitrate in their roots than normal plants by absorbing and transporting less NO_3^- to the shoot. Zhang et al. (2018) used this term SINAR ("Stress-initiated nitrate allocation to roots") to describe NO_3^-allocation to the root during biotic and abiotic stress.

Nitrate Transporters genes are upregulated during pathogen infection

There is a lot of agronomic evidence showing how using excess N fertilizer affects the frequency of plant diseases (Fagard et al., 2014; Fan et al., 2017). A biotrophic disease that depletes plant nutrients, for instance, powdery mildew, and excessive N fertilizer use increases its severity. It's interesting to note that Arabidopsis tolerance to *Erwinia amylovora* is reduced with decreased N fertilizer use. These results suggest an intricate interplay between N absorption, metabolism, and disease infection mechanisms. As a result, it is clear that N status influences plant disease susceptibility or tolerance under particular environmental circumstances (Fagard et al., 2014). The molecular mechanism underlying how NO_3^-transporters affect fungal infection or pathogenic assault is unfortunately not copiously known.

Pike et al., (2014) studied the low-affinity transporter *NPF3.2* (in grapevine) and cloned the Arabidopsis ortholog *NPF3.1* in order to examine potential processes behind N uptake by the biotrophic pathogen. In this work, it was demonstrated that the major and minor veins of leaves' vascular tissues and the powdery mildew pathogen infection upregulate the expression of *NPF3.2* and *NPF3.1*. Under N-deficient conditions, the deletion of *NRT2.1* and *NRT2.2* led to an increase in resistance to infection by *Pseudomonas syringae*pv tomato DC3000 (Camanes et al., 2012). Additionally, using single and double Arabidopsis mutants, the roles of two potential high-affinity NO_3^- transporters in the NRT2 family, *NRT2.5* and *NRT2.6*, were examined in response to the rhizospheric bacterium *STM196* (Kechid et al., 2013).

According to the study, *STM196*-induced root system architecture was eliminated and plant growth was hindered by mutations in *NRT2.5* and *NRT2.6*. Consequently, Arabidopsis leaves expressing *NRT2.5* and *NRT2.6* seem to be essential for the plant to respond to *STM196* in a way that is independent of NO_3^- absorption. Both *NRT2.5* and *NRT2.6* must be expressed for *STM196* to function properly and stimulate plant development (Kechid et al., 2013). The importance of *NRT2.5* in plant biotic defense was recently demonstrated by T-DNA mutants of *NRT2.5*, which displayed greater resistance to *Pseudomonas syringae*pv. tomato DC3000 inoculation than their wild-type counterparts (Du Toit et al., 2020; Devanna et al., 2021).

These results have shown the functions of NO_3^- transporters in the plant response to biotic stress and have also proposed safe, novel, and long-term approaches to agricultural disease management.The expression of a putative nitrate transporter, *OsNPF4.5*, appears to be enhanced by mycorrhizal colonization of rice roots (Wang et al., 2020c).This outcome enhanced the host plant's growth and yield characteristics. However, OsNPF4.5 inactivation decreased rice's ability to take up symbiotic nitrogen and reduced the incidence of arbuscules (Wang et al., 2020c).

Clustered Regularly Interspaced Palindromic Repeats (CRISPR)/Cas9 combined with the Cas9 nuclease (CRISPR/CAS9) system was created to further discover the genes boosting NUE. The use of CRISPR/CAS9 has made it simple and reliable to modify genes for better plant N uptake. The CRISPR/CAS9 technique has been used in numerous applications in important crops, such as sorghum, rice, and tomatoes (Ito et al., 2015; Ma et al., 2015). It is noteworthy that CRISPR/CAS9 primarily alters negative growth regulators rather than overexpressing positive regulators, opening up possibilities for agricultural breeding (Tiwari et al., 2020).

Conclusion:-

Nitrate transporters have not only been demonstrated to play important roles in plant intake and transport capacity, but also in enhancing plant N utilization, which has ensured the possibility of fulfilling future global food demands. Increased NUE and plant development are, in fact, dependent upon enhanced NO_3^- absorption and use (NO_3^- transport, remobilization, and assimilation) through transporters activity. Majority of studies have successfully demonstrated how nitrate transporters affect stressful environmental conditions (biotic and abiotic stress both). They have also discussed how they interact with other plant nutrients, in lab; there aren't many field studies that support

their roles.The established NO_3^- transporters were discovered to serve diverse physiological roles in environmental and dietary stressors, although comparatively few NO_3^- transporters performing complicated interaction functions have been identified. Among all nitrate transporters, NRT2.1, NRT2.2, NRT2.5 and NPF2.6 have potential roles in plant defense. The fundamental processes driving these multipurpose roles in plant defenseare still unknown, and it is also unclear.

Declaration
Authors declare no conflict of interest.

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