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BNI-enabled Wheat in Nepal Toward Social Implementation

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Table of Contents

Abstract.....	iv
1: Preface.....	1
2: What is BNI-enabled Wheat?	3
3: Wheat Production and Its Challenges in Nepal	7
4: Significance of Introducing BNI-enabled Wheat in Nepal	9
5: Multi-location Trials of BNI-enabled Wheat in Nepal.....	10
i. Experimental Sites	10
ii. Method	11
iii. Results.....	12
6: Leveraging Activities in Nepal through an International Network.....	15
7: Toward the Introduction of BNI-enabled Wheat in Nepal	18
Author contribution statement	20
Acknowledgements	20
References.....	20

Abstract

JIRCAS developed BNI (Biological Nitrification Inhibition)-enabled wheat to enhance nitrogen use efficiency and reduce environmental impact. BNI suppresses soil nitrification, reducing nitrate leaching and nitrous oxide emissions, offering a sustainable solution to nitrogen-related agricultural challenges. Wheat is vital in Nepal, especially in the Terai (a lowland region), but yields remain low due to limited fertilizer access and reliance on imports. Fertilizer shortages and high costs prevent farmers from achieving optimal yields. BNI-enabled wheat helps narrow the yield gap by improving nitrogen use efficiency, making it suitable for Nepal's conditions. JIRCAS, in collaboration with local institutions, is testing BNI varieties adapted to Nepal's agro-ecological zones, aiming to replace existing CIMMYT lines. This initiative supports strengthening food security, reduces environmental burden, and promotes sustainable wheat production. Initial trials in Nepal used "Munal," but issues with yellow rust shifted the focus to the Terai region, where "Borlaug 2020" was introduced and its BNI-enabled version tested. Nepal plans to adopt and eventually breed its own BNI varieties, supported by international collaboration and research.

1: Preface

Modern agriculture has met the growing demand for food-driven by rapid population growth, through the "Green Revolution," which combined industrial nitrogen fixation technologies with high-yielding varieties and chemical fertilizers. However, this approach has drastically altered the global nitrogen cycle, surpassing ecological thresholds (Rockström et al. 2009). It is estimated that approximately half of the nitrogen found in the human body now originates from industrial sources (Tian et al. 2020). Notably, the portion of applied nitrogen that is not absorbed by crops contributes to serious environmental and health issues, including water pollution from nitrate nitrogen and the emission of nitrous oxide, a greenhouse gas roughly 300 times more potent than carbon dioxide (Fig. 1).

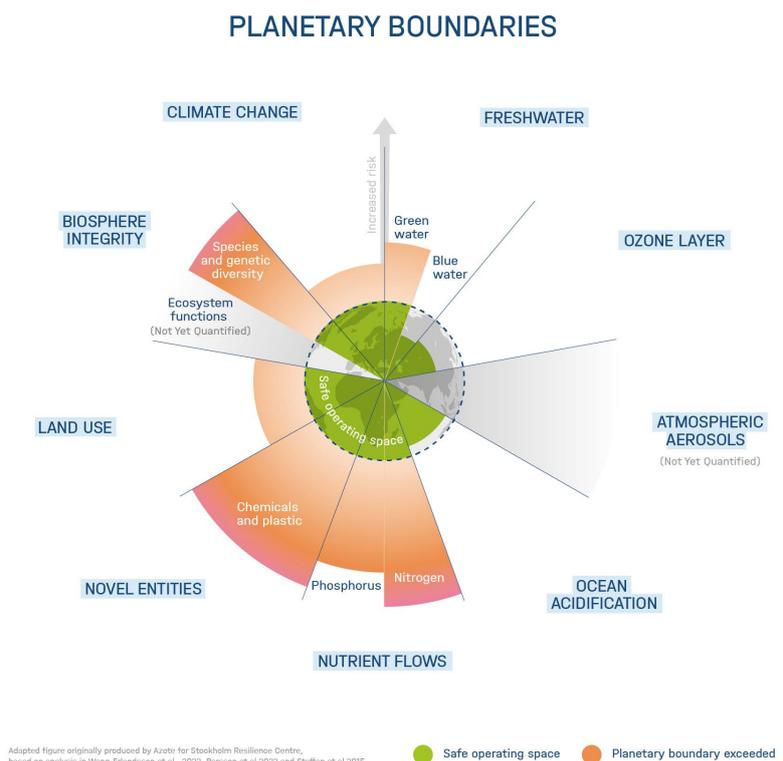


Figure 1. Planetary boundaries (the 2022 report by the Stockholm Resilience Centre)

In response to these challenges, the Japan International Research Center for Agricultural Sciences (JIRCAS) successfully developed a BNI-enabled wheat variety

based on the international cultivar "Munal" for South Asia, and confirmed its effectiveness through trials conducted in Tsukuba, Japan. BNI (Biological Nitrification Inhibition) is a technology that enhances nitrogen use efficiency and reduces environmental impact by suppressing the activity of nitrifying bacteria in the soil through compounds secreted by crop roots, thereby promoting the retention of ammonium nitrogen.

