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Biological nitrification inhibition: A review

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Abstract
Everything in this nature has a role to play and whenever there is a problem for its natural pathway, the nature finds its own way for mitigating it. N fertilizers acts as backbone for higher food production and helps to overcome the darkness of poverty and hunger. But the nitrogen (N) use efficiency of cultivated plant species is very low ranging from 30-50% only. The nitrogen leakage causes environmental problems like NO_3^- leaching, eutrophication and N_2O gas emission. In order to keep a check on it nature has its own mechanism. Certain plant species known to release root exudates which will control the nitrification process known as biological nitrification inhibition (BNI). Pasture plant species such as *Brachiaria*, *Andropogon* and cultivated plant species like sorghum and rice are known to having the BNI capacity through release of chemicals like brachialactone, sorgoleone and 1,9 decanediol. These chemicals hamper the activity of ammonia monooxygenase and hydroxylamine oxidoreductase enzyme activity including electron transfer pathway. BNI in plants is detected by using bioluminescence assay of *Nitrosomonas europea*. BNI has several advantages like alternative to synthetic nitrification inhibitors, reduced N_2O emission, usage as green manure crops and in crop rotations and decreasing N loss from cow urine and manures. Genetic exploration will help to transfer BNI trait to other cultivated plant species and to develop crops with inbuilt nitrification inhibition capacity.

Keywords: Biological nitrification inhibition, ammonium, nitrification, *Brachiaria*, nitrosomonas

Introduction
Today though we have touched the surface of moon, we are presently unknown about the processes taking place below centimeters beneath the surface of soil. Many technologies the mankind developed today are known to the nature from the time of immemorial and they are occurring without the notice of human being. Everything in this nature has a role to play and whenever there is a problem for its natural pathway, the nature finds its own way for mitigating it. There is a saying that there will be darkness beneath the lamp. It is true in case of fertilizers also. To meet our food demand fertilizers acted as boon and they helped to overcome the darkness of hunger and poverty. But fertilizers have its own problems. In case of nitrogenous fertilizers which acts as backbone for our food security, only 30-50% is used by the plant, remaining 50-70% is leaked into the environment. So, farmers have to spend more on fertilizers to meet crop demand.

This problem is not ended here. The nitrogen (N) lost to environment causes nitrogen cascade in atmosphere, its effects continuous further for long time. Whatever the N fertilizer we applied it is finally converted to ammoniacal and nitrate forms. The ammoniacal form being positively charged is adsorbed to the soil cation exchange capacity, so it is less prone to leaching. But the nitrate form being negatively charged prone to leaching and denitrification losses. The nitrate lost through leaching accumulates in the aquatic system and causes eutrophication which will hamper the aquatic life system. Even human can't escape from this because excess nitrate in drinking water causes blue baby disease in human being. Usually, fertilizer application is followed by irrigation by our farmers. It may create partial anaerobic condition. In this situation the nitrate is reduced to NO and N_2O through denitrification. The nitrous oxide produced is a potential greenhouse gas which is nearly 300 times stronger than CO_2 for causing global warming (Kothari *et al*., 2022) [6].

So to overcome this issue the wisest animal developed the synthetic nitrification inhibitors. But the adaptation of this technology remained poor due to its own lacunas. They are costly chemicals and found difficult to adapt by poor farmers. The proper availability is one more issue. Even it had degradation and pollution issues. Lack of awareness about these compounds is also a major issue.

