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Special Feature

Biological Nitrification Inhibition (BNI) Research

JIRCAS Japan International Research Center For Agricultural Sciences

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Special Feature

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The Significance of "BNI Technology"

As global issues such as climate change become evident, many activities are being carried out to achieve the Sustainable Development Goals (SDGs), which are shared goals of the international community. In 2021, the United Nations Food Systems Summit was held, confirming that a series of activities surrounding food should be transformed into a sustainable system. Japan has also formulated a long-term policy, the Strategy for Sustainable Food Systems, MIDORI, to achieve both productivity improvement and sustainability in agriculture, forestry, fisheries, and food industries through innovation. The biological nitrification inhibition (BNI) technology pioneered by JIRCAS is one of the most promising technologies to address these issues.

JIRCAS has produced many research results in the fields of soil microorganisms and plant nutrition from early on. Research on BNI began in 1995 with a focus on the characteristics of ammonia nitrogen utilization in tropical grasses (*Brachiaria*) in South America. From that time on, the research has been cognizant of environmental problems caused by nitrates and nitrous oxide. After that, research papers on the nitrification inhibitory function of *Brachiaria* and the chemical identification of substances released from the roots progressed, and in 2006, Dr. G. V. Subbarao and others clarified the concept of BNI. The 5-year project continued for 3 periods (15 years) at JIRCAS and during that time, the target crops increased and a BNI consortium was formed with CGIAR research centers, expanding the circle of international collaboration.

Under such circumstances, wheat with enhanced BNI ability was developed by crossing wheat with a wild relative. The resulting research article by Dr. Subbarao and colleagues was honored as one of the best papers of the year 2021 by the prestigious scientific journal *Proceedings of the National Academy of Sciences* (*PNAS*). Dr. Subbarao was also invited to the popular TED Talks event to speak about "BNI technology," giving it the global recognition it deserved. In April this year, BNI-enhanced wheat was introduced at the G20 Meeting of Agricultural Chief Scientists in India, and was also exhibited and introduced at the G7 Agriculture Ministers' Meeting in Japan. Research funds have been obtained one after another, and field demonstrations have begun in Japan, India, Nepal, and other countries. In just a few years since the scientific paper was written, field trials have been conducted in several countries across continents, and good results have been achieved. It is attracting a great deal of attention from around the world not only because of the research impact but also because of the speed at which it is spreading.

The survival of humankind depends on whether food systems can be sustainably maintained over the long term while maintaining balance in a fragile global ecosystem. To that end, in addition to understanding soil, climate, and other environmental facts, it is essential to develop technologies based on scientific understanding of the relationships between organisms such as crops, microorganisms, and insects. "BNI technology" is a future-oriented technology that was developed based on the interdependence of soil, microorganisms, and crops. It is friendly to the global environment and can be easily adopted by producers. JIRCAS is currently in the fourth term of the project on BNI technology, and research is still ongoing. In the future, we believe that new collaborations in various fields will be necessary as research progresses and technologies spread. I sincerely ask all those who read this newsletter for their support and cooperation in promoting "BNI technology."

KOYAMA Osamu
President, JIRCAS

What is the BNI Research that Originated at JIRCAS?

Modern agriculture, which relies on the application of nitrogen fertilizer to farmland, is responsible for the abundance of food we enjoy. The nitrogen fertilizer applied to farmland comes from chemical fertilizers produced industrially from the air using fossil fuels. It is estimated that more than half of the nitrogen in the human body comes from industrial nitrogen fixation. This combination of chemical fertilizers and high-yielding crop varieties, better known as the "Green Revolution," helped eliminate the food shortages that faced humanity and earned Dr. Norman Borlaug the Nobel Peace Prize in 1970.

On the other hand, it has been found that more than half of the nitrogen fertilizer applied is lost because it is not used by crops. Much of this loss is attributed to nitrification, the process by which microorganisms in agricultural soils extract energy from nitrogen fertilizer. Nitrification is the pathway by which ammonium, a component of nitrogen fertilizer, is converted to nitrate, which is the energy source for some soil microorganisms. This pathway is an important process in the global nitrogen cycle, but excessive application of nitrogen fertilizer to farmland not only improves crop production but also unilaterally activates these soil microorganisms. Nitrate produced by nitrification has a negative charge and is not easily adsorbed by soil particles, which also have a negative charge, making it difficult to remain in the soil. The nitrate that leaches from the soil is toxic and causes groundwater contamination and eutrophication, which leads to the formation of large blue-green algae blooms. In addition, nitrification produces N_2O, a greenhouse gas 298 times more potent than CO_2. Much of the nitrogen fertilizer used for food production feeds soil microorganisms rather than crops, polluting the environment, and more nitrogen fertilizer is needed to provide needed staple foods, further activating soil microorganisms and creating a global crisis. (Fig. 1)

In 1986, the International Center for Tropical Agriculture (CIAT) in Colombia reported a strange phenomenon, saying that "in certain tropical pasture fields, there was no nitrate in the soil at all." In 1995, JIRCAS began a collaborative research project to scientifically investigate this phenomenon. We found that not only was there no soil nitrate in the tropical pasture grass, but also that the number of microorganisms that cause nitrification of the soil was low. We hypothesized that the grass itself had the ability to inhibit nitrification, and our research showed that substances released from the roots selectively inhibit the microorganisms responsible for soil nitrification. This, in turn, reduces the environmental impact of nitrogen. We named this phenomenon "Biological Nitrification Inhibition (BNI)," and our goal has since been to develop "BNI-enabled crops," i.e., crops that have this function enabled (Fig. 2).

Fertilization is the key to a good harvest. Because wheat takes up the largest amount of nitrogen fertilizer in intensive cropping systems, it is referred to as a "fertilizer harvester." However, current modern varieties do not have BNI capacity, so we searched among wild wheats and found one species, *Leymus racemosus*, with BNI capacity. This wild species can be crossed with wheat, and we spent more than 10 years introducing this function into high-yielding wheat varieties. Now, a prototype "BNI-enabled wheat" has been successfully established, and field trials around the world are demonstrating that this wheat uses nitrogen fertilizer efficiently and reduces the environmental impact of nitrogen.

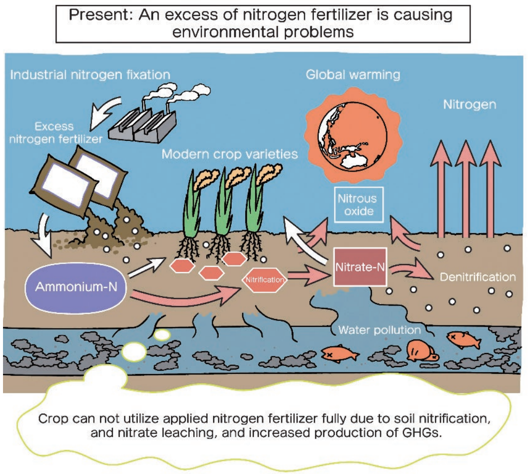
JIRCAS is promoting the "BNI-System Project," which aims at using BNI in various crop production systems. In addition to tropical pasture and wheat, the project is targeting sorghum, maize, and some minor cereals to establish BNI-enabled crops and utilize BNI in their production systems. After nearly 20 years of research, the BNI research concept that originated at JIRCAS has begun to move toward social implementation.

JIRCAS leads the "BNI International Consortium" and holds a biennial international consortium meeting to foster collaboration with researchers from around the world. BNI research is beginning to expand into crop development, plant nutrition, and all soil-related research fields, as well as chemistry to identify BNI compounds, their biosynthesis and mode of action, and social sciences to evaluate the adoption of BNI-enabled crops. The 4th BNI International Consortium Meeting was held at the Tsukuba International Congress Center in November 2022 after a four-year absence due to COVID-19, with 60 participants (including 40 from overseas) (Photo 1). This was the first International Consortium Meeting since the establishment of BNI-enabled crop prototypes and the confirmation of the effects of BNI-enabled wheat in field trials. Many researchers from international organizations and wheat-producing countries attended to share the excitement of finding solutions to the world's food problems through the use of BNI-enabled, planet-friendly crops.

The SATREPS (Science and Technology Research Partnership for Sustainable Development) project in India is testing BNI-enabled wheat and introducing BNI capacity to local elite lines for social adoption in the Indo-Gangetic Plains, India's breadbasket. In addition, the Green Asia project is conducting multi-site trials for social implementation in Nepal in response to the Strategy MIDORI formulated by the Ministry of Agriculture, Forestry and Fisheries (MAFF) on May 12, 2021. In April 2023, the MAFF project, “Accelerating the Development of BNI-enabled Wheat for Japan,” which aims to introduce BNI capacity into elite wheat in Japan, was launched and is being implemented. In addition, BNI-enabled wheat was introduced as a planet-friendly food production technology at the G7 Agriculture Ministers’ Meeting in Miyazaki in April and the G20 Agriculture Ministers’ Meeting in Hyderabad, India, in June, and discussions are underway with G7 and G20 countries on collaborative research (Photo 2).

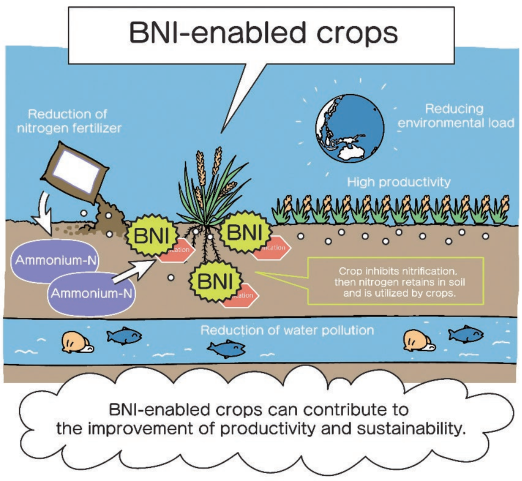
With the support of our partners and stakeholders, we are eager to move forward and realize the “Second Green Revolution” needed for a sustainable future.

YOSHIHASHI Tadashi, Biological Resources and Post-harvest Division
Guntur V. Subbarao, Crop, Livestock and Environment Division



Science Manga Studio (2021)

Fig. 1. Nitrogen cycle in upland fields
Most of the industrially produced nitrogen fertilizers are not absorbed by crops, but are used as an energy source by microorganisms, causing environmental burden and impacting the field.



Science Manga Studio (2021)

Fig. 2. BNI-enabled crops
BNI compounds secreted from crop roots inhibit soil nitrification, allowing the fertilizer applied to be retained in the soil and be efficiently absorbed by the crop.

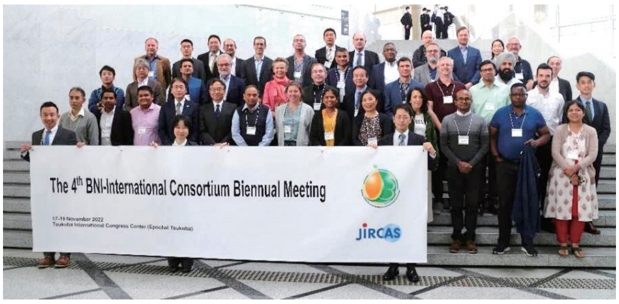


Photo 1. The 4th BNI-International Consortium Biennial Meeting in Tsukuba (17-19 November 2022)
Nearly 60 participants representing 20 countries (both developed and developing) participated and exchanged opinions on BNI research.

Photo 2. Visit of H.E. Francesco Lollobrigida, Minister of Agriculture, Food Sovereignty and Forestry of Italy, to the JIRCAS booth (top) and presentation by Mr. Osamu Koyama, President of JIRCAS, at the G7 Agriculture Ministers’ Meeting in Miyazaki (bottom)

Wheat Improvement Using Genetic Resources of Wild Species —Introduction of BNI traits from wild wheat—

One of the important characteristics of wheat is that it has three genomes, which makes it easy to utilize wild wheat species for wheat breeding. Other major crops such as rice and maize each have only one genome, and the deletion of even one chromosome is lethal to the plant. On the other hand, wheat, with the presence of three similar genes and chromosomes, can avoid lethality even if one of them is lost. Hybridization between cultivar and wild species often changes the number of chromosomes. Diploid species of rice and maize cannot usually tolerate this change, while wheat can overcome the change in chromosome number. Due to this characteristic, wild wheat species have often been used for wheat breeding.

The utilization of wild species offers great potential for wheat improvement. Some wild species can grow in arid areas where cultivated wheat cannot survive (without human assistance), and others can grow on abandoned land with little nutrition. If these characteristics can be introduced into wheat, it may be possible to develop a groundbreaking, never-seen-before wheat variety.

Plant species in the genus *Leymus* are distantly related wild wheat species. These plant species inhabit the waterfront of coast lines and grow extremely vigorously even in sandy beaches with almost no nutrients, and therefore it could be expected that they would have excellent properties in terms of nutrient absorption and utilization (Photo 1).

Not surprisingly, when JIRCAS analyzed the BNI activity of one *Leymus* species, namely *L. racemosus*, we found that the BNI activity was several times higher than that of wheat. To introduce the BNI ability of *L. racemosus* into wheat, we first produced a hybrid between wheat and this species and obtained a set of *L. racemosus* chromosome addition lines of wheat, in which a pair of *L. racemosus* chromosomes were introduced into wheat. Then we further developed chromosome translocation lines in which a half chromosome arm of *L. racemosus* is added into wheat. Among these lines, we found a high BNI activity on the short arm of *L. racemosus* N chromosome (*Lr-N* short arm), revealing the existence of high BNI ability in this part (Photo 2).

Photo 1. A *Leymus* species found near Abashiri, Hokkaido
Although it grows naturally on sandy seashore, it shows vigorous growth (left). The rhizomes of *Leymus* plants are exposed due to erosion caused by waves (right).

To see the effects of BNI on the *Lr-N* short arm, we conducted a low-nitrogen field experiment. The left side of Photo 2 is the line 'BNI-enabled Munal' with *Lr-N* short arm, and the right side is the wheat cultivar 'Munal.' The bottom row is a chromosome diagram, and the only difference between the two is the introduced N short arm segments which are stained pink. Comparing the growth of the plants, the parent wheat variety 'Munal' showed yellowing of the leaves due to nitrogen deficiency and poor growth, while 'BNI-enabled Munal' did not show any of that. The fresh weight and yield of 'BNI-enabled Munal' is much higher than that of the parent product 'Munal,' indicating that plants with BNI traits can grow vigorously even under low nitrogen conditions. The BNI trait on a chromosomal segment of wild wheat, *L. racemosus*, was found to have such a tremendous and surprising effect.

KISHII Masahiro
Biological Resources and Post-harvest Division

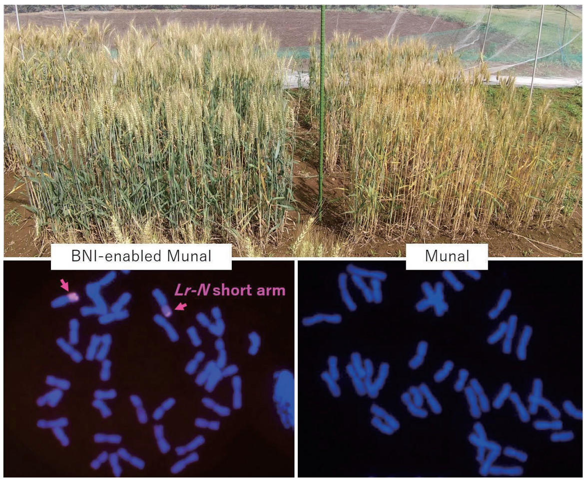


Photo 2. BNI-enabled wheat
BNI-enabled wheat 'Munal' (left) and parent wheat cultivar 'Munal' (right). The bottom row shows chromosome images. In BNI-enabled wheat 'Munal,' the short arm of the N-chromosome (*Lr-N* short arm; pink part) with BNI ability was introduced.
The top row is a field test. Whereas BNI-enabled 'Munal' retains green parts, the parent wheat cultivar 'Munal' turns yellow due to nitrogen deficiency. BNI-enabled 'Munal' also shows much higher yield than the parent wheat cultivar 'Munal.'

Establishment of Nitrogen-efficient Wheat Production Systems in Indo-Gangetic Plains by the Deployment of BNI-technology — A SATREPS Project

Located in the northern part of the Indian subcontinent, the Indo-Gangetic Plains (IGP) is a large plain that stretches 2,000 km from Pakistan through India and Nepal to Bangladesh and covers an area of more than 700,000 square kilometers. The IGP is also known for the success story of the Green Revolution in the 1960s led by Dr. Norman Borlaug, who introduced high-yielding dwarf wheat varieties along with irrigation and nitrogen fertilization. Currently, the IGP is a major producer of wheat, thereby supporting the food security of more than 1.4 billion people in India, which became the world's most populous nation in 2023. Wheat is a crop that requires plenty of fertilizers, and this is the case in India. In the IGP, farmers annually apply 200 kg of N per hectare on average, and more quantity, around 300 kg of N per hectare, is applied in the northwestern states of Punjab and Haryana. However, nitrogen use efficiency (NUE, the rate of nitrogen absorbed/utilized by the crop to the total applied N) is merely 30% or less. Unutilized N is rapidly lost to the environment as nitrate-N, contaminating aquatic ecosystems, and as nitrous oxide (N_2O) gas, contributing to global warming. Moreover, the Government of India has been spending a great deal of money importing urea and subsidizing the cost of chemical fertilizers for wheat farmers since 2005. Therefore, there is a strong need to improve NUE and reduce the amount of fertilizer application in wheat production systems in the IGP.

JIRCAS and CIMMYT (The International Maize and Wheat Improvement Center) have jointly succeeded in cultivating a BNI-enabled wheat line (BNI-Munal) from the original international wheat variety, Munal. The BNI-Munal line has the ability to reduce nitrification potential in rhizosphere soils and shows higher yield and N uptake, thus improving NUE, in a field experiment in Japan as compared with the parent variety, particularly under low-N conditions. With this promising result, JIRCAS launched a SATREPS (Science and Technology Research Partnership for Sustainable Development) project in India in 2022 for the deployment of BNI technology to the IGP with the aim of improving NUE in wheat production systems in the area.

The counterparts on the Indian side are, chiefly, the Borlaug Institute for South Asia (BISA), which is under the umbrella of CIMMYT and has committed profoundly to wheat research and development in India, and three national research institutions, i.e., the Indian Agricultural Research Institute (IARI), the Indian Institute for Wheat and Barley Research (IIWBR), and the Central Soil Salinity Research Institute (CSSRI), which are under the Indian Council of Agricultural Research (ICAR). In this project, we will demonstrate that yield and quality of wheat are maintained even when N application was reduced by 10% to 20% in production systems that adopt BNI technology. The goal of the 5-year project is to propose new wheat production systems with less N load to the environment in the IGP.

To this end, we have started introducing BNI-capacity to Indian elite wheat varieties from different locations in India since last season by crossing with BNI-Munal (and other BNI-enabled international lines) and through selection of backcrossed progenies. We have also started multi-locational field trials in Ludhiana (Punjab), Jabalpur (Madhya Pradesh), Samastipur (Bihar), and the National Capital Territory of Delhi (NCTD), which differ in climate and soil conditions as well as N fertilizer recommendation (Photo). An experiment using lysimeters in India will investigate the temporal and spatial changes of the chemical species of N in the soil, and a new N dynamics model in the soil-plant-air continuum that incorporates the BNI function will be constructed. This will enable us to make a more precise ex-ante evaluation of the broader and longer-term benefits of BNI technology.

BNI technology is simply a combination of transforming conventional varieties into BNI-enabled ones, applying the core technology, and the practice of using less N fertilizers, so it seems that the technology is not highly challenging for farmers. However, BNI is a novel concept and for conservative farmers in particular, there is hesitation that fertilizer reduction would lead to yield drop and quality degradation of their harvest. Apparently, the benefit of BNI technology is difficult to see for farmers, though the benefit to the environment is more visible. Therefore, to promote understanding of BNI technology, we will engage the relevant stakeholders (federal and local governments, fertilizer manufacturers, environmental organizations, and so on) and offer incentives for farmers to adopt BNI technology. It would take more than 5 years for BNI-enabled Indian varieties to be officially released, so their full dissemination in different areas of the IGP could be achieved several years after the end of this project, followed by the realization of the agronomical and environmental benefits of BNI technology. Thus, the overall goal of this project is for BNI technology to be viewed and understood as a valuable and indispensable technology for modern agriculture.

TOBITA Satoshi
Professor, Nihon University
Specially Assigned Investigator, JIRCAS

Photo. Field performance of the international wheat variety "Munal" (left) and the BNI-enabled "BNI-Munal" (right) with no N applied at BISA-Ludhiana, Punjab, one of the sites for the multi-location trials. Photo taken in March 2023.

Life-Cycle Assessment of Greenhouse Gas Reduction Potential of BNI-enabled Wheat

Keeping the increase in global average temperature well below 2°C above pre-industrial levels and pursuing efforts to limit the temperature increase to 1.5°C are the common goals among the 195 countries that, as of February 2023, have joined the Paris Agreement. Japan is aiming to achieve carbon neutrality by 2050, which has been formulated in its long-term low greenhouse gas emission development strategies as requested in the Paris Agreement. On the other hand, global demand for food is expected to increase by 1.7 times in 2050 as compared to 2010. Therefore, it is necessary to establish sustainable food systems that combine high productivity with less greenhouse gas (GHG) emissions and low environmental impact.

To prevent nitrogen (N) pollution while increasing food production, JIRCAS has proposed the use of ammonium through Biological Nitrification Inhibition (BNI). In collaboration with the International Maize and Wheat Improvement Center (CIMMYT), JIRCAS has developed BNI-enabled wheat with a 30% nitrification inhibition rate ("BNI30%"), which increases food production with less N fertilizer use than conventional wheat. Aiming to reach carbon neutrality by 2050, the research team is currently developing BNI-enabled elite wheat varieties with a 40% reduction in nitrification ("BNI40%").

How much GHG emissions could be reduced by BNI-enabled wheat? This was estimated by using the life cycle assessment (LCA) method. This method calculates the life cycle greenhouse gas (LC-GHG) emissions by summing up emissions from agricultural material production to wheat cultivation stages (Fig.1). This method allows the evaluation of fertilizer-induced GHG emissions reduction by BNI-enabled wheat. According to the LCA method, if "BNI30%" is disseminated by 2030, it could reduce LC-GHG emissions by 12.3% and N fertilization by 11.7%, and improve nitrogen use efficiency (NUE) by 12.5% (Fig. 2, 30%). If "BNI40%" is disseminated by 2050, it could reduce LC-GHG emissions by 15.9% and N fertilization by 15.0%, and improve NUE by 16.7% (Fig. 2, 40%). In addition, fertilizer-induced GHG emissions could be reduced by 9.5% across wheat-harvested areas worldwide by 2050 if "BNI40%" is introduced only in areas suitable for BNI wheat. However, GHG emissions could be reduced further as some BNI effects are expected to work even in non-suitable areas.

The development and dissemination of BNI-enabled crops that adapt to various food systems will contribute to mitigating GHG emissions in regions around the world. It will also contribute to the establishment of an agricultural system that combines high productivity with reduced environmental impact from agriculture while being expected to have an effect on mitigating global warming.

LEON Ai
Social Sciences Division

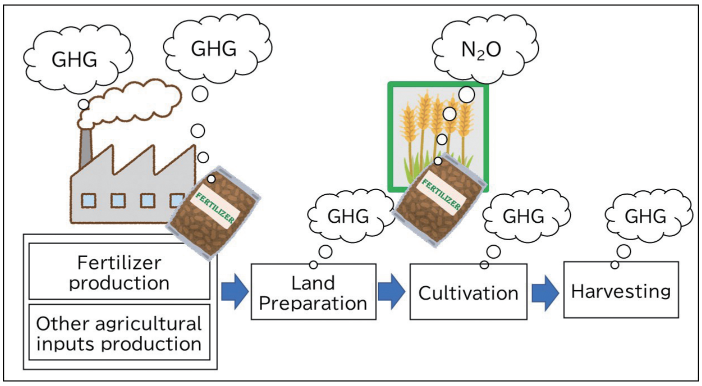


Fig. 1. Life cycle greenhouse gas emissions from BNI-enabled wheat cultivation

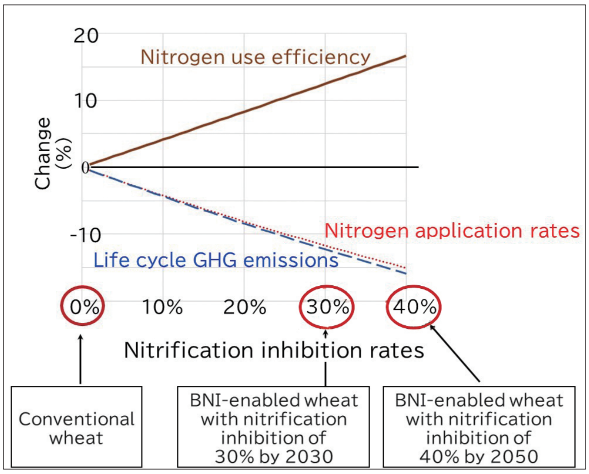


Fig. 2. Changes in life cycle GHG emissions, nitrogen fertilizer application rates, nitrogen use efficiency, and nitrification inhibition rates resulting from the introduction of BNI-enabled wheat

Towards the Use of BNI Technology in Maize, the World's Most Widely Produced Field Crop

Maize, the world's most widely grown field crop with a production volume of 1.03 billion metric tons per year, is of great importance as a food crop and as an industrial raw material for starch, animal feed, etc. However, its nitrogen use efficiency is very low (~50%), so we are conducting research to establish a maize production system that utilizes its biological nitrification inhibition (BNI) function. To this end, it is necessary to elucidate the BNI compounds released from the roots of maize in order to gain a foothold for BNI utilization and develop BNI-enabled maize for the production system.

Root-secreted compounds are classified as hydrophobic or hydrophilic based on their solubility in water. Hydrophobic compounds are less soluble in water and tend to remain in the rhizosphere, whereas hydrophilic compounds are soluble in water and disperse more widely in the soil to exert their effects. We collected hydrophobic and hydrophilic exudates released from maize roots to detect BNI compounds, using BNI activity (the ability of nitrifying bacteria to inhibit nitrification) as an indicator, and undertook the painstaking task of isolating and identifying each BNI compound one by one from root exudates containing a variety of compounds. As a result, we identified "zeanone" and "HDMBOA" as hydrophobic BNI compounds and "MBOA" as a hydrophilic BNI compound. Among them, zeanone was the first to be found in nature. The strength of BNI activity among the three compounds, from strongest to weakest, was found to be in the following order: MBOA > zeanone > HDMBOA. Two weak BNI compounds, HMBOA and HDMBOA-β-glucoside, were also identified from the root tissue.

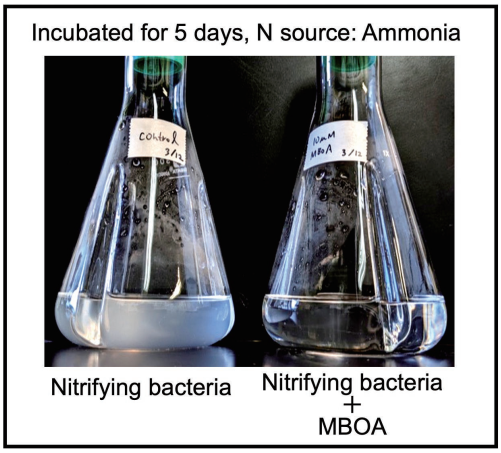


Photo. Effect of MBOA on the growth of nitrifying bacteria
Liquid medium clouded white by proliferating bacteria (left flask) and liquid medium remaining clear after strong growth inhibition in the presence of MBOA (right flask).

Subsequent functional analysis of MBOA revealed that MBOA strongly inhibits nitrification and the growth of nitrifying bacteria (Photo), that the addition of MBOA inhibits nitrification in soil, and that the nitrification inhibitory activity of MBOA in soil decreases with time as MBOA is degraded by soil microbes.

From the above, the mechanism of BNI expression in maize can be considered as shown in Figure 1. The hydrophobic BNI compounds, zeanone and HDMBOA, released from the maize root surface can inhibit nitrification in the rhizosphere, while the hydrophilic BNI compound, MBOA, can inhibit nitrification over a wider area of the soil. HDMBOA produced at the root surface is converted to MBOA with more potent BNI activity in the soil. In addition, HDMBOA-β-glucoside accumulated inside the roots is also converted by glycolytic enzymes to HDMBOA with high BNI activity and then spontaneously becomes MBOA. The MBOA produced is degraded by soil microorganisms, but is constantly supplied by maize roots, thus maintaining BNI activity. From the above, it is clear that MBOA is a key compound for BNI in maize.

We will use our findings to develop BNI-enabled maize suitable for actual agricultural use and promote the development of a sustainable maize production system with reduced environmental impact by comparing the amounts of BNI compounds among genetic stocks, clarifying the secretion mechanism of BNI compounds, and conducting field trials.

OTAKA Junnosuke
Biological Resources and Post-harvest Division

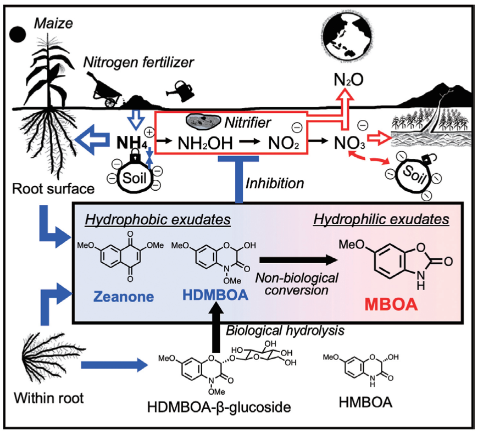


Fig. 1. Plausible BNI mechanism in maize
Maize can release three BNI-active compounds both in hydrophobic and hydrophilic exudates. HDMBOA and HDMBOA-β-glucoside in the root tissue are eventually converted to MBOA in the soil; therefore, MBOA is the key BNI compound in maize.

JIRCAS TODAY

【Research Highlights】

Increased Rice Yield Improves Nutrition of Farmers in Madagascar —Towards solving malnutrition problem in Africa by improving productivity of staple crops—

JIRCAS, in collaboration with the University of Tokyo and the National Office of Nutrition of Madagascar, has used econometric methods to show that increasing the yield of rice, the main staple food in Madagascar, is effective in improving farmers' nutrition in rural areas where serious nutrition problems exist.

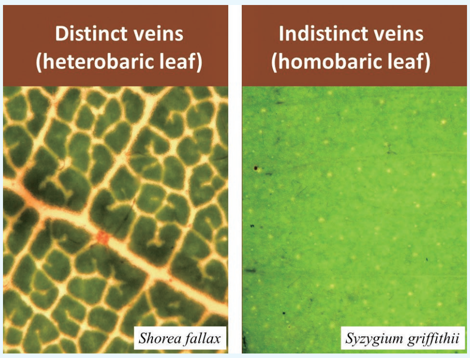
The analysis in this study showed that increased rice yield resulted not only in increased rice consumption but also in increased purchase of nutritious foods such as vegetables, fruits, and meat/fish due to the cash income from rice sales. It was suggested that the diversification of foods consumed through these purchase behaviors contributes to improved nutrition, not only in terms of energy supply, but also in terms of the supply of micronutrients such as vitamin A, zinc and iron, i.e., in both quantity and quality.

Until now, there have been limited studies evaluating the impact of increased productivity of staple food crops on farmers' nutritional supply in rural areas of sub-Saharan Africa. This study found that technological interventions to improve paddy rice productivity can lead to diversification of purchasing behavior through consumption and markets, leading to improved nutrition for poor farmers, and is expected to contribute to Goal 2 of the Sustainable Development Goals (SDGs), "Zero Hunger."

Elucidating the Leaf Vein Structure and Function of Tropical Rainforest Trees —Understanding leaf vein structure contributes to the selection of trees with high adaptability to the environment—

JIRCAS has been evaluating the adaptive capacity of tropical forest trees to the environment and developing afforestation technology to enhance forestry productivity and environmental adaptability through the combination of tree species suitable for the plantation environment. In collaboration with Kochi University and the Sarawak Forest Department of Malaysia, JIRCAS has now revealed that the leaf vein structure of trees in Malaysian tropical rainforest is closely related to leaf toughness and photosynthetic capacity.

Plant eaters such as insects and herbivores, and light intensity increase from the ground toward the forest canopy. Therefore, trees need to have sturdier leaves as they grow taller, and higher photosynthetic capacity is more advantageous. Leaves are classified by species into two types: leaves with transparent fibrous tissue (bundle sheath extensions) around the vascular bundles that clearly show the veins when the leaf is exposed to light (heterobaric leaves), and leaves without this tissue, in which the veins are difficult to see (homobaric leaves). Heterobaric leaves have fibrous tissue, which makes them sturdier, but also allows more light to penetrate into the leaf and increase the photosynthetic capacity of the leaf as a whole. In other words, trees with heterobaric leaves have both high photosynthesis and leaf defense, which is advantageous in bright, high-coverage environments such as forest canopies. On the other hand, homobaric leaves, with their gapless arrangement of chloroplast-bearing cells instead of fibrous tissue, are able to utilize weak light more efficiently, which is advantageous in darker environments near the forest ground surface.



In order to enhance environmental adaptability in afforestation, it is important to select tree species that are suitable for the environment of the plantation site. Understanding of the leaf vein structure is expected to enable the selection of tree species suitable for the environment among diverse tropical rainforest tree species and the implementation of afforestation that considers the functional characteristics of the tree species.

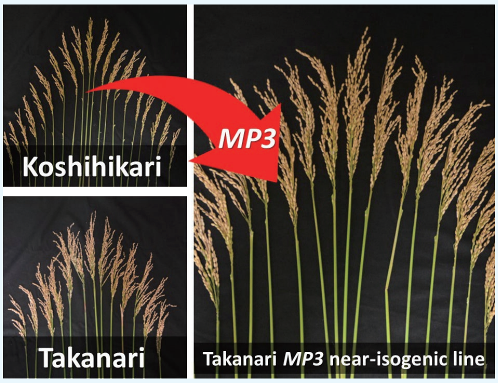
JIRCAS TODAY

【Research Highlights】

Discovery of Koshihikari-derived Gene that Increases Rice Yield in High CO_2 Environment —Contributing to sustainable rice cultivation under climate change—

A joint research group consisting of researchers from JIRCAS, National Agriculture and Food Research Organization (NARO), Nagoya University, Yokohama City University, RIKEN, Meiji University, and Kazusa DNA Research Institute has identified *MP3* (*MORE PANICLES 3*), a gene in 'Koshihikari' rice cultivar that promotes the growth of axillary buds that form the base of rice panicles and increases the number of panicles.

The *MP3* gene sequence (genotype) differs among rice cultivars, and some Japanese rice cultivars, such as 'Koshihikari,' have a genotype that increases the number of panicles, which is not found in indica rice cultivars overseas. Since the high-yielding Japanese rice cultivar 'Takanari' has *MP3* of the indica type, we developed a near-isogenic rice with the Koshihikari-derived *MP3*, which increased the number of panicles by 20 to 30%. Furthermore, in a paddy field test that reproduced high atmospheric CO_2 levels expected in the future, it was found that the developed rice increased its yield by 6% compared to Takanari.



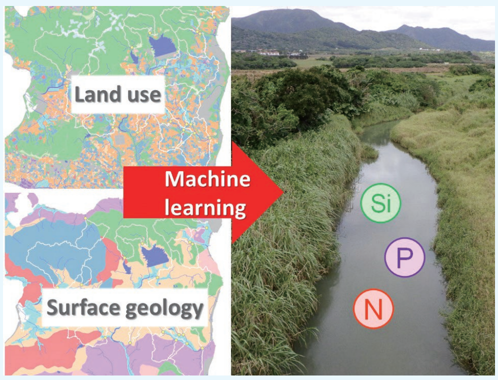
With the ongoing global climate change, the development of technologies to realize sustainable crop production is an urgent issue, and rice breeding strategy using *MP3* is one of such technologies expected to contribute to the stable production of rice under high CO_2 conditions in the future.

Machine Learning Predicts Nutrient Concentrations in Tropical Rivers —Implications for coastal ecosystem conservation efforts—

JIRCAS has created a new model to accurately predict the concentration of nutrients (nitrogen, phosphorus, and silicon) in river water on Ishigaki Island, Okinawa Prefecture, using a machine learning method.

This model predicts riverine nutrient concentrations based on the watershed characteristics such as land use and surface geology using the Random Forest machine learning algorithm. It can also identify the watershed characteristics that have significant impacts on each nutrient. While most of the conventional simulation models target nitrogen and phosphorus, this model can predict the concentration of silicon as well, making it possible to assess the risk of coral reef decline due to excessive nitrogen and phosphorus loads and that of harmful algal blooms such as dinoflagellates due to reduced silicon.

While estimation and prediction of terrestrial nutrient loadings usually rely on the efforts and expertise in conducting hydrologic and water quality monitoring and operating mathematical simulation models, this model makes it possible to predict nutrient concentrations with relatively simple operations provided that data on land use, surface geology and population density in the target watersheds are available.



As well as the changes in marine environment due to climate change, there are growing concerns about the adverse effects on coral reefs and other coastal ecosystems around tropical islands caused by excessive nutrient inputs from lands. The results of this study are expected to aid in developing policies to conserve healthy coastal ecosystems by appropriately controlling terrestrial nutrient loadings.

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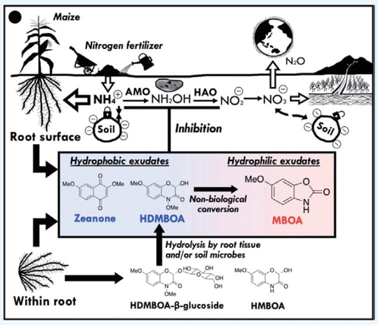
【Research Highlights】

Successful Identification of Key Compound for Biological Nitrification Inhibition in Maize
—Progress in developing BNI-enabled maize to reduce the use of nitrogen fertilizer—

JIRCAS, in collaboration with the National Agriculture and Food Research Organization (NARO), has succeeded in identifying MBOA (6-methoxy-2(3H)-benzoxazolone), a water-soluble compound produced by maize roots that is the key to biological nitrification inhibition (BNI).

In modern agriculture, industrially produced ammonia nitrogen fertilizers are applied to farmland in large quantities and are converted by soil microorganisms (nitrifying bacteria) through nitrification to nitrate nitrogen, causing various problems such as water pollution and greenhouse gas emissions.

MBOA is known to be a secondary metabolite produced by certain grass species (maize and wheat). Incubation experiments and chemical analyses with soil and root exudates showed that MBOA inhibits nitrification and the growth of nitrifying bacteria. Furthermore, it was also found that HDMBOA, a hydrophobic BNI compound, is chemically converted into MBOA with more stable and potent BNI activity in soil and exhibits BNI.



Using MBOA, an important indicator of BNI expression, to enhance the BNI capacity of maize, the world's most widely produced field crop (1.03 billion tons produced, compared with 0.74 billion tons for wheat), will reduce nitrogen fertilizer runoff and environmental pollution, thereby improving the global nitrogen cycle. In this way, we will increase the productivity and sustainability of agriculture and contribute to the promotion of the Strategy for Sustainable Food Systems, MIDORI.

P-dipping Rice Fertilizer Application Technique is Effective in Avoiding Flood Damage
—Contributes to stable and sustainable rice production in Sub-Saharan Africa—

The effect of P-dipping, a localized fertilizer application technique for lowland rice production, was tested in farmers' fields under different climatic and topographical conditions in Madagascar, in collaboration with the National Center for Applied Research on Rural Development (FOFIFA) of Madagascar and the Radio-isotope Laboratory of the University of Antananarivo. The results showed that the technology not only significantly improved fertilizer use efficiency, but was also effective in avoiding damage due to flooding that occurs in the early stages of growth.

Rising international fertilizer prices and more frequent extreme weather events associated with climate change are making agricultural production more difficult for poor farmers with low purchasing power and weak production infrastructure, including those in Madagascar.

The results of this study show that P-dipping has the potential to improve fertilizer use efficiency and help farmers cope with frequent flooding. The technology is expected to contribute to stable and sustainable rice production in sub-Saharan Africa as its use expands.

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