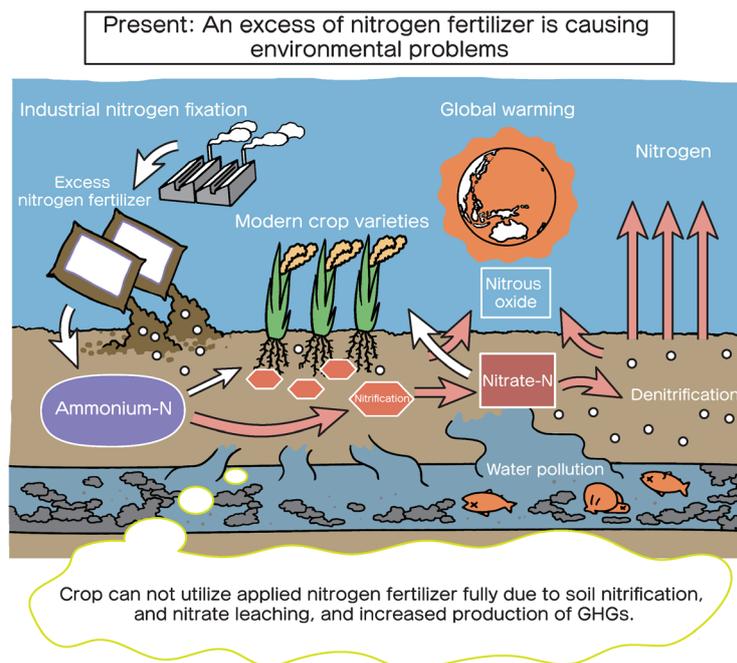
The parent variety "Munal" was selected for its resistance to wheat black stem rust (Ug99) and was introduced in several South Asian countries during the 2010s. In particular, "Munal" has a history of cultivation in Nepal. Nepal consists of two major agro-ecological zones: the mountainous region and the Terai plains. While Nepal's main wheat-producing region is the Terai, "Munal" has been cultivated in mountainous regions. Given these backgrounds, the availability of new BNI-enabled variety as a prototype, BNI-enabled Munal was deemed suitable for local field trials in Nepal. While the main wheat-producing region in Nepal is the Terai, which differs in environmental conditions and cultivated varieties from the mountainous areas, the initial objective under the Green Asia project was to verify the effectiveness of BNI technology. However, yellow rust remains a serious issue in mountainous regions, and "Munal" has recently begun to be considered insufficiently resistant to it due to new races in certain region.

Furthermore, with a view toward the practical implementation of BNI-enabled wheat, the potential for variety development and introduction in the Terai region was explored. As the Green Asia Project progressed, the current state of wheat production across Nepal was assessed. It became evident that, compared to the mountainous areas, where production and impact are limited, the introduction of BNI-enabled varieties in the Terai region would make a more significant contribution to Nepal's food security.

In 2020, CIMMYT's international variety "Borlaug 100" was introduced to the Terai region under the local name "Borlaug 2020." After temporary disruptions caused by the COVID-19 pandemic, it is now being promoted to farmers as a recommended variety. In response, multi-location trials using BNI-enabled Borlaug 100, a 1st generation BNI-enabled wheat variety, were conducted across Nepal under the Green Asia project, with the aim of replacing "Borlaug 2020" with its BNI-enabled counterpart.

2: What is BNI-enabled Wheat?

Over the past 50 years, the amount of nitrogen fertilizer applied to farmland has increased approximately tenfold, while food production has only tripled (Ray et al., 2013). This imbalance has led to serious environmental consequences. In soil, nitrifying bacteria convert ammonium nitrogen from fertilizers into nitrate nitrogen. However, nitrate nitrogen is poorly retained in soil and easily leaches into aquatic systems, causing eutrophication and further converting into nitrous oxide (N_2O), a potent greenhouse gas approximately 300 times more powerful than carbon dioxide. Due to anthropogenic nitrogen pollution, the global nitrogen cycle has already exceeded its safe operating limits and is now in a high-risk state (Rockström et al. 2009). Nevertheless, as global food demand continues to rise, the introduction of sustainable solutions is urgently needed (Fig. 2).

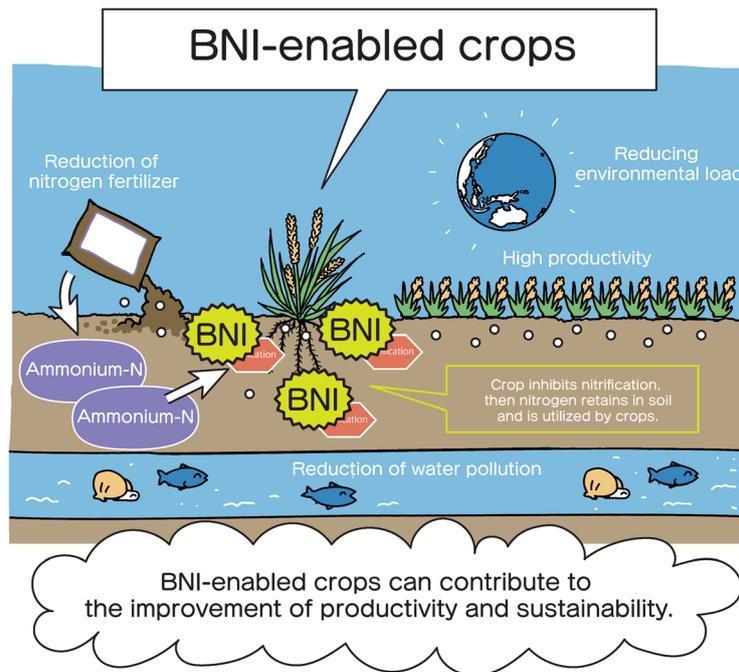


Science Manga Studio (2021)

