BIOLOGICAL NITRIFICATION INHIBITION (BNI) -POTENTIAL FOR REDUCING NITRIFICATION AND N_2O EMISSIONS FROM AGRICULTURAL SYSTEMS

Guntur V. Subbarao

Japan International Research Center for Agricultural Sciences (JIRCAS),
1-1 Ohwashi, Tsukuba, Ibaraki 305-8686, Japan

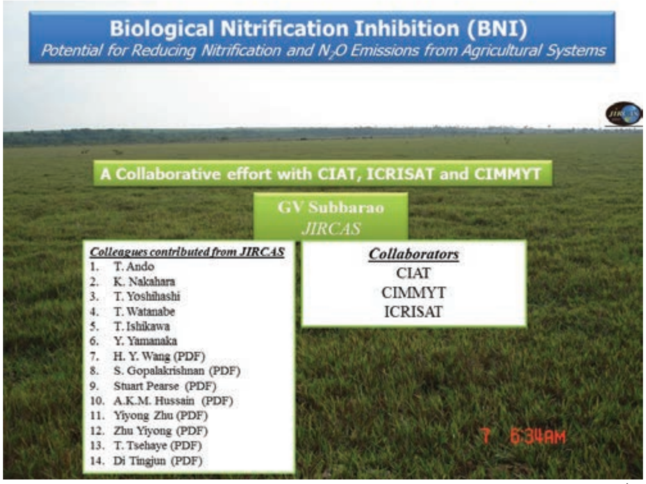
....

Guntur Venkata Subbarao holds a Doctorate degree in Crop Physiology and Plant Nutrition from Indian Institute of Technology (IIT), Agricultural Engineering Department, Kharagpur, India, and is a Senior Researcher at JIRCAS, Japan. His expertise is in the area of crop physiology and nutrition. He is a subject expert on biological nitrification inhibition (BNI) research. Before joining JIRCAS, he has worked at Kennedy Space Center, NASA, USA.

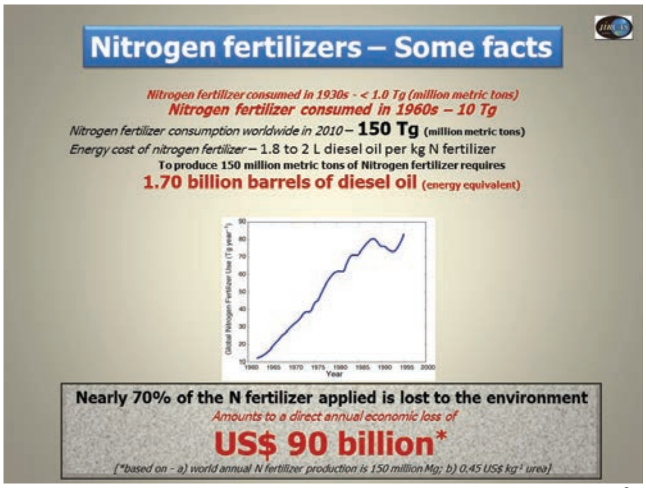
ABSTRACT

Nitrification, the biological oxidation of ammonium (NH_4^+) to nitrate (NO_3^-), weakens the soil's ability to retain N and facilitates N-losses [from N-fertilizer and SOM-N (soil organic matter derived N) through NO_3^- -leaching and denitrification from production agriculture. Modern production systems have become high nitrifying due to accelerated soil-nitrifier activity and this results in low NUE. Nearly 70% of the nitrogen fertilizer applied (about 175 Tg N) to agricultural systems is lost (through NO_3^- leaching and gaseous emissions (N_2O, NO and N_2) from denitrification] before the crop can absorb and convert into plant-protein, causing enormous cost to the world's economy, harming ecosystems- and human health. Annual economic loss from the lost N-fertilizer amounts to 90 US$ billion. Globally, nitrification and denitrification are the primary drivers for generation of nitrous oxide (N_2O), the most powerful greenhouse gas with global warming potential 300 times greater than that of CO_2, and is the third most important contributor to global warming. Plants ability to produce and release nitrification inhibitors from roots and suppress soil-nitrifier activity is termed 'biological nitrification inhibition' (BNI). With recent developments in in-situ measurement of nitrification inhibition, it is now possible to characterize BNI function in plants. The current status of BNI research in JIRCAS, in particular, on production, release, regulation and genetic control of BNI-trait using *Brachiaria* sp. pastures, sorghum and wheat as model systems, will be presented during this talk. The effectiveness of BNI function in suppressing soil nitrifier activity, soil nitrification and N_2O emissions has been shown in the field using *Brachiaria humidicola* pasture grasses. As a plant function, BNI is regulated by a range of genetic, soil and environmental factors. The BNI-capacity in root systems of major food- and feed-crops can be exploited as a plant-trait using conventional and modern breeding tools. BNI-enabled cultivars of major food and feed crops will be an integral part of next-generation production systems that must be low-nitrifying, low-N_2O emitting and have enhanced NUE to benefit both agriculture and environment.

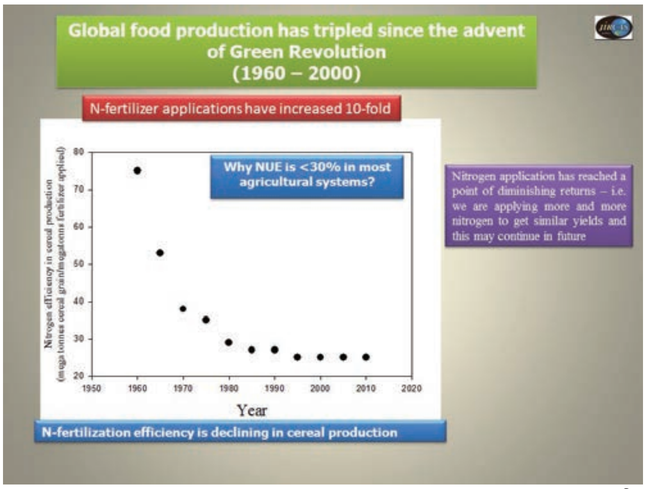
KEYWORDS
Biological nitrification inhibition (BNI), Greenhouse gases, Nitrous oxide emissions, Nitrogen use efficiency (NUE), Nitrification inhibitors.