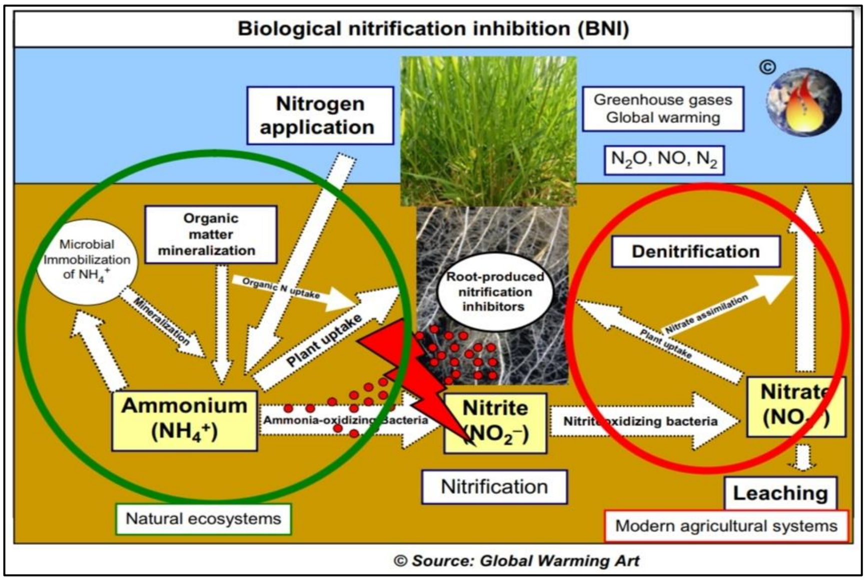

Fig 1: A schematic representation of the biological nitrification inhibition interfaces with the nitrogen cycle. The BNI produced by the root inhibits the process that converts ammonium to nitrate. In ecosystems with large amounts BNI such as Brachiaria pastures, the flow of nitrogen from ammonium to nitrate is restricted and ammonium accumulates in soil and root systems. In systems with little or no BNI such as modern agricultural systems, nitrification occurs at a rapid rate and ammonium is converted to nitrate-N, which is highly susceptible to loss from the soil and root systems (Subbarao *et al*., 2009b) [21].

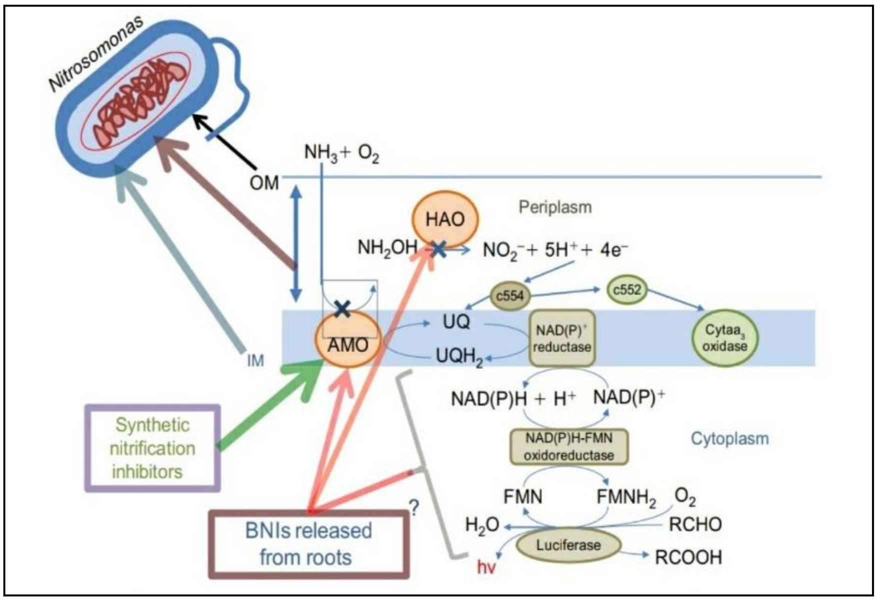

Fig 2: The mechanisms involved in the inhibitory effects of selected synthetic nitrification inhibitors and of the BNIs released from the roots of *Brachiaria humidicola* (Subbarao *et al*., 2007a) [9].

Nitrification is the conversion of ammonia into nitrate. It is carried out by obligate chemoautotrophs like nitrosomonas and nitrobacter. Nitrosomonas converts ammonia to nitrite using ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) enzyme system, whereas nitrobacter converts nitrite to nitrate by using nitrite oxidoreductase enzyme. And on the other hand, plant can uptake N in both nitrate and ammoniacal forms. Even though majority of plants uptake NO_3^- form it has to be converted to ammoniacal form within plant system for assimilation. This conversion and 0020 assimilation require four times more energy than NH_4^+ uptake. Uptake of NH_4^+ also helps in rhizosphere acidification which will help in nutrient availability. So it will be beneficial for plant also to uptake N in ammoniacal form.

Nature is the best architect. Problems and its solutions both are hidden in it. Many times we face the problems but the solutions are kept unnoticed, it is true in case of nitrification also. Nature has gifted its own key to open this lock of over nitrification system by a phenomenon called biological nitrification inhibition (BNI). But as usual this key was left unnoticed by mankind up to beginning of this century.

Plants can modify its root surrounding for its growth. Plants are known to release certain phytochemicals into their rhizosphere. Immediate vicinity of roots surrounded by soil which is under the influence of plant roots is known as rhizosphere. Mainly this rhizosphere secretion helps the plants for nutrient availability. But it was proved that the root exudates have influence on the nitrification process and nitrifiers. The controlling of nitrification process by plants through their root exudates is known as biological nitrification inhibition (Subbarao *et al*., 2006a) [17].