Figure 2. An excess of nitrogen fertilizer is causing environmental problems

In 1983, researchers at the International Center for Tropical Agriculture (CIAT) observed that tropical grasses in the Poaceae family maintained extremely low levels of nitrate nitrogen in the soil. This observation led to the discovery of BNI, a natural

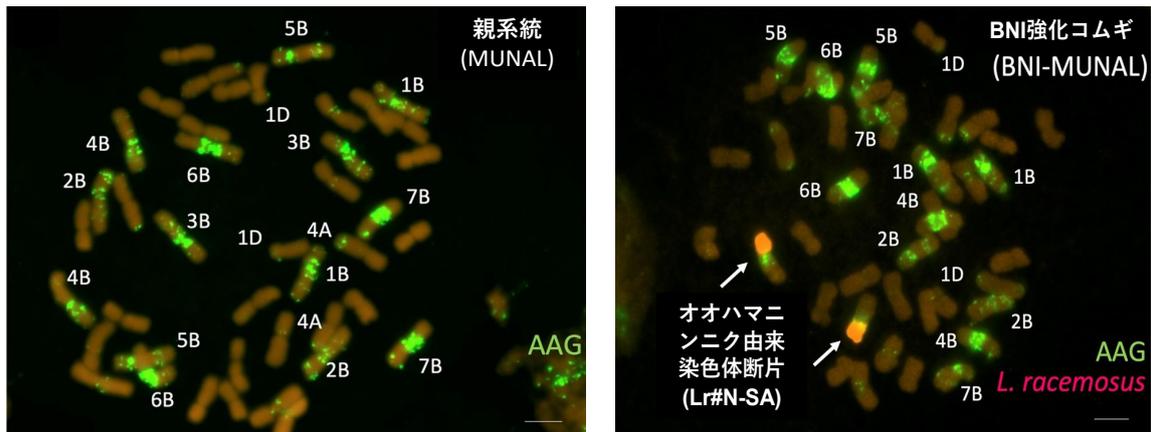
mechanism by which plants suppress the activity of nitrifying bacteria in the soil, thereby reducing nitrification. By inhibiting the formation of nitrate nitrogen, BNI enhances nitrogen use efficiency and reduces the environmental burden of agriculture (Fig. 3).



Science Manga Studio (2021)

Figure 3. BNI-enabled crops

Focusing on wheat, a crop highly dependent on nitrogen fertilization, researchers investigated the genetic resources of the International Maize and Wheat Improvement Center (CIMMYT) but found no lines with high BNI capacity (Subbarao et al., 2007). However, strong BNI activity was identified in *Leymus racemosus*, a wild relative of wheat. This BNI trait was found to originate from the short arm of the N chromosome (Lr#N-SA). Through conventional breeding, this chromosome segment was substituted for the short arm of wheat chromosome 3B. As a result, BNI activity nearly doubled, increasing from 92.7 to 181.7 ATU (Allylthiourea Equivalent Unit) per gram of root dry matter per day (Fig. 4; Subbarao et al., 2021).



**Figure 4. Karyogram of BNI-enabled wheat
A chromosome segment derived from *Leymus racemosus* (Lr#N-SA) has been translocated into the parental line**

Field trials in Tsukuba, Japan demonstrated that BNI-enabled wheat significantly suppressed the activity of nitrifying bacteria in the rhizosphere soil, reducing nitrification by approximately 30% and N₂O emissions by about 25%. These results indicate that BNI technology has the potential to serve as a climate change mitigation strategy by reducing the environmental impact of agriculture while maintaining or improving productivity (Fig. 5 & 6).



**Figure 5. BNI-enabled wheat (BNI-Munal) at the grain-filling stage in Tsukuba experimental field
While the parental line shows signs of nitrogen deficiency, the BNI-enabled wheat remains green.**

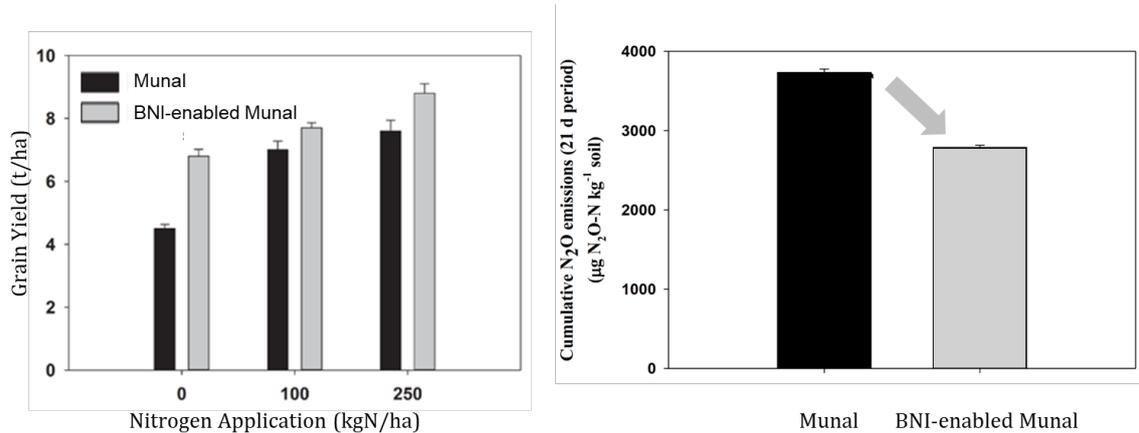


Figure 6. Results from field trial of BNI-enabled wheat
BNI-enabled wheat achieved the same yield as the parental line with 60% less nitrogen fertilizer, while reducing N₂O emission by 25%.