Biological Nitrification Inhibition (BNI)
Potential for Reducing Nitrification and N_2O Emissions from Agricultural Systems
A Collaborative effort with CIAT, ICRISAT and CIMMYT
GV Subbarao
JIRCAS
Colleagues contributed from JIRCAS
1. T. Ando
2. K. Nakahara
3. T. Yoshihashi
4. T. Watanabe
5. T. Ishikawa
6. Y. Yamanaka
7. H. Y. Wang (PDF)
8. S. Gopalakrishnan (PDF)
9. Stuart Pearse (PDF)
10. A.K.M. Hussain (PDF)
11. Yiyong Zhu (PDF)
12. Zhu Yiyong (PDF)
13. T. Tsehaye (PDF)
14. Di Tingjun (PDF)
Collaborators
CIAT
CIMMYT
ICRISAT
6:34AM

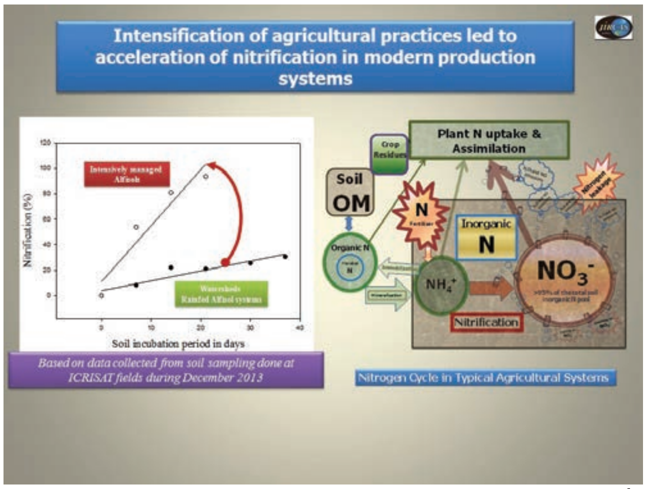
1


Nitrogen fertilizers – Some facts
Nitrogen fertilizer consumed in 1930s - < 1.0 Tg (million metric tons)
Nitrogen fertilizer consumed in 1960s – 10 Tg
Nitrogen fertilizer consumption worldwide in 2010 – 150 Tg (million metric tons)
Energy cost of nitrogen fertilizer – 1.8 to 2 L diesel oil per kg N fertilizer
To produce 150 million metric tons of Nitrogen fertilizer requires
1.70 billion barrels of diesel oil (energy equivalent)
Year
Nearly 70% of the N fertilizer applied is lost to the environment
Amounts to a direct annual economic loss of
US$ 90 billion*
[*based on - a) world annual N fertilizer production is 150 million Mg; b) 0.45 US$ kg⁻¹ urea]

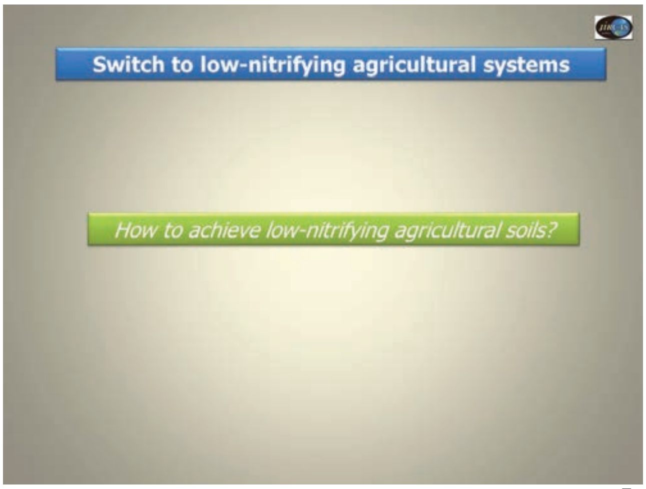
2


Global food production has tripled since the advent of Green Revolution
(1960 – 2000)
N-fertilizer applications have increased 10-fold
Why NUE is <30% in most agricultural systems?
Nitrogen efficiency in cereal production
(mega tonnes cereal grain/mega tonnes fertilizer applied)
Year
Nitrogen application has reached a point of diminishing returns – i.e. we are applying more and more nitrogen to get similar yields and this may continue in future
N-fertilization efficiency is declining in cereal production

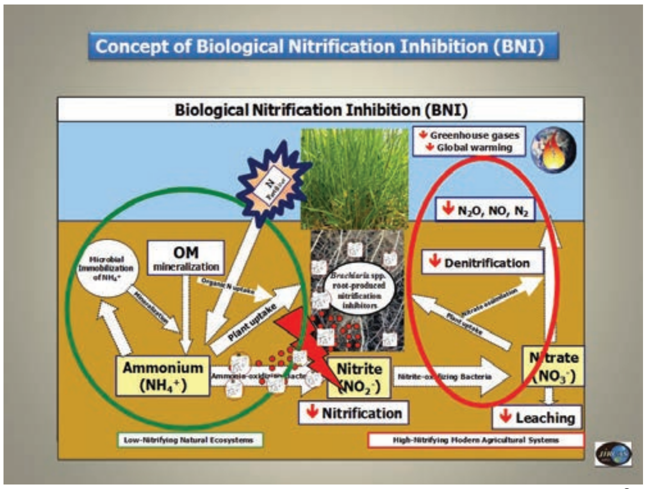
3


Intensification of agricultural practices led to acceleration of nitrification in modern production systems
Nitrification (%)
Intensively managed Alfisols
Watersheds Rainfed Alfisol systems
Soil incubation period in days
Based on data collected from soil sampling done at ICRISAT fields during December 2013
Plant N uptake & Assimilation
Crop Residues
Soil OM
Organic N
N
Inorganic N
NH_4^+
NO_3^-
Nitrification
Nitrogen Cycle in Typical Agricultural Systems

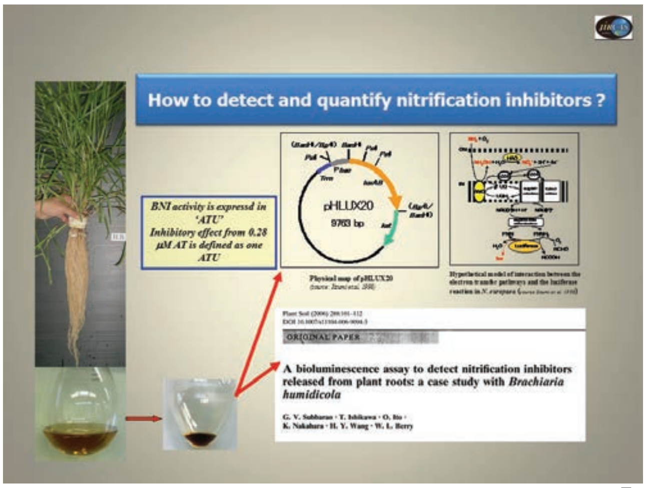
4


Switch to low-nitrifying agricultural systems
How to achieve low-nitrifying agricultural soils?