2. Who possess this BNI capacity?

Plants growing under N limited conditions usually show biological nitrification inhibition. Pasture plants such as *Andropogon species*, *Brachiaria species* and *Hyparrhenia* species are known to have biological nitrification inhibition capacity (Lata *et al*., 1999) [18]. Among these *Brachiaria humidicola* found to have highest BNI capacity. Even the cultivated plant species such as rice, sorghum also possess this inhibitory action. Wild relatives of wheat *Leymus racemosus* also have good nitrification inhibition capacity (Subbarao *et al*., 2007) [19].

The roots of *Brachiaria humidicola* known exude compounds like 2-phenyl propanoids, methyl p-coumarate, methyl ferulate from its roots which are collectively called as brachialactone. Whereas the aerial parts contain unsaturated free fatty acids, linoleic acid and linolenic acid which inhibits the nitrification process (Gopalkrishnan *et al*., 2007; Subbarao *et al*., 2008) [1, 24]. In sorghum compounds like sorgoleone, methyl 3-4 hydroxyphenyl propionate (MHPP) and sakuranetin known to have nitrification inhibition action. Different genetic stocks of sorghum have different sorgoleone release capacity and hence different BNI (Zakir *et al*., 2008; Sarr *et al*., 2020) [24, 14]. Karanjin in pongamia seeds known to prevent the nitrification (Sahrawat *et al*., 1981) [13].

In roots of rice 1,9 decanediol acts as nitrification inhibitor and it is superior over DCD in inhibitory action (Lu *et al*., 2019) [9]. Australian native plants such as *Hibiscus splendens*, *Solanum echinatum* have high inhibitory action against nitrification (Janke *et al*., 2018) [3]. Certain weed species such as wild radish, brome grass, wild oats and annual rye grass also have good nitrification inhibition ability (Sullivan *et al*., 2017) [11].

3. How it happens?

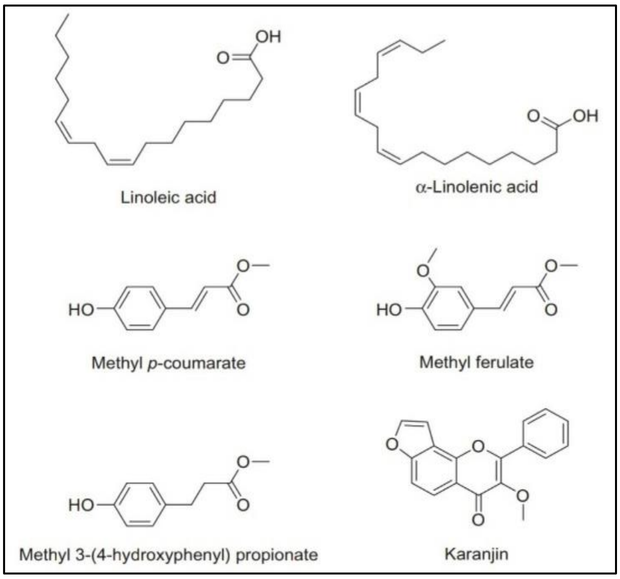

Fig 3: Chemical structures of compounds reported to show BNI activity in plants

Synthetic nitrification inhibitors hampers the activity of ammonia monooxygenase enzyme activity, whereas the BNI compounds like linoleic and linolenic acids known to suppress the activity of both AMO and HAO activity including the electron transfer pathway HAO to ubiquinone and cytochrome (Subbarao *et al*., 2007) [19].

Nitrification process is carried out by both ammonia oxidizing archea (AOA) and ammonia oxidizing bacteria (AOB) depending on the prevailing conditions. In rice roots 1, 9 decanediol changes the community structure of AOB more pronounced than the community structure of AOA (Lu *et al*., 2019) [9].

But in sorghum sorgoleone found to shape the community of nitrifying organisms which affects the population of AOA rather than AOB (Sarr *et al*., 2020) [14]. Where as in temperate forest tree species it was found that nitrobacter population is affected by biological nitrification inhibition rather than AOA or AOB (Laffite *et al*., 2020) [7]. Another theory suggests that BNI may be due to higher nitrogen immobilization rates. The nitrate or ammonical form assimilated by soil microorganisms is converted to organic forms and prevented from loss. It leads to temporal storage of N. So, it reduces the substrate availability to nitrifiers and prevents nitrification (Vazquez *et al*., 2019) [23].