It has been confirmed through research that the BNI trait is expressed by the short arm of the *Leymus racemosus* N chromosome (Lr#N-SA), and the wheat line in which this segment has been substituted for chromosome 3B is the first-generation BNI-enabled wheat currently undergoing field trials. The Lr#N-SA is genetically stable and follows Mendelian inheritance, making it possible to introduce the BNI trait into various wheat varieties through breeding. Efforts are currently underway to incorporate BNI capacity into elite wheat varieties in India and Japan through crossbreeding.

BNI-enabled wheat improves nitrogen use efficiency by suppressing soil nitrification, reducing nitrate leaching and nitrous oxide emissions. Developed using a wild wheat relative, it nearly doubles BNI activity. Field trials show 30% less nitrification and 25% lower emissions, offering a sustainable solution to agriculture's environmental challenges.

3: Wheat Production and Its Challenges in Nepal

Although rice is the staple food in Nepal, wheat is the second most important cereal crop. In particular, in the Terai region, which accounts for more than half of Nepal's agricultural production, wheat plays a highly significant role. As Nepal relies heavily on food imports, wheat production within the rice–wheat cropping system contributes approximately 2.3% to the national GDP and is vital to the livelihoods of many people.

The agricultural conditions in Nepal's Terai region are very similar to those in northeastern India. The soil belongs to the Indo-Gangetic Plain, and the environmental conditions are comparable. However, the average wheat yield in Nepal remains lower than in India: 3.0 t/ha in Nepal compared to 4.0-5.0 t/ha in Panjab, India (FAOSTAT, 2023).

To meet domestic demand, Nepal is forced to import 260,000 tons of wheat annually, amounting to 69 million USD (FAOSTAT, 2023). With population growth and a dietary shift toward wheat-based foods, demand is expected to increase further.

To respond to this rapid rise in demand, it has been suggested that Nepal must increase its domestic wheat yield to 3.9 t/ha by 2030. Achieving this goal requires narrowing the yield gap through scientific approaches, while also improving farmers' profitability and reducing dependence on imported agricultural inputs.

Although the Nepalese government provides subsidies for fertilizers, the country lacks domestic facilities for industrial nitrogen fixation. As a result, all fertilizers must be imported, making Nepal highly vulnerable to international market fluctuations of fertilizers and exchange rate volatility. Being a landlocked country without seaports, Nepal faces competition with India, where fertilizer demand peaks at similar times, making it difficult for farmers to obtain necessary fertilizers (especially urea) when needed. Fertilizers not covered by government subsidies are extremely expensive, leading to low input levels at the farm level and preventing wheat varieties from reaching their full yield potential.

A report based on field trials and model analysis (Devkota et al., 2024) identified the

gap between recommended fertilizer application standards and actual farmer practices as a major limiting factor. Addressing this gap is expected to improve both wheat productivity and farmer profitability. Increasing government subsidies and introducing varieties with higher nitrogen use efficiency, such as BNI-enabled wheat, could significantly narrow the yield gap and potentially transform wheat production in Nepal.

4: Significance of Introducing BNI-enabled Wheat in Nepal

BNI-enabled wheat improves nitrogen use efficiency by suppressing soil nitrification, thereby extending the retention period of ammonium nitrogen in the soil. This allows for reduced nitrogen fertilizer application while maintaining equivalent yield levels. As a result, agricultural environmental burdens, such as nitrous oxide (N₂O) emissions and nitrate nitrogen leaching into ecosystems, can be significantly reduced. In Nepal, however, narrowing the yield gap caused by fertilizer application levels below recommended standards remains a major challenge, and improving nitrogen use efficiency is urgently needed.

The BNI-enabled wheat developed by JIRCAS is based on international varieties bred by CIMMYT and is currently being tested as a prototype, with data being accumulated in Japan. However, most trials have been conducted only in Mexico, where CIMMYT is located, and in Japan, where JIRCAS is based. Despite the fact that these international varieties have already been introduced in Nepal, field trials had not yet been conducted there.

Under the Green Asia Project, efforts have been underway to replace CIMMYT's international varieties with BNI-enabled wheat in Nepal. This initiative is being carried out as a collaborative research project with the Nepal Wheat Research Program (NWRP), the Borlaug Institute for South Asia (BISA) Nepal Office, and the Nepal Agricultural Research Council (NARC), which are responsible for public wheat breeding in Nepal.

Nepal consists of two major agro-ecological zones: the mountainous region and the Terai plains. These regions require wheat varieties with significantly different traits. Therefore, BNI-enabled Munal, developed by JIRCAS, was designated for the mountainous region, while BNI-enabled Borlaug 100 (locally known as Borlaug 2020) was designated for the Terai region. The nitrogen response characteristics of each parent variety were examined to determine whether improvements in nitrogen use efficiency could be effectively utilized in each zone.

5: Multi-location Trials of BNI-enabled Wheat in Nepal

In Nepal, multi-location trials of BNI-enabled wheat have been conducted to evaluate its adaptability and effectiveness under diverse agro-ecological conditions. These trials aim to assess the performance of BNI-enabled wheat varieties in terms of yield, nitrogen-use efficiency, and soil health improvement across different regions, particularly in the Terai plains, where wheat is a major crop.