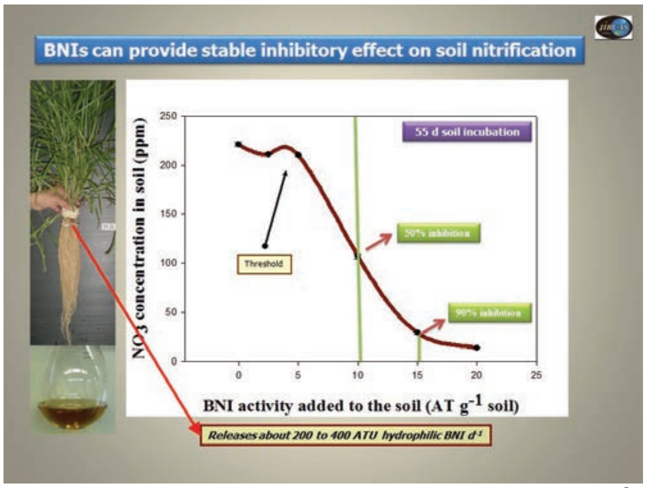
5


Concept of Biological Nitrification Inhibition (BNI)
Biological Nitrification Inhibition (BNI)
Greenhouse gases
Global warming
N_2O, NO, N_2
Denitrification
OM mineralization
Microbial immobilization of NH_4^+
Plant uptake
Brachiaria spp. root-produced nitrification inhibitors
Ammonium (NH_4^+)
Nitrite (NO_2^-)
Nitrate (NO_3^-)
Nitrification
Leaching
Low-Nitrifying Natural Ecosystems
High-Nitrifying Modern Agricultural Systems

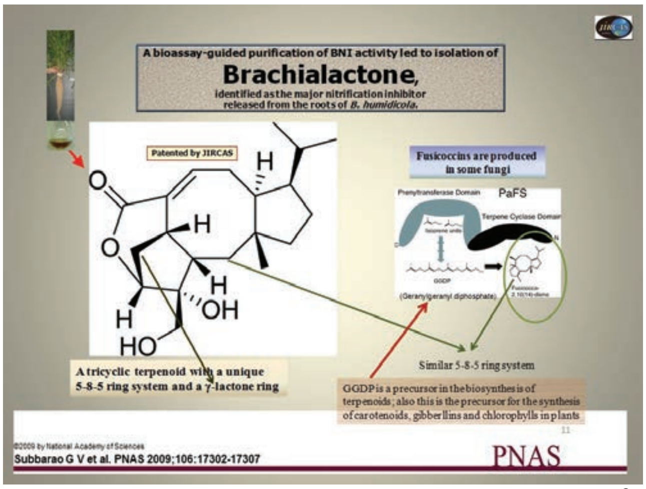
6


How to detect and quantify nitrification inhibitors ?
BNI activity is expressd in 'ATU' Inhibitory effect from 0.28 μM AT is defined as one ATU
pHLUX20
9783 bp
Physical map of pHLUX20
ORIGINAL PAPER
A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with Brachiaria humidicola

7


BNIs can provide stable inhibitory effect on soil nitrification
NO3 concentration in soil (ppm)
55 d soil incubation
Threshold
50% inhibition
90% inhibition
BNI activity added to the soil (AT g-1 soil)
Releases about 200 to 400 ATU hydrophilic BNI d-1

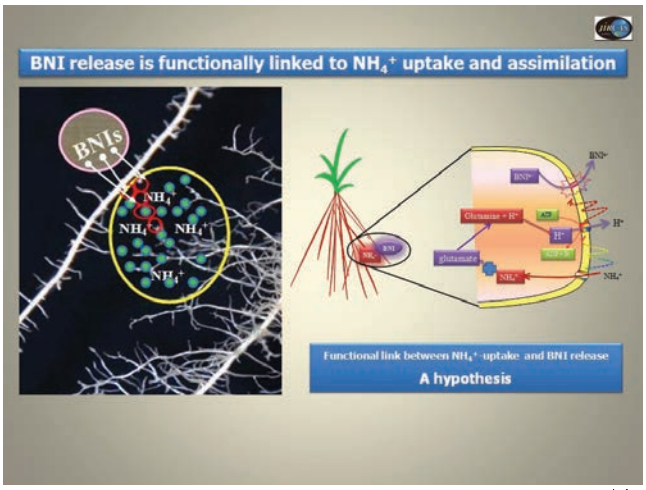
8


A bioassay-guided purification of BNI activity led to isolation of
Brachialactone,
identified as the major nitrification inhibitor released from the roots of B. humidicola.
Patented by JIRCAS
Fusicoccins are produced in some fungi
PaFS
A tricyclic terpenoid with a unique 5-8-5 ring system and a γ-lactone ring
Similar 5-8-5 ring system
GGDP is a precursor in the biosynthesis of terpenoids; also this is the precursor for the synthesis of carotenoids, gibberllins and chlorophylls in plants
©2009 by National Academy of Sciences
Subbarao G V et al. PNAS 2009;106:17302-17307
PNAS

9


BNIs release from roots is not a constitutive process
What triggers the release of BNIs from roots?

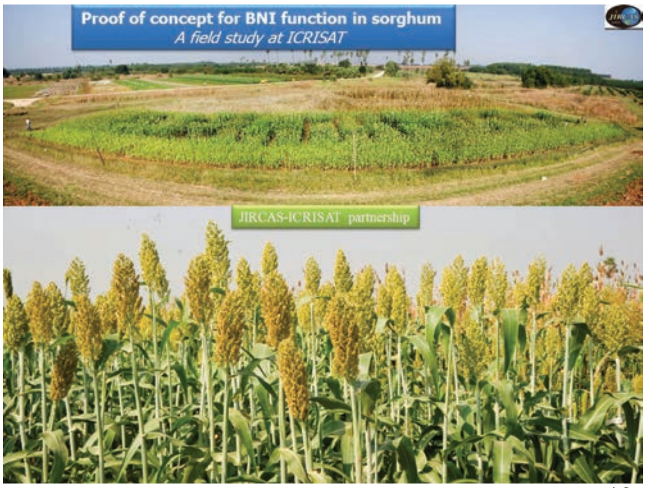
10


BNI release is functionally linked to NH4+ uptake and assimilation
BNIs
Functional link between NH4+-uptake and BNI release
A hypothesis