4. How to detect BNI in plants?

To explore the BNI phenomenon at first it has to be identified. There is a chance that many plant species having BNI capacity left unnoticed. So, identification is the major task. A recombinant strain of *Nitrosomonas europea* is used for bioluminescence assay which will detect BNI. If the plant possesses the nitrification inhibition, then the growth of nitrosomonas is inhibited and luminescence is affected (Subbarao *et al*., 2007) [19]. Recently it was found that nitrate reductase activity in leaves is a good indicator of biological nitrification inhibition under greenhouse and field (Karwat *et al*., 2019) [5].

5. How long biological nitrification inhibition can inhibit microorganisms from nitrification?

Nitrifications process in soil my last for several weeks. So, to prevent nitrification the inhibitory compounds should also release for several weeks for stable nitrification inhibition. Recombinant luminescent *Nitrosomonas species* is generally used for studying stability of BNI compounds in soil. Some other studies emphasized the need of threshold level of biological nitrification inhibitory compounds in soil. In *Brachiaria* species the compounds released may last for 80-100 days to prevent nitrification. This is also affected by edaphic and climatic factors like property of inhibitory compound (solubility, volatility, degradation, adsorption capacity), soil properties like clay and organic matter content, texture, pH, microbial diversity and environmental factors like temperature and moisture availability (Gopalkrishnan *et al*., 2009) [2]. In rice roots the release of 1,9 decanediol was accelerated by low ammoniacal nitrogen concentration, low pH, better aeration of rhizosphere and presence of nitrifiers (Zhang *et al*., 2019) [9]. Where as in *Brachiaria humidicola* plants supplemented with ammoniacal fertilizers showed higher nitrification inhibition (Subbarao *et al*., 2007) [19].

6. How BNI is helpful?

1. Synthetic nitrification inhibitors are having degradation and pollution issues where as biological nitrification inhibitors are natural compounds will not have these problems.
2. It was found that when cattle and sheep feeds on *Plantago lanceolata* the nitrification from their urine was reduced when applied to soil. It had even controlled

nitrate leaching and nitrous oxide emissions. So, in dairy-pasture system where nitrate loss from animal urine was major problem BNI plants can be mixed with other fodder and can mitigate nitrate leaching and nitrous oxide emissions (Judson *et al.*, 2019; Simon *et al.*, 2019) [4, 15].
3. BNI plants such as *Brachiaria humidicola* can be used as green manure crops. In *Brassica rapa* it was found that nitrate accumulation in leaf was reduced by using *Brachiaria humidicola* as green manure crop (Shin *et al*., 2019) [16].
4. It can improve the quality of manure when used in composting. It was found that when 0.6% MHPP used in dairy manure composting emission of NH3, N_2O, and CH_4 were reduced by 65.4%, 62.3% and 61.9% from manure. The ammonical N form increased and nitrate form N content reduced. It prevented N loss from manure and the final product had higher total N (Ren *et al*., 2019) [12].
5. BNI helps to reduce nitrate leaching loss and indirectly helps in preventing N_2O emission.
6. Through genetic intervention and exploration BNI trait can be transferred to cultivated plant species from their wild forms which have this trait and inbuilt nitrification inhibition crops can be developed.
7. It was found that BNI compounds exuded have this inhibitory action in succeeding crop also. So, it can be used in crop rotation (Moreta *et al*., 2020) [10].
8. It has potential to increase nitrogen use efficiency of plants and increase crop yields which will help to reduce the requirement of N (Sun *et al*., 2016) [22].
9. BNI compounds may be extracted and can be used in other crops. 1,9 decanediol has superiority over DCD in nitrification inhibitory action. So, it will be a promising compound in future.

7. Is there any limitation for BNI?

The major problem lies within its identification. At present our knowledge is limited to only few crops possessing this capacity. We have to add more crops to this gallery. Soil properties like pH and clay content found to have influence on BNI. High microbial load in soil may degrade the BNI compounds and its stability may be affected.

8. Conclusion

At present though we are advanced in modern technologies we are presently unable to completely understand some natural phenomenon. One such phenomenon is biological nitrification inhibition, where plant will control the nitrification process through release of root exudates. Modern agricultural system depends heavily on N fertilizers which have environmental consequences such as eutrophication and greenhouse gas emission. In this regard BNI is a boon to mankind because BNI has potential to mitigate these issues. It should be properly identified and explored. At present we have just scratched the surface of BNI with our limited knowledge we have to scratch more for better shining of this subject. More and more crops should be added to the gallery of BNI crops. Their active chemical ingredients and mode of action should be understood.

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