The trials are being carried out in collaboration with local research institutions such as the Nepal Agricultural Research Council (NARC) and the National Wheat Research Program (NWRP), with support from the Borlaug Institute for South Asia (BISA) and JIRCAS. Through these trials, researchers are collecting data on agronomic traits, environmental impact, and farmer acceptance, which will contribute to the development of sustainable wheat production systems in Nepal.

The results from these trials are expected to inform future scaling-up strategies and regional adaptation plans. They also serve as a foundation for international collaboration, particularly with institutions in South Asia working on climate-smart agriculture and sustainable intensification.

i. Experimental Sites

BNI-wheat trials were conducted at six different locations in Nepal: three in the mountainous region (Khumaltar near Kathmandu, Surkhet, and Doti) and three in the Terai region (Bhairahawa, Nepalgunj, and Dhanusha) (Fig. 7). The number and selection of field stations were designed not only to represent the two major wheat-growing regions of Nepal (mountainous and plain) but also to capture the country's diverse climatic zones, spanning from west to east and across a wide range of altitudes—from approximately 93 meters in Dhanusha to 1320 meters in Khumaltar. Altitude plays an important role in rust disease infestation, as higher elevations with cooler temperatures during the growing season tend to experience more severe yellow rust outbreaks.

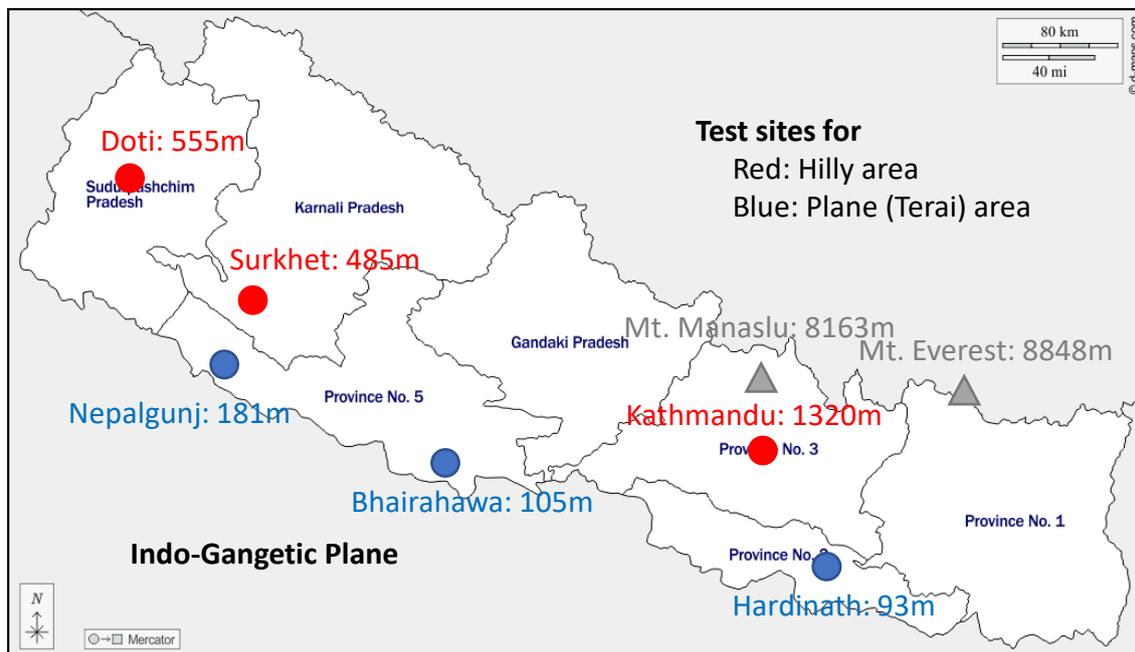


Figure 7. Field test sites of BNI-enabled wheat (Red dots show mountainous region, and Blue dots show Terai region)

ii. Method

The plot size for the field tests was 6 m² (six rows of 4 meters) at Khumaltar and 4.5 m² (six rows of 3 meters) at the other five sites, each a local standard pot size. The BNI-enabled wheat variety 'BNI-Munal' and its parental line 'Munal' were tested during the 2022-23 crop season. Three nitrogen input levels were applied-100%, 50%, and 0% of the recommended amount-with urea used as the nitrogen source.

Harvesting began in mid-April 2023, starting with the Terai region sites. Due to the different climatic zones of the six field stations, harvest times varied by location. The final harvest took place at Khumaltar at the end of May. Post-harvest procedures at all six sites were completed by June 24.

In the 2023-24 crop season, BNI-Borlaug 2020 was introduced following discussions with JIRCAS scientists. This variety is the BC₉ generation of BNI-Borlaug 100, derived from the parental line Borlaug 100, and was officially released as Borlaug 2020. BNI-Borlaug 2020, sourced from CIMMYT, Mexico, was tested using the same methodology as BNI-Munal in the previous season. However, due to limited availability of BNI-Borlaug 2020 BC₉ seeds, it was introduced only at Bhairahawa, a

representative Terai agro-climatic zone. Since Borlaug 2020 was introduced exclusively in the Terai region, BNI-Borlaug 2020 was also limited to that region for comparison.

For the 2024-25 crop season, BNI-Munal and Munal were tested in the mountainous region, while BNI-Borlaug 2020 and Borlaug 2020 were tested in the Terai region, with the aim of promoting their social implementation.

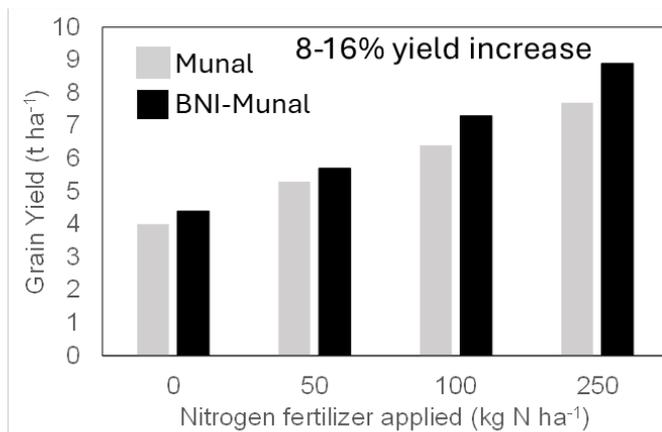
For the BNI field trials, ammonium sulfate was recommended as the nitrogen fertilizer, and pesticide application was discouraged during the crop season to avoid disrupting soil microbial communities. However, due to limited availability, urea-which may increase soil pH-was used instead. Additionally, conventional practices were applied to manage pest infestations during the trials.

iii. Results

The yield data of BNI-Munal and Munal were obtained in 2022-2023 crop season (Fig. 8). Clearly, we observed better yield performance in all nitrogen conditions (0, 50, 100 and 250 kg N/ha) in Doti in mountainous region (up to 16%) and in Bhairahawa in Terai region (up to 19%). Kathmandu (mountainous region) had serious yellow rust outbreak, and we could not obtain meaningful data. It is interesting that we could see higher yield in Bhairahawa where the soil pH of is 8.5. According to the BNI hydroponic tests in JIRCAS, BNI activities became zero in pH8.0. Since soil pH condition is very complex with various micro-soil pH compared to uniform hydroponic condition, BNI could have effects at higher pH in soil than that in hydroponic tests. The 19% yield increase even in Doti (mountainous region) indicates that BNI wheat will be very promising in the entire Nepal, not only mountainous regions where the soil is slightly acidic but also in Terai regions where is the country's main wheat production area and the soil is slightly alkaline.



Doti, Sudurpaschim (Hilly)
Soil pH6.0



Bhairahawa, Lumbini (Terai)
Soil pH8.5

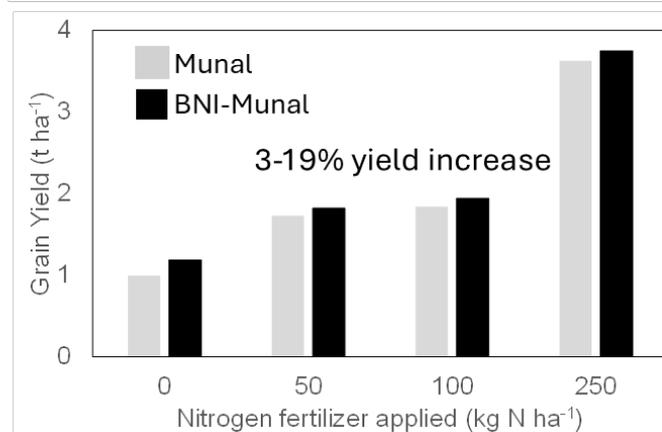


Figure 8. Field trial and yield data of BNI-MUNAL and MUNAL taken in FY22-23 cycle

In 2023-2024 crop season, we became ready to test actual wheat cultivar of Terai region, BNI-Borlaug 2020 and Borlaug 2020 (Fig. 9). Same as the results of 2022-2023 crop season, BNI-Borlaug 2020 showed better yield performance in all nitrogen conditions we observed (0, 50, 100 and 250 kg N/ha). The maximum percentage in yield increase of BNI-Borlaug 2020 over Borlaug 2020 was 11% which is less than that of BNI-Munal (2022-2023 crop season). It may be due to the grain yield at all of nitrogen conditions that were higher in BNI-Borlaug 2020 than BNI-Munal (2024-2025 crop season, Fig. 10).

In March 2024, this trial was witnessed by many visitors during the first stakeholder meeting for BNI wheat in Nepal. Personnels from Nepali seed companies were very interested in BNI-Borlaug 2020 as replace of Borlaug 2020.

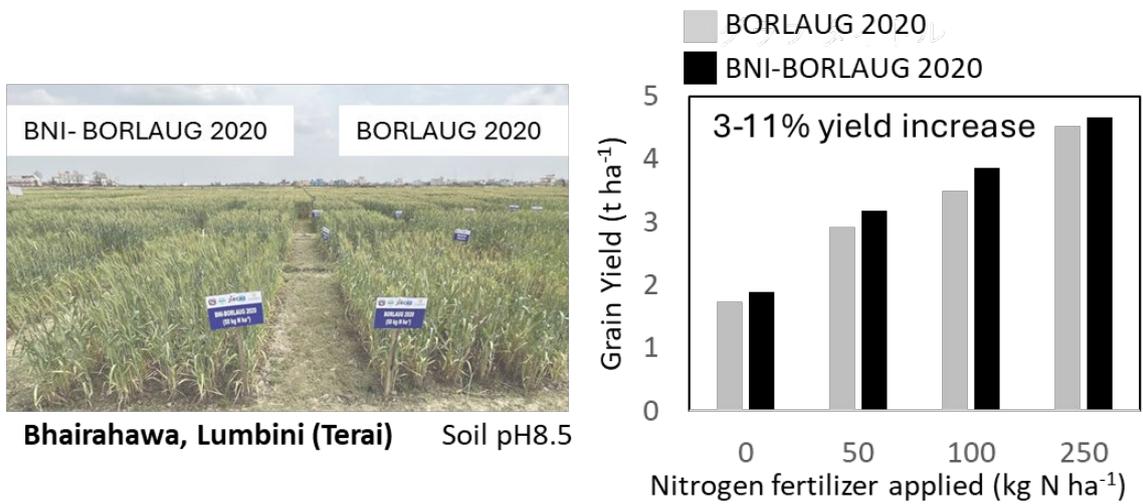


Figure 9. Field trial and yield data of BNI-BORLAUG 2020 and BORLAUG 2020 taken in 23-24' cycle

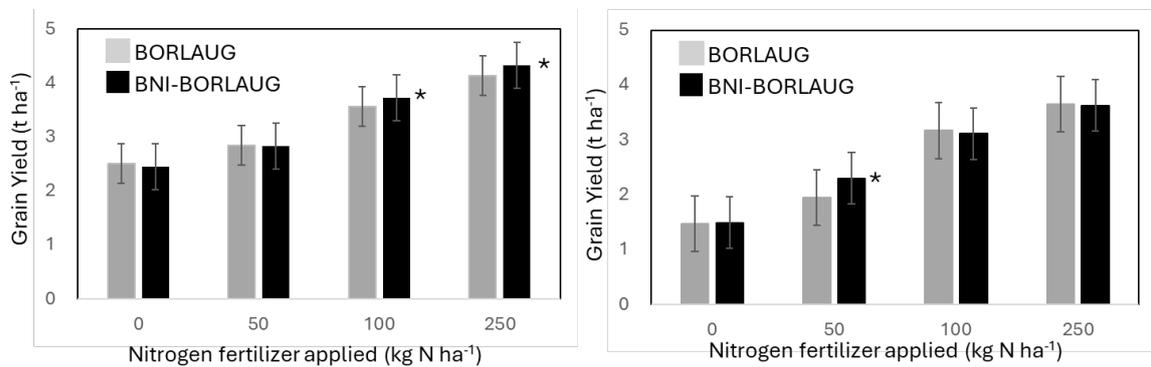


Figure 10. Field trial and yield data of BNI-BORLAUG and BORLAUG taken in 24-25' cycle at Bhairahawa (left) and Nepalgunj (right)

6: Leveraging Activities in Nepal through an International Network

In addition to its activities in Nepal under the Green Asia project, JIRCAS has been implementing initiatives aimed at social implementation, including efforts under the SATREPS program targeting the Indo-Gangetic Plain in India and the introduction of BNI-enabled wheat in Japan. The Indo-Gangetic Plain, which spans the Ganges River basin, is a key breadbasket for India's food production. Since Nepal's Terai region shares similar agro-ecological characteristics, collaboration is anticipated with BISA which is actively working to improve food production in this region.

In 2023, two representatives from Nepal (Dr. Mahesh Subedi from NARC and Dr. Khem Raj Pant from NWRP) were invited to participate in the joint steering committee meeting for the SATREPS project, where they contributed to discussions and shared insights on the introduction of BNI-enabled wheat and lessons learned. Additionally, the interim results of field trials in Nepal and activities of the Green Asia project were presented at COP28 in the UAE during the Tech & Innovation Hub's knowledge-sharing session titled *"Accelerating Application of Climate-Smart and Resilient Agricultural Technologies within Asia-Monsoon Countries,"* together with Dr. Khem Raj Pant from NWRP (Fig. 11).



Figure 11. Presentation at COP28 *"Accelerating Application of Climate Smart and Resilient Agricultural Technologies within Asia-Monsoon Countries"*

The introduction of BNI-enabled wheat has garnered significant attention and has been recognized as a valuable contribution by JIRCAS to Nepal. It has also gained support as a technology that simultaneously enhances food security and agricultural sustainability. Furthermore, three representatives from Nepal (Dr. Mahesh Subedi

from NARC, Dr. Deepak Pandey, and Dr. Khem Raj Pant from NWRP) were invited to the 5th BNI International Consortium Meeting, Tsukuba in 2024, which covers global BNI research and is hosted biennially by JIRCAS. They shared the current status of BNI-enabled wheat introduction in Nepal and participated in discussions on its global deployment (Fig. 12).

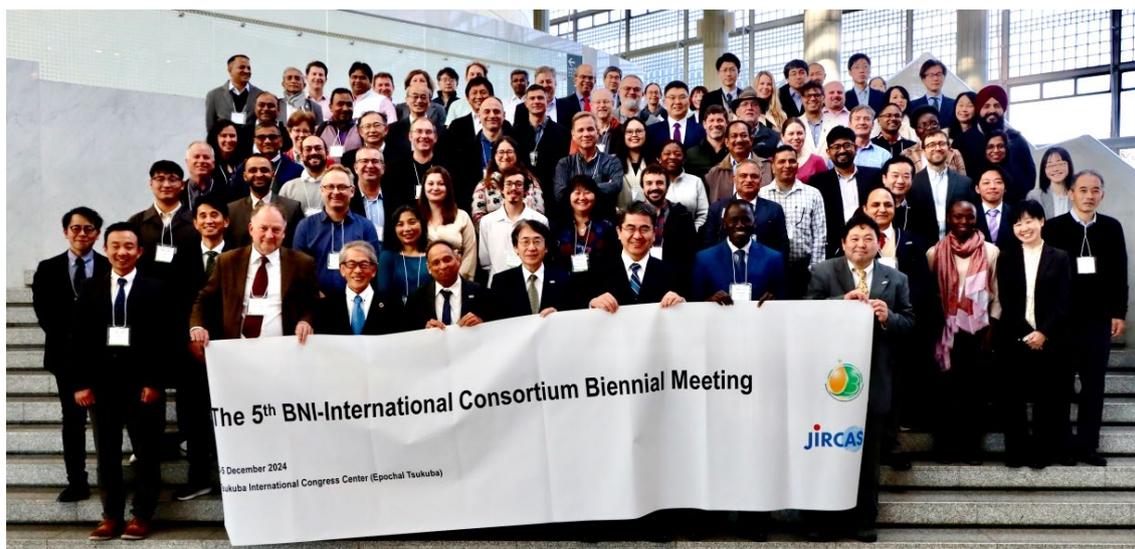


Figure 12. 5th BNI International Consortium Meeting (December 2024, Tsukuba International Congress Center (Epochal Tsukuba))

Although the conference was hosted by JIRCAS, it was held jointly with CIMMYT's CropSustain initiative partly, facilitating broader international networking. For example, collaboration with the Indian Institute of Wheat and Barley Research (IIWBR) has been strengthened, contributing to the development of cooperative frameworks for the future utilization of BNI-enabled wheat.

On March 21, 2024, the first stakeholder meeting on the "Deployment of Biological Nitrification Inhibition (BNI)-enabled wheat into the Nepali wheat production system," under the Green Asia project, was held at the National Wheat Research Program in Bhairahawa, Nepal. The meeting was attended by 20 participants, including representatives from seed companies and the Ministry of Agriculture and Livestock Development (MoALD), Nepal (Fig. 13).



Figure 13. Stakeholders meeting at NWRP (Bhairahawa in March 21, 2024)

During the meeting, we discussed a roadmap for introducing BNI-enabled wheat lines into the Nepali wheat production system. A field visit to the Bhairahawa site was conducted to enhance understanding and acceptance of BNI-enabled wheat among stakeholders.

Participants recognized the importance and potential value of BNI-enabled wheat in Nepal's agricultural system and urged the National Wheat Research Program (NWRP) to accelerate its social implementation. MoALD also shared recent developments in the variety registration system, which now allows the registration of "derived varieties." This change paves the way for BNI-enabled wheat to be officially registered and introduced within the next few years.

7: Toward the Introduction of BNI-enabled Wheat in Nepal

Among the BNI-enabled wheat varieties based on CIMMYT international lines, it was found in the Green Asis project that BNI-enabled Munal suffered significant damage in some areas of Nepal's mountainous regions due to recent outbreaks of yellow rust disease. The parent variety, Munal, had already been replaced in Nepal for the same reason. It is known that the short arm of wheat chromosome 3B, which was replaced with Lr#N-SA, originally carries rust resistance genes *Sr2* and *Sr12*. These genes are believed to enhance the effectiveness of other resistance genes, but Lr#N-SA is thought not to carry such resistance genes. While this issue did not arise in trials conducted by JIRCAS and CIMMYT, it may pose a problem in Nepal, where outbreaks are more severe. On the other hand, most sites in the mountainous region did not experience damage, suggesting that BNI-enabled Munal may still be usable in those areas, though caution is warranted.

BNI-enabled Borlaug 2020, developed for the Terai region, has shown excellent performance in its BC₉ generation. Based on current results, it is considered feasible to proceed with variety registration as a derivative of Borlaug 2020. In Nepal, where wheat production suffers from inefficiencies due to the yield gap, the introduction of BNI-enabled wheat could be a breakthrough. It would contribute to food security and significantly reduce the environmental impact per unit of production by improving nitrogen use efficiency. This makes BNI-enabled wheat particularly suitable for regions where fertilizer application by farmers falls below recommended levels and yield gaps are large.

This approach differs from that of India, where government subsidies ensure that farmers apply fertilizer at nearly recommended levels. In India, the main benefit of introducing BNI-enabled wheat would be a substantial reduction in government spending, providing strong incentives for policymakers. In contrast, in Nepal, the key significance lies in improving farmers' productivity and profitability by narrowing the yield gap through enhanced nitrogen use efficiency.

In any case, the environmental burden of wheat production will be reduced, and it is hoped that BNI-enabled wheat will be adopted in more production sites.

While the current initiative involves replacing CIMMYT international varieties selected by Nepal's public breeding program with BNI-enabled wheat, it is expected that Nepal will eventually utilize BNI wheat technology independently and develop its own BNI-enabled wheat varieties suited to its unique environmental conditions.

To support this foundation, CIMMYT's CropSustain project has been launched with funding from the Novo Nordisk Foundation. Building on the experience gained through the Green Asia Project, the initiative aims to pave the way for actual variety registration and dissemination. JIRCAS is a strategic partner in this effort and plans to continue its involvement in wheat breeding in Nepal.

Author contribution statement

MK and TY contributed equally to leading project activities, drafting the manuscript, and conducting systematic reviews. GVS contributed to conceptualization and manuscript review. DP, MS and KRP contributed to data collection, analysis, and assembly, as well as activities related to multilocation trials in Nepal.

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