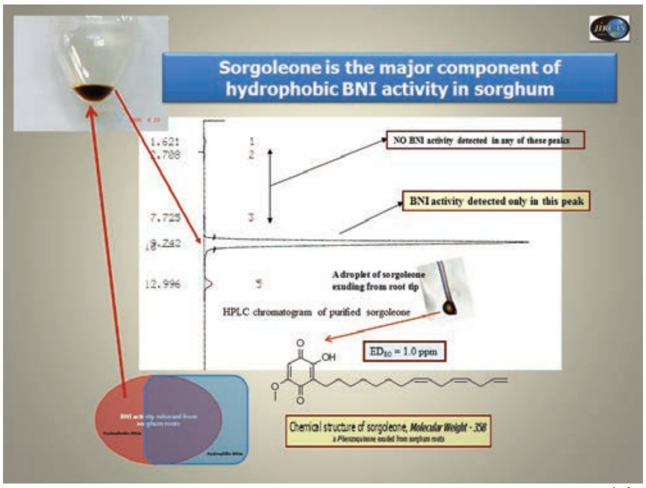
11


Is there potential for genetic improvement of BNI capacity?
Sorghum and Wheat as case studies
Genetic variability is the primary requirement for genetic improvement in trait/s of interest

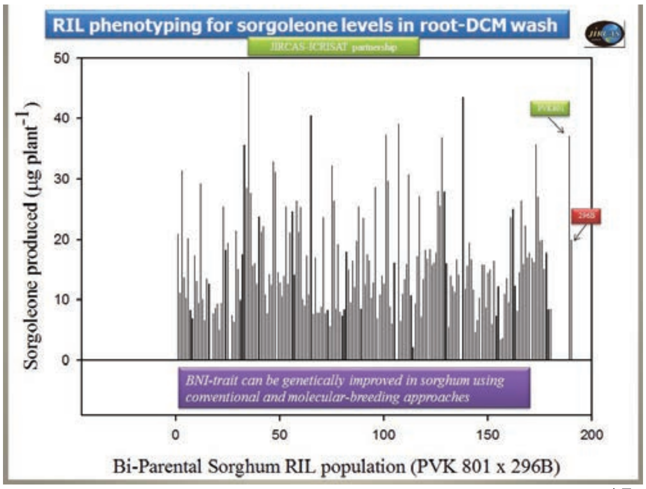
12



13

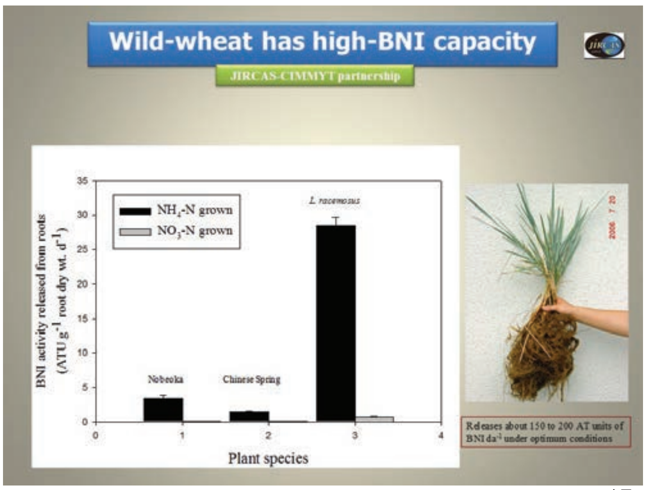


14

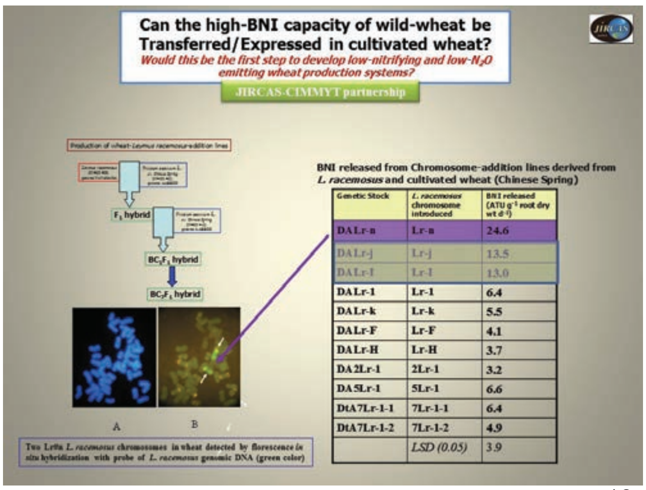


15

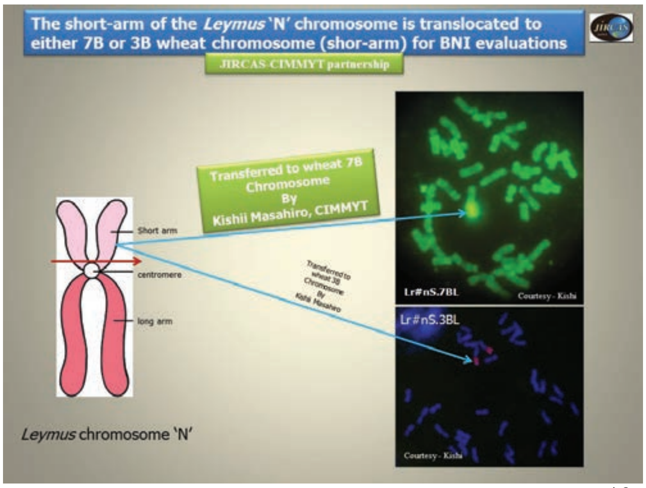
16



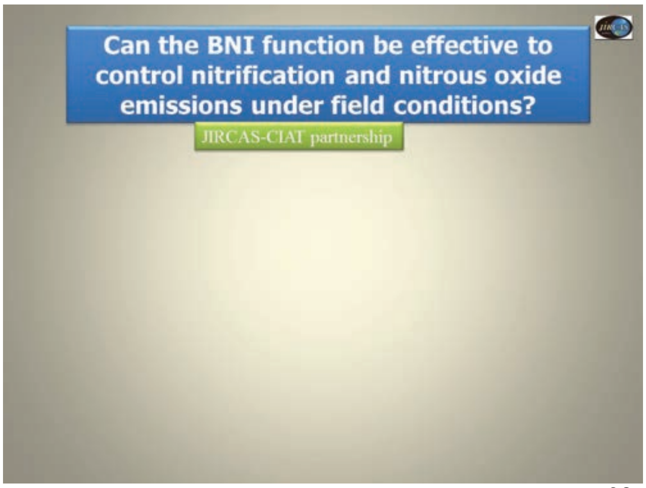
17



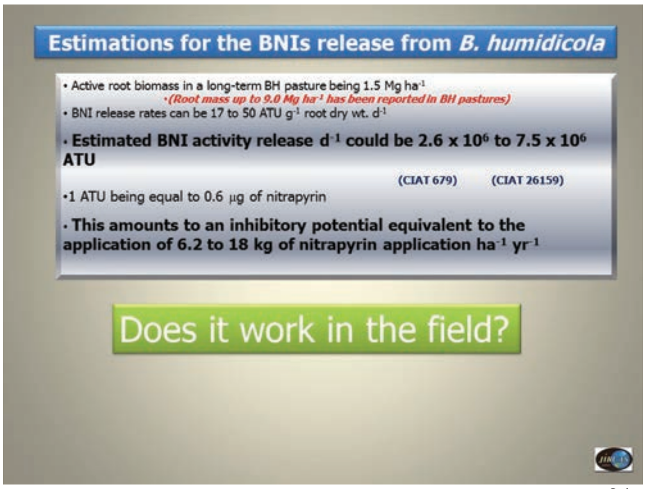
18



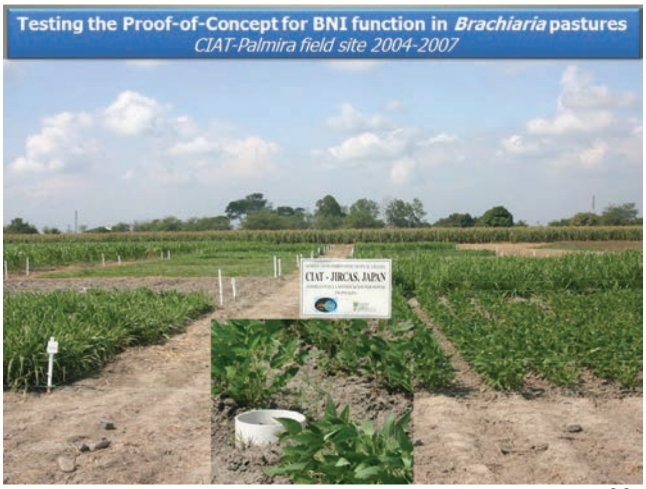
19



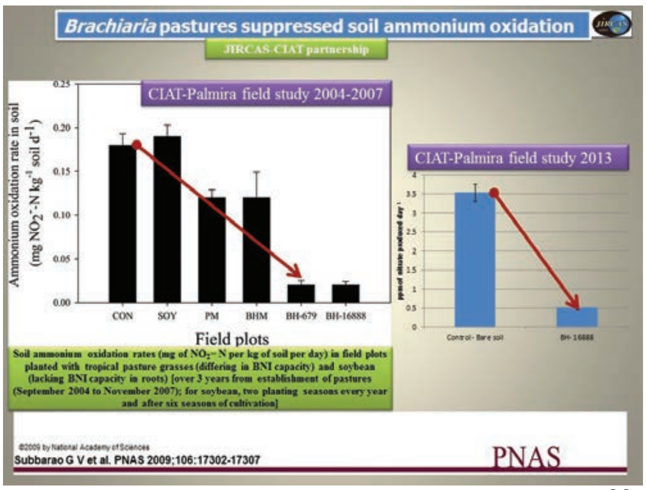
20



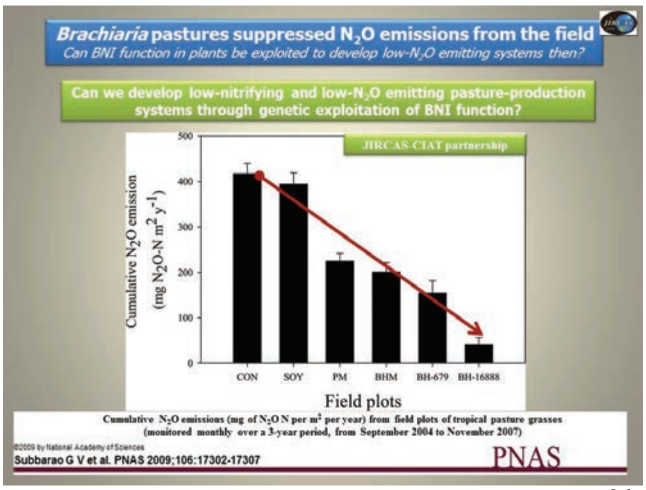
21



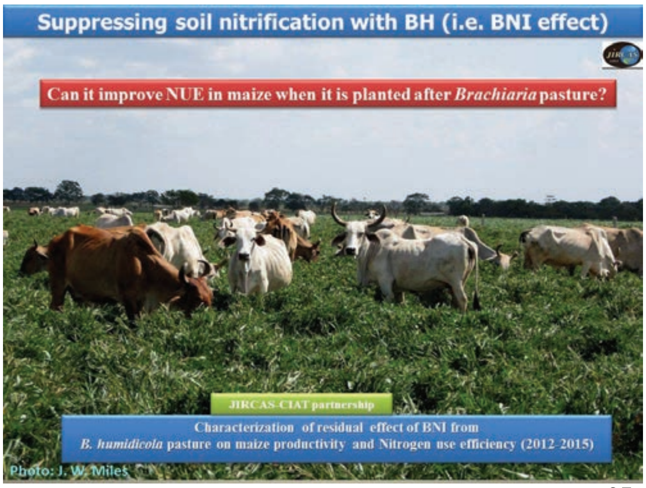
22



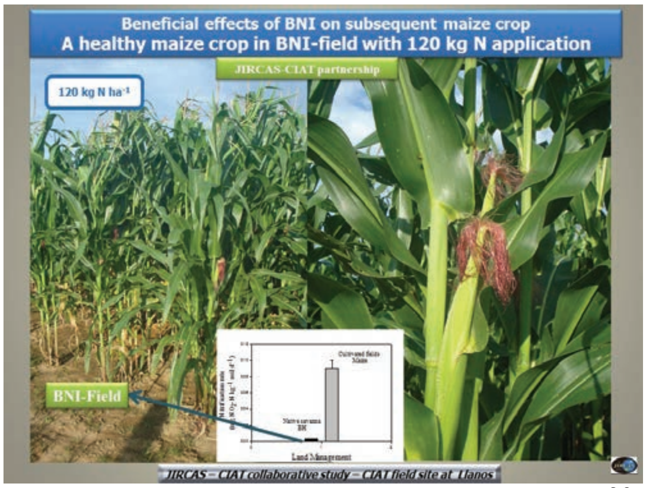
23



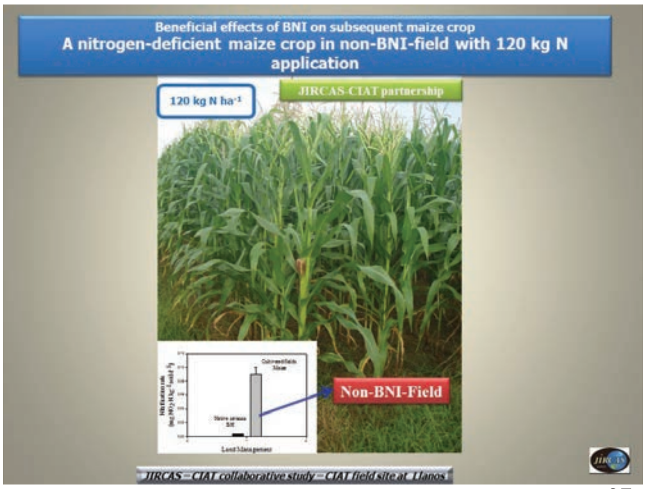
24



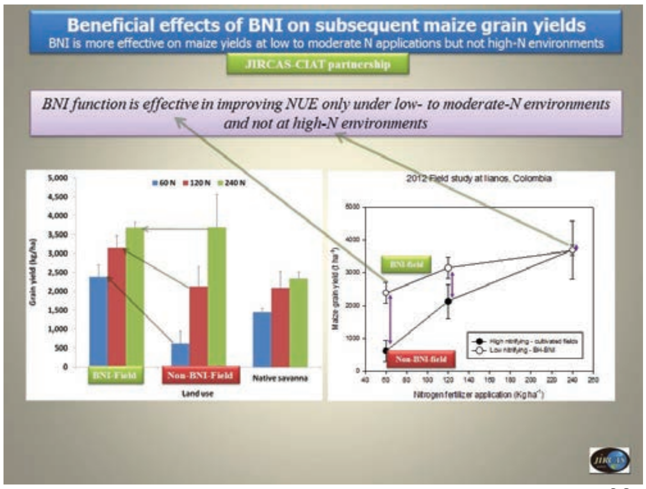
25



26



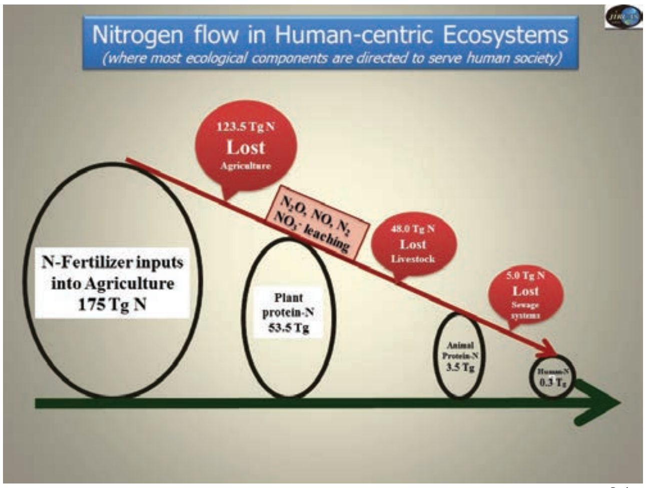
27



28

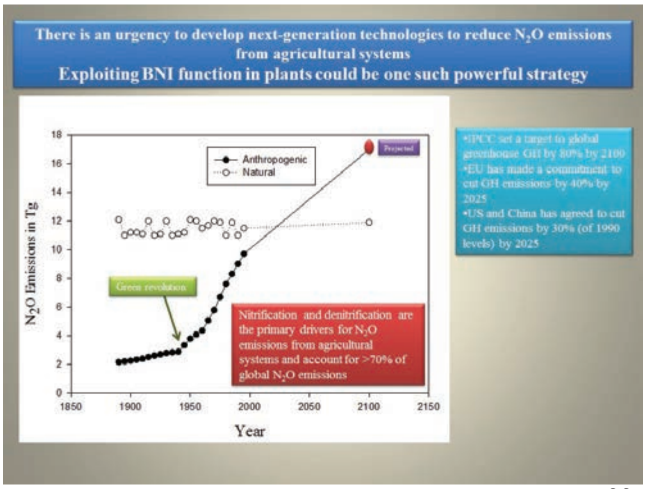
29

30

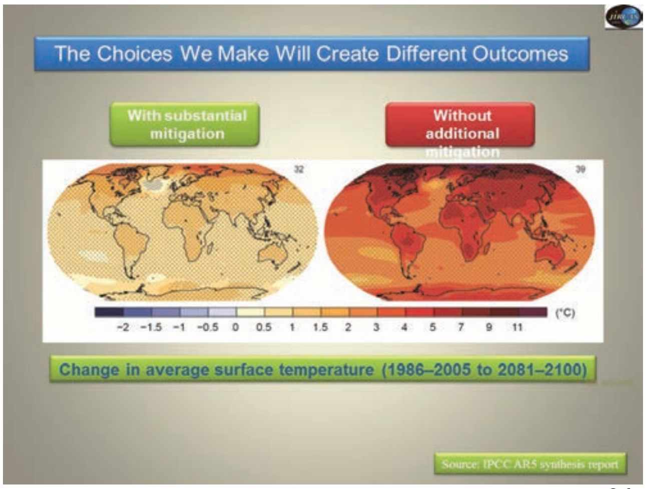


31

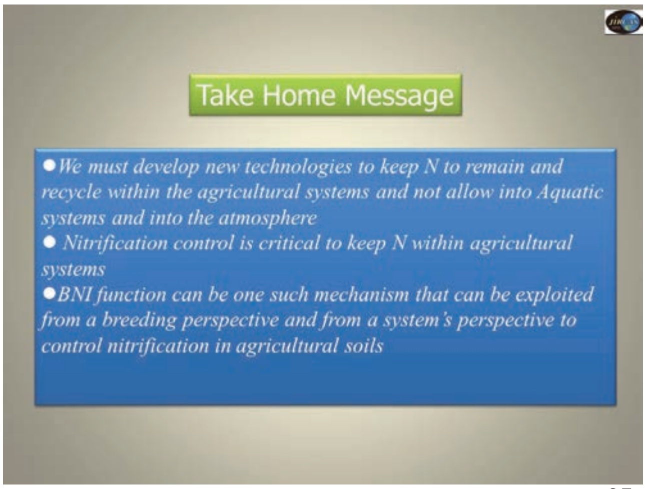
32



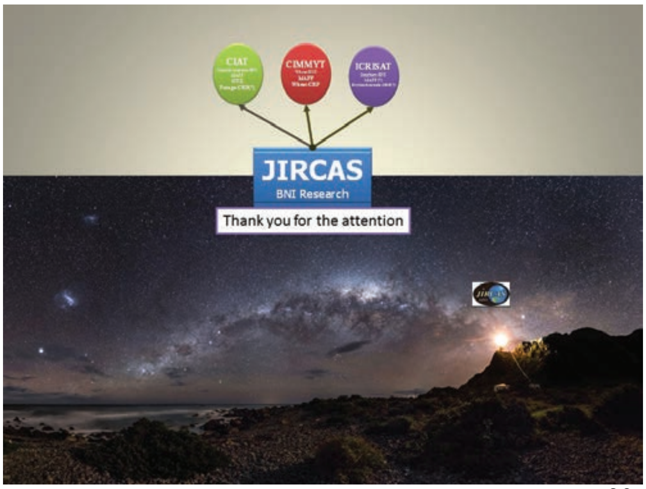
33



34



35



36

Chair Matsumoto: The next speaker is Dr. Guntur Subbarao. He holds a doctorate degree in Crop Physiology and Plant Nutrition from Indian Institute of Technology. He is now the Senior Researcher at JIRCAS. His expertise is in the area of crop physiology and nutrition. He is a subject expert on biological nitrification inhibition, BNI. Today, his topic is about BNI. His title is "Biological Nitrification Inhibition (BNI) - Potential for Reducing Nitrification and N_2O Emissions from Agricultural Systems." Dr. Subbarao, please.

Dr. Guntur Subbarao: Thank you, Mr. Chairman. Good afternoon everybody. So there are number of my colleagues who have contributed to the development of this work and also we work closely with three CG Centers, starting from CIAT on tropical pastures, CIMMYT on wheat and sorghum, with ICRISAT.

Just to give you an idea about the amount of nitrogen fertilizers we are using, currently we are using about 150 million metric tons of nitrogen fertilizer compared to about 10 million metric tons of nitrogen fertilizer we were using in 1960s. So, there is almost a 15-fold increase in the last five decades in terms of nitrogen fertilizer use. And just to give you an idea about the amount of energy that goes into producing this nitrogen fertilizer is enormous, almost 1.7 billion barrels of diesel oil energy equivalent is required to produce just 150 million metric tons of fertilizer. And nearly 70% of this nitrogen fertilizer is lost from the agricultural systems without having a chance for the plants to convert it into plant protein. The economic loss from the nitrogen fertilizer alone comes to about $90 billion per annum. The ecological cost and the cost associated on the human health is enormous, probably it's not even estimated until now. Probably I would cover that point towards the end, but it is quite enormous, the amount of nitrogen loss in terms of ecological and economic cost.

It's often said that the green revolution has tripled the food production from 1960 until now. Of course, what people don't tell you is the nitrogen fertilizer applications have increased 10-fold just to maintain or just to keep this 3-fold increase in food production. That is one of the reasons why we have a continuous decline in nitrogen's efficiency for agricultural production systems and we are reaching a point where it's almost coming to a point of diminishing return. So, we are applying more and more nitrogen fertilizer just to maintain the same yields.

One of the reasons why the nitrogen's efficiency is going down with time despite all these agronomic improvements is because here. This is the dataset we collected from ICRISAT or our collaborated work with ICRISAT. The nitrification rates are going up tremendously with the intensification. Here the nitrification rate in the rainfed watershed and this is the nitrification rates in the same ICRISAT fields Alfisols where the system is intensified. The nitrification rates are almost five to six times higher in the intensified systems compared to the rainfed watershed systems, the same type of fields. Because of that all the nitrogen fertilizer we are applying in agricultural systems are rapidly getting converted into nitrate; and once it gets converted into nitrate, then it is subjected to loss, either through leaching or denitrification.

How do we achieve low-nitrifying agricultural systems? That is a key to improve the nitrogen's efficiency in agricultural systems.

One of the ideas we have been working in JIRCAS is how can we harness the power of the production of nitrification inhibitors from the plant root systems to control this nitrification process. So, we termed it as BNI, Biological Nitrification Inhibition. These nitrification inhibitors are produced by the plants to control this nitrified activity. So, this is plant rate which is only available in certain plants, certain crops. How we wanted to characterize, how we wanted to develop, how we wanted to exploit this function to control the nitrified activity in agricultural systems as a means to improve the nitrogen's efficiency is one of our research goals in JIRCAS. If we control nitrification and the nitrogen would remain in ammonium form or it will move into this pathway which is not subjected to loss, but once you allow the nitrogen to go into this path then most of the nitrogen is lost. That is one of the reasons why the nitrogen's efficiency is less than 30% in most of the agricultural systems.

At JIRCAS, we had developed an assay system using the recombinant Nitrosomonas concept that can detect and quantify the amount of nitrifiers produced by the plants. So, we can quantify in terms of allylthiourea units that's a unit we developed to quantify the amount of inhibitor activity coming from the plants. Most of our work initially is focused on the tropical pasture grasses where the function has been the strongest. This is Brachiaria grass, the tropical grass, grown mostly in South America and in parts of Africa.

A plant like this can produce up to 200 to 400 units of inhibitor activity per day. To get 50% inhibition in the soil, the soil has to accumulate about 10 units of activity per gram of soil then the nitrification is inhibited up to 50%. That means the nitrifier activity is suppressed up to 50%. If it goes up to 15 units of activity, then we can have almost 70% to 80% inhibition.

We have isolated and identified the active component responsible for the inhibitor activity in Brachiaria. So, it's named as Brachialactone. Now, we have a possibility to develop the Brachiaria pastures into high levels of Brachialactone using the breeding mean, so that is one of the genetic improvement strategies you can develop in terms of produce, developing new types of crops that has ability to control nitrification in the soil systems to improve the nitrogen's efficiency.

What triggers the release of these nitrification inhibitors from the plants?

One is the presence of ammonia. When the ammonia is present in the soil, the plant sense and the plants that have the ability to produce these inhibitors then they start releasing these inhibitors. The release of these inhibitors is so closely linked with the uptake and assimilation of ammonia in the plant root systems. Also, the release of these inhibitors is confined to only the regions where the plants sense ammonia so it is very, very localized. Because it is localized in nature, the inhibitor concentration can be substantial to stop the nitrification process. So, the concentration can be high to stop the nitrifier activity.

Is there any potential for genetic improvement of BNI capacity in crops or pasture? So, this is the question we have been asking. The idea is whether we can develop the next generation of crops, particularly the staple crops, where the root systems have the ability to produce these nitrification inhibitors to suppress nitrified activity so that the nitrogen can be utilized more effectively.

One of the model systems we are working is sorghum. This is the collaboration with ICRISAT where we are trying to see the proof-of-concept, whether we can detect and quantify the strength of this function in the plants grown in the field at a field level.

We have identified the active component responsible for the inhibitor activity coming from the sorghum roots. This is called Sorgoleone. We have identified quite a substantial amount of genetic variability for the sorghum varieties to produce this compound.

This is RIL population. The sorgoleone production variability and these are the parental lines, the highest sorgoleone producing line and the lowest sorgoleone producing line. This is RIL population. Now, we are trying to develop the molecular markers for sorgoleone production in sorghum. So, this would make it possible in future to develop sorghum varieties that can produce very, very high levels of sorgoleone from the root systems as a way to control nitrification.

Also, we work closely in partnership with CIMMYT to understand and develop or improve the BNI producing capacity of the root systems in wheat system. So wheat is very important, wheat and maize are the most important because they consume almost 70% of the total nitrogen fertilizer that is used in agricultural systems.

Our initial work did not show much inhibitor production in the cultivated wheat. That's the reason we looked at one of the wild relatives, the Leymus racemosus. That has a very high capability to produce nitrification inhibitors from the root systems.

Now we have located a chromosome in Leymus so that is chromosome N that is the controlling the nitrification inhibitor production in Leymus. When we introduced into the wheat, the wheat started producing the inhibitors. Now we are trying to see which is the main inhibitor production region in the chromosome of chromosome N? So, we have transferred this short-arm of the chromosome N into the wheat in two translocation points here.

This is the inhibitor production when we introduced the whole chromosome compared to the cultivated wheat. This is the inhibitor production in the short-arm is translocated to the 3B position in the wheat. Now at least a major portion of the inhibitor production is controlled by the short-arm of the chromosome N that is confirmed. So now we are trying to see whether we can reduce the chromosome size to such a stage where we can have a normal wheat growth with high inhibitor production in wheat. That is underway.

The important thing is does it work in the field, so whether these plants can produce sufficient amount of inhibitors in the field. So, we calculated the amount of inhibitor production from the Brachiaria grasses on a yearly basis which comes to about 2.6 million units per day inhibitor production and we can calculate how much in terms of the commercial application of inhibitor.

This is the experiment we have set up to test the proof-of-concept. This is with CIAT's help – CIAT collaboration, where we looked at by growing Brachiarias whether we can reduce the nitrification in the soil.

Here we looked at a variety of tropical grasses. That's the soybean is our negative control. We can see after 3 years the nitrification rates in the soil comes down substantially, almost close to 90% of nitrification rates what you see in the normal control soil.

The nitrous oxide emissions, which is a part of the nitrification denitrification process, also suppressed substantially by Brachiaria. Now how can we exploit this, can we exploit this from a cropping system's perspective?

We reduced the nitrification by growing Brachiaria and then we planted maize.

Here when the nitrification is controlled, the maize grows very nicely with 120 kilograms of nitrogen.

The field next to that where the nitrification is not controlled, the same amount of fertilizer nitrogen, it shows nitrogen deficiency symptoms.

This is what came in Nature last year in news Focus where this emphasized, the concept of BNI function and the possibility of developing crops with this function to control the nitrification and improve the nitrogen's efficiency.

To conclude this, currently we are using about, as I said, 175 million metric tons of nitrogen fertilizer in agricultural system. When it comes to the humans, we only retain less than 1 teragram of nitrogen in our body so the rest is all lost up in the air. That is the reason we are forced to apply the same amount of nitrogen fertilizer every year because nothing is remaining in the soils that soils are not able to retain any of this nitrogen.

Where all this nitrogen is going into? The water, a part of this nitrogen is going into water and causing enormous problems with pollution. China was mentioned about the intensification and which is mostly driven by the nitrogen fertilizer application and this is what happening. A lot of this nitrogen is going into the water.

Also, this is the nitrous oxide emissions. You can see the graph here. This is the beginning of the green revolution and you can see the nitrous oxide emissions are going up; and by 2100, it is expected to double, reach almost 19 million metric tons by then. But IPCC has set a target in its recent report that the greenhouse gas emissions should be reduced by 80% and which the US and China have committed that they wanted to reduce, cut the greenhouse gas emissions by 40% by 2025.

The choices we make will create different outcomes. This is not my quote. This comes from the IPCC. So with substantial mitigation, we can control the global warming, if not, we probably would end up like that.

We must develop new technologies to keep nitrogen to remain and recycle in agricultural systems and not allow into aquatic and atmospheric systems. So, the nitrification control is critical to keep nitrogen within agricultural systems and BNI function can be one such mechanism that can be exploited from a breeding perspective and from a system's perspective to control nitrification in agricultural soils. Thank you.

Chair Matsumoto: Thank you, Dr. Subbarao. We've so easy to understand what is BNI and function of BNI and so your group try to relate it to the breeding and use the function of BNI under the cropping system. I'm sorry that we have so short and few minutes to – we have one question or comment and do you have other some comment? Please.

Male Questioner: Yeah. I just want to know about the biochemical compound, did you identify in wheat already or…

Dr. Guntur Subbarao: No, for wheat we have not yet.

Male Questioner: Okay and that is…

Dr. Guntur Subbarao: So it's in the progress.

Male Questioner: Okay, thank you.

Chair Matsumoto: Please. Dr. Karanja?

Dr. Nancy Karanja: I am just wondering around. Since you know the chemical that is being secreted, do you see any potential of possibly harvesting or coming up with ways of producing that chemical and then it is incorporated in fertilizer as a shortcut?

Dr. Guntur Subbarao: Yeah, that is always a possibility. In fact, most of the chemical compounds that are developed as pesticides or as antibiotics for humans all come from the natural sources initially. Yeah, this is a possibility. But because the soil nitrification happens inside the soil, so the best way to deliver the nitrification inhibitors is through the root systems. Because you can develop a chemical nitrification inhibitor, but how do you deliver it into the soil which is happening deep into the soil, almost within the 30 to 50-centimeter zone, isn't it? So probably the roots could be a powerful system to deliver this nitrification inhibitor, otherwise you have to spray on fertilizer band or you have to coat the nitrogen fertilizer with the nitrification inhibitor then there are other problems coming in. But it is possible.

Chair Matsumoto: Okay, the time is up. Thank you, Dr. Subbarao. The morning session is finished